Thyroid Function and the Risk of Atherosclerotic Cardiovascular Morbidity and Mortality

Arjola Bano, Layal Chaker, Francesco U.S. Mattace-Raso, Aad van der Lugt,
M. Arfan Ikram, Oscar H. Franco, Robin P. Peeters, Maryam Kavousi

Adapted from Circ Res. *2018;122(3):e18*

ABSTRACT

Background Thyroid hormones have been linked with various proatherogenic and antiatherogenic processes. However, the relationship of thyroid function with manifestations of atherosclerosis remains unclear. We therefore aimed to investigate the association of thyroid function with atherosclerosis throughout its spectrum, from subclinical atherosclerosis to incident atherosclerotic cardiovascular (ASCV) events to ASCV mortality.

Methods This population-based study was embedded within the Rotterdam Study. The risk of atherosclerosis was evaluated by measuring: (1) Presence of subclinical atherosclerosis, assessed by coronary artery calcification (CAC) score >100 AU; (2) ASCV events, defined as fatal and nonfatal myocardial infarction, other coronary heart disease mortality or stroke; (3) ASCV mortality, defined as death because of coronary heart disease, cerebrovascular or other atherosclerotic diseases. Associations of thyroid-stimulating hormone (TSH) and free thyroxine (FT_4) with the outcomes were assessed through logistic regression and Cox proportional hazard models, adjusted for potential confounders including cardiovascular risk factors.

Results A total of 9420 community-dwelling participants (mean age ± standard deviation, 64.8±9.7 years) were included. During a median follow-up of 8.8 (interquartile range, 4.5 to 11.8) years, 934 incident ASCV events and 612 ASCV deaths occurred. FT_4 levels were positively associated with high CAC score (odds ratio [OR], 2.28; 95% confidence interval [95% CI], 1.30 to 4.02) and incident ASCV events (hazard ratio [HR], 1.87; 95% CI, 1.34 to 2.59). The risk of ASCV mortality increased in a linear manner with higher FT_4 levels (HR, 2.41; 95% CI, 1.68 to 3.47 per 1 ng/dL) and lower TSH levels (HR, 0.92; 95% CI, 0.84 to 1.00 per 1 logTSH). Results remained similar or became stronger among euthyroid participants.

Conclusions FT_4 levels in middle-aged and elderly subjects were positively associated with atherosclerosis throughout the whole disease spectrum, independently of cardiovascular risk factors.

INTRODUCTION

Atherosclerosis progresses insidiously from a subclinical condition to the clinical onset of vascular events to death.[1] Despite advances in prevention and treatment, atherosclerotic disease remains a leading cause of death, with a considerable clinical and economic burden worldwide.[2] Hence, the identification of additional modifiable risk factors for atherosclerosis is of major importance.

Thyroid function has a complex relation with various contributors to atherogenesis. Higher thyroid hormone concentrations have commonly been linked with systolic hypertension[3,4] and hypercoagulation,[5] whereas lower levels of circulating thyroid hormones can instigate hyperlipidemia and inflammation.[6] Although atherosclerosis is a continuous process, prospective epidemiological studies to date have mainly focused on the relation between specific ranges of thyroid function and distinct atherosclerotic events, such as coronary heart disease (CHD) or stroke.[7-12] Results have been inconsistent, including studies that find no association between thyroid function and atherosclerotic outcomes,[8,12] as well as other studies reporting an increased risk of atherosclerotic outcomes with either lower[7,10] or higher thyroid function.[9,11,13] Differences in study designs, follow-up period, and age range of participants may partly explain the inconsistencies across studies. In addition, these inconsistencies can also stem from the heterogeneity in the assessment of atherosclerosis. To date, a comprehensive investigation exploring the link between the full range of thyroid function and atherosclerosis throughout its spectrum, from subclinical atherosclerosis to overt atherosclerosis to atherosclerotic mortality, within the same cohort is lacking.

Therefore, in a large population-based cohort study of middle-aged and elderly individuals, we examined the association of thyroid function with atherosclerosis throughout its spectrum, including coronary artery calcification (CAC: as a well-documented marker of subclinical atherosclerosis),[14] atherosclerotic cardiovascular (ASCV) events (as a measure of clinical atherosclerosis) and ASCV mortality.

METHODS

Study population

This study was embedded within the Rotterdam Study, a prospective population-based cohort study that investigates the determinants, occurrence and progression of chronic diseases in the middle-aged and elderly. The objectives and study design

have been described in detail previously.[15] The Rotterdam Study was initiated in 1989, including 7983 participants aged 55 years or older (RS I) residing in Ommoord district of Rotterdam, the Netherlands. In 2000, the study was extended with a second cohort of 3011 subjects (RS II). In 2006, a third cohort of 3932 subjects aged 45 years or older was added (RS III). Study participants undergo extensive follow-up medical examinations every 3 to 5 years.

Baseline measurements for our study were performed during the third visit of the first cohort (n=4797) and the first visit of the second (n=3011) and third (n=3932) cohorts of the Rotterdam Study. The original cohort during these three visits included a total of 11740 participants, of which 10063 had available blood measurements. Thyroid function measurements were performed at baseline in a random sample of 9683 participants of the Rotterdam Study, during the third visit of the first cohort and the first visit of the second and third cohorts. Of these, 9420 participants had complete information on prevalent ASCV disease status and complete follow-up data; and were considered eligible for the analysis (Supplemental Figure 1). Follow-up started at the date of thyroid function assessment. In the analysis of incident ASCV events, the end date of follow-up was considered the date of incident ASCV event, the date of death or 1 January, 2012, whichever came first. In the analysis of ASCV mortality, the end date of follow-up was considered the date of death or 1 January, 2012, whichever came first.

The Rotterdam Study has 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. All participants have given written informed consent.

Assessment of thyroid function

Thyroid function was assessed at baseline in 3 study cohorts using the same method and assay. Measurements of thyroid-stimulating hormone (TSH), free thyroxine (FT_4), and thyroid peroxidase antibodies (TPOAb) were performed in baseline serum samples stored at -80°C using the electrochemiluminescence immunoassay ECLIA Roche. The reference ranges of serum TSH (0.40–4.0 mIU/L) and serum FT_4 (0.86–1.94 ng/dL; equivalent to 11–25 pmol/L) were determined based on national guidelines and our previous studies.[16]

Assessment of CAC, ASCV events, and ASCV mortality

The risk of atherosclerosis was evaluated by measuring CAC, ASCV events and ASCV mortality. Hard outcomes were included to avoid misclassification bias.

CAC measurements were performed at baseline in a random sample of 1999 participants, during the third visit of the first cohort and the first visit of the second cohort. CAC was measured by electron beam computed tomographic scans (C-150 Imatron GE) of the coronary arteries.[15] Calcification of the coronary arteries was quantified through AccuImage software (AccuImage Diagnostics Corp), displaying all pixels with a density >130 Hounsfield units and using the Agatston's method.[17] CAC score of ≥100 Agatson units (AU) suggests clinically significant atherosclerotic plaque [2] and has been used in past consensus statements.[18] Therefore, we grouped participants into CAC score <100 AU and CAC score ≥100 AU.

ASCV events were defined as fatal and nonfatal myocardial infarction, other CHD mortality, or stroke, as described previously.[19] Prevalent ASCV disease was defined as history of myocardial infarction, stroke, and coronary or other arterial revascularization.[19,20] Prevalent ASCV disease at baseline was assessed through interview and verified in medical records.

ASCV mortality was defined as death because of CHD, cerebrovascular disease, or other atherosclerotic diseases, as described previously.[19,21] Non-ASCV mortality was defined as death because of causes other than atherosclerotic disease. Ascertainment of ASCV mortality in the Rotterdam Study has been described in detail previously.[21] In short, information on ASCV 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. The completeness of follow-up for ASCV mortality was 99%.

Additional measurements

Information on medical history and medication use was obtained from questionnaires in combination with medical records. Information on the history of thyroid disease, thyroid surgery and thyroid medication use was obtained from questionnaires in combination with pharmacy records. During the baseline home interview, participants provided information on smoking habits and the number of alcoholic beverages they consumed weekly. Smoking habits were categorized as current, former and never smoking. Serum glucose and lipid levels were measured by an automated enzymatic procedure (Mannheim System). Estimated glomerular filtra-

tion rate (eGFR) was calculated according to the CKD Epidemiology Collaboration (CKD-EPI) formula.[22] Physical activity was measured by questionnaires and expressed in metabolic equivalent hours (METh)/week. Body mass index (BMI) 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 ≥7 mmol/L, non-fasting plasma glucose level ≥11.1 mmol/L (when fasting samples were absent) or the use of antidiabetic medications.[15] Atrial fibrillation (AF) cases were ascertained by two research physicians and a cardiologist utilizing: 1) electrocardiograms recorded at baseline and during follow-up; 2) additional medical information obtained from general practitioners files, from outpatient clinics and from a national registry of all hospital discharge diagnoses.[15,16] Heart failure (HF) was defined as the presence of typical symptoms and signs (ie, breathlessness at rest or during exertion, ankle edema, and pulmonary crepitations), confirmed by the objective evidence of cardiac dysfunction (ie, chest X-ray, echocardiography) or a positive response to the initiated treatment.[15] The adjudication of HF was performed in accordance with the guidelines of the European Society of Cardiology.[23] Measurements of thyroid peroxidase antibodies (TPOAb) were performed in baseline serum samples stored at -80°C using the electrochemiluminescence immunoassay ECLIA Roche. Levels of TPOAb >35 kU/ml were regarded as positive, as recommended by the assay manufacturer.

Statistical analysis

The association of thyroid function with atherosclerosis spectrum was assessed by using logistic regression and Cox proportional hazard models. We used logistic regression models to investigate the cross-sectional association of thyroid function with the risk of having CAC score of ≥100 AU, among participants who were free of ASCV disease. The association of thyroid function with incident ASCV events was prospectively investigated through Cox proportional hazard models. The analysis on incident ASCV events comprised individuals who were free of any ASCV event at baseline and only first events during follow-up were analyzed. The association of thyroid function with ASCV mortality was also examined through Cox proportional-hazard models. We further compared the hazard ratios (HR) of ASCV mortality with those of non-ASCV mortality. To account for multiple comparisons, the analyses investigating the association of TSH or FT_4 with subclinical atherosclerosis, incident

ASCV events, and ASCV mortality were additionally corrected for the false discovery rate using the Benjamini and Hochberg method.[24]

Analyses were stratified by sex (men versus women) and age. The latter was grouped based on the cutoff of 65 years, which is closer to the mean and median age of our population.

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 status, alcohol intake, BMI, total cholesterol, triglycerides, systolic blood pressure, prevalent diabetes mellitus and use of antihypertensive and lipid-lowering medications. The analyses of ASCV mortality were additionally adjusted for presence of prevalent ASCV disease.

TSH was naturally log transformed, due to its skewed distribution. The proportional hazards assumption was assessed by Schoenfeld test and plots. No violation of the proportional hazards assumption was observed. Potential departure from linearity was explored by adding quadratic and cubic terms of covariates in the multivariable model, but none of these terms were significant. We checked for effect modification by separately adding product interaction terms of the exposure (TSH or FT_4 or TPOAb) with each of the covariates of the most adjusted model.

Multiple imputations were performed for missing data (<5% missings for all covariates). Schoenfeld test and plots were performed using R (survival package R project, Institute for Statistics and Mathematics, R Core Team, version 3.2.3). All other statistical analyses were performed using IBM SPSS version 21 (IBM Corp). Results of this study are reported according to the STROBE statement guidelines (Supplemental Material).[25]

Sensitivity analyses

We performed several sets of sensitivity analyses to explore the robustness of our findings: (1) We limited the study participants to only those with thyroid function within the reference range, without history of thyroid disease and not using thyroid medications; (2) Thyroid function-altering medications, physical activity and estimated glomerular filtration rate (eGFR) can influence the metabolism of thyroid hormones. Therefore, we excluded participants using thyroid function-altering medications, such as thyroid medications, analgesics (including nonsteroidal anti-inflammatory drugs, paracetamol and muscle relaxants), corticosteroids or amiodarone. Besides, we additionally adjusted our analyses for physical activity or estimated glomerular

filtration rate; (3) We restricted the analyses to participants without history of AF and censored the incident AF cases during follow-up; (4) We restricted the analyses to participants without history of HF at baseline and censored the incident HF cases during follow-up; (5) To account for possible reverse causation, we investigated the association of thyroid function with incident ASCV events and ASCV mortality, after excluding the events that occurred during the first 2 years of follow-up; (6) We investigated the association of thyroid function with CAC score as a continuous variable, among participants who were free of ASCV disease. Because of its skewed distribution, CAC score was log transformed after adding 1 (ln[CAC+1]); (7) To explore the role of thyroid autoimmunity on atherosclerotic outcomes, we investigated the association of TPOAb levels with the risk of incident ASCV events and ASCV deaths.

RESULTS

We included 9420 participants with a maximum follow-up time of 14.7 years and a median of 8.8 years (interquartile range, 4.5 to 11.8 years). Baseline characteristics are presented in Table 1. The mean age of participants was 64.8 (±9.7) years and 56.7% were women (Table 1). A total of 934 incident ASCV events (incidence rate, 12.6 per 1000 person-years) and 612 ASCV deaths (incidence rate, 7.9 per 1000 person-years) occurred during follow-up. Results did not change substantially after primary and additional adjustments for potential confounders; therefore we further report the most adjusted model (Model 2).

The characteristics and determinants of thyroid function in the Rotterdam Study population have been described in detail previously.[26] In brief, the main determinants of FT_4 in the Rotterdam Study population are age, BMI, sex, and TPOAb levels, whereas the main determinants of TSH are age, smoking, and TPOAb levels.

Thyroid function and CAC score

Increasing FT_4 levels were associated with higher odds of having CAC score ≥100 AU (odds ratio [OR], 2.28; 95% confidence interval [95% CI], 1.30 to 4.02 per 1 ng/dL; Table 2). The association remained statistically significant after controlling for the false discovery rate (false discovery rate-corrected *P* value, 0.008; Table 2). However, TSH levels were not associated with having a CAC score ≥100 AU (OR, 0.94; 95% CI, 0.84 to 1.05 per 1 logTSH; Table 2). No evidence of nonlinearity was observed (*P* for nonlinearity for FT_4 and TSH 0.8 and 0.6, respectively; Supplemental Figure 2).

Table 1. Baseline characteristics of 9420 participants*

Age, years	64.8 (9.7)
Women, n (%)	5342 (56.7)
Smoking, n (%)	
current	2001 (21.2)
former	4481 (47.6)
never	2938 (31.2)
TSH, mIU/L	1.9 (1.2-2.8)
FT_4, ng/dL	1.2 (0.2)
TPOAb positive, n (%)	1251 (13.3)
Use of thyroid medication, n (%)	303 (3.2)
Thyroid surgery, n (%)	170 (1.8)
History of thyroid disease, n (%)	765 (8.1)
Body mass index, kg/m^2	27.2 (4.2)
Total cholesterol, mmol/l	5.7 (1.0)
Triglycerides, mmol/l	1.5 (0.8)
Use of lipid-lowering medications, n (%)	1518 (16.1)
Systolic blood pressure, mm Hg	139.4 (21.0)
Use of antihypertensive medications, n (%)	2176 (23.1)
History of diabetes mellitus, n (%)	1077 (11.4)
History of atherosclerotic cardiovascular disease, n (%)	922 (9.8)
Follow-up time for atherosclerotic events, years	7.1 (4.3-11.6)
Follow-up time for atherosclerotic mortality, years	8.8 (4.5-11.8)

Abbreviations: TSH, thyroid-stimulating hormone; FT_4, free thyroxine; TPOAb, thyroid peroxidase antibodies (cutoff 35 kU/ml). *Data are presented as mean (standard deviation) or median (25^{th}-75^{th} percentiles), unless otherwise specified.

Restricting the analyses to participants with thyroid function within the reference ranges resulted in similar or increased point estimates, with wider 95% CIs (OR, 2.43; 95% CI, 1.14 to 5.16 per 1 ng/dL FT_4; OR, 0.91; 95% CI, 0.72 to 1.16 per 1 logTSH; Table 2). Results were consistent in the analyses with CAC score as a continuous variable (β, 0.54; 95% CI, 0.01 to 1.08 per 1 ng/dL FT_4; β, 0.01; 95% CI, -0.10 to 0.12 per 1 log TSH; Supplemental Figure 2). Furthermore, we found no association of TPOAb with the odds of having CAC score ≥100 AU (OR, 0.85; 95% CI, 0.63 to 1.15; Supplemental Table 1). In the analyses of CAC score, the interaction terms between the exposure (TSH or FT_4) and each covariate in the most adjusted model were not statistically significant. In particular, there were no significant sex or age differences (*P* for interaction of TSH and FT_4 with sex [men versus women], 0.96 and 0.88, respectively; *P* for interaction of TSH and FT_4 with age [<65 versus ≥65 years], 0.88 and 0.67, respectively; Supplemental Table 4).

Table 2. Cross-sectional association of thyroid function with high CAC score*

	High CAC score /TN, %	OR (95% CI) Model 1	*P* value	OR (95% CI) Model 2	*P* value
All participants					
TSH	817/1763 (46.3%)	0.98 (0.88; 1.10)	0.79	0.94 (0.84; 1.05)	0.29‡
FT_4	817/1763 (46.3%)	**2.15 (1.22; 3.77)**	0.008	**2.28 (1.30; 4.02)**	0.004§
Thyroid function within the reference ranges†					
TSH	626/1336 (46.8%)	1.00 (0.78; 1.27)	0.98	0.91 (0.72; 1.16)	0.48
FT_4	626/1336 (46.8%)	**2.50 (1.18; 5.29)**	0.01	**2.43 (1.14; 5.16)**	0.02

Model 1: age, sex, and cohort. Model 2: Model 1, smoking, alcohol intake, body mass index, total cholesterol, triglycerides, systolic blood pressure, prevalent diabetes mellitus, use of antihypertensive medications, and use of lipid-lowering medications. *CAC ≥100 AU was defined as high CAC score. All included participants were free of atherosclerotic cardiovascular disease. †Normal reference ranges of thyroid function were defined as serum TSH levels of 0.4 to 4.0 mIU/L and serum FT_4 levels of 0.85 to 1.95 ng/dL, after excluding thyroid medication users and participants with history of thyroid disease. ‡False discovery rate-corrected *P* value, 0.34. §False discovery rate-corrected *P* value, 0.008. ORs of TSH are denoted per 1 unit increase of natural log transformed TSH (mIU/L). ORs of FT_4 are denoted per 1 unit increase in FT_4 (ng/dL). Abbreviations: CAC, coronary artery calcification; TN, total number; OR, odds ratio; CI, confidence interval; TSH, thyroid-stimulating hormone; FT_4, free thyroxine.

Thyroid function and incident ASCV events

There was a positive association of FT_4 levels with the risk of incident ASCV events (HR, 1.87; 95% CI, 1.34 to 2.59 per 1 ng/dL), which remained statistically significant after controlling for the false discovery rate (false discovery rate-corrected *P* value, 0.0006; Table 3). There was no association of TSH levels (HR, 0.96; 95% CI, 0.89 to 1.03 per 1 logTSH) with the risk of incident ASCV events (Table 3). No evidence of nonlinearity was observed (*P* for nonlinearity for FT_4 and TSH, 0.6 and 0.8, respectively; Supplemental Figure 2). Restricting the analyses to participants with thyroid function within the reference ranges resulted in similar or increased point estimates, with wider 95% CIs (HR, 2.50; 95% CI, 1.58 to 3.94 per 1 ng/dL; HR, 0.96; 95% CI, 0.82 to 1.11 per 1 logTSH; Table 3). This corresponds to a 2.70–times higher risk of incident ASCV events, for a participant with an FT_4 in the higher limit of the reference range (1.94 ng/dL), compared with a participant with an FT_4 in the lower limit of the reference range (0.86 ng/dL). The associations became slightly stronger after excluding participants using thyroid function-altering medications; and slightly attenuated after censoring the analyses at the time of incident AF (Supplemental Table 2). The associations remained similar after censoring the analyses at the time of incident HF; or after additionally adjusting for physical activity or estimated glomerular filtration rate. Results did not change substantially after excluding events that occurred during the first 2 years of follow-up (Supplemental Table 3). Furthermore, we found no

Table 3. Association of thyroid function with incident atherosclerotic cardiovascular events*

	Events/TN, %	HR (95% CI) Model 1	*P* value	HR (95% CI) Model 2	*P* value
All participants					
TSH	934/8498 (11.0%)	0.96 (0.89; 1.03)	0.34	0.96 (0.89; 1.03)	0.35‡
FT_4	934/8498 (11.0%)	**1.89 (1.37; 2.61)**	<0.0001	**1.87 (1.34; 2.59)**	0.0002§
Thyroid function within the reference ranges†					
TSH	736/6826 (10.8%)	0.95 (0.81; 1.10)	0.50	0.96 (0.82; 1.11)	0.59
FT_4	736/6826 (10.8%)	**2.67 (1.69; 4.20)**	<0.0001	**2.50 (1.58; 3.94)**	<0.0001

Model 1: age, sex, and cohort. Model 2: Model 1, smoking, alcohol intake, body mass index, total cholesterol, triglycerides, systolic blood pressure, prevalent diabetes mellitus, use of antihypertensive medications, and use of lipid-lowering medications. *All included participants were free of atherosclerotic cardiovascular disease at baseline. †Normal reference ranges of thyroid function were defined as serum TSH levels of 0.4 to 4.0 mIU/L and serum FT_4 levels of 0.85 to 1.95 ng/dL, after excluding thyroid medication users and participants with history of thyroid disease. ‡False discovery rate-corrected *P* value, 0.35. §False discovery rate-corrected *P* value, 0.0006. 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). Abbreviations: TN, total number; HR, hazard ratio; CI, confidence interval; TSH, thyroid-stimulating hormone; FT_4, free thyroxine.

association of TPOAb with the risk of incident ASCV events (HR, 0.95; 95% CI, 0.78 to 1.16; Supplemental Table 1). In the analyses of incident ASCV events, the interaction terms between the exposure (TSH or FT_4) and each covariate in the most adjusted model were not statistically significant. In particular, there were no significant sex or age differences (*P* for interaction of TSH and FT_4 with sex [men versus women], 0.92 and 0.62, respectively; *P* for interaction of TSH and FT_4 with age [<65 and ≥65 years], 0.30 and 0.34, respectively; Supplemental Table 4).

Thyroid function and ASCV mortality

Higher FT_4 levels were associated with a higher risk of ASCV mortality (HR, 2.41; 95% CI, 1.68 to 3.47 per 1 ng/dL; Table 4). The association remained statistically significant after controlling for the false discovery rate (false discovery rate-corrected *P* value, <0.0001; Table 4). In line, higher TSH levels were associated with a lower risk of ASCV mortality (HR, 0.92; 95% CI, 0.84 to 1.00 per 1 logTSH), although the association was borderline significant (Table 4). No evidence of nonlinearity was observed (*P* for nonlinearity for FT_4 and TSH, 0.8 and 0.8, respectively; Supplemental Figure 2).

In the analysis of ASCV mortality, we found statistically significant differences by prevalent ASCV disease at baseline and by sex (*P* for interaction of FT_4 with prevalent ASCV disease [present versus absent], 0.002; *P* for interaction of TSH and FT_4 with sex [men versus women], 0.03 and 0.002, respectively), but no statistically

Table 4. Association of thyroid function with atherosclerotic cardiovascular mortality

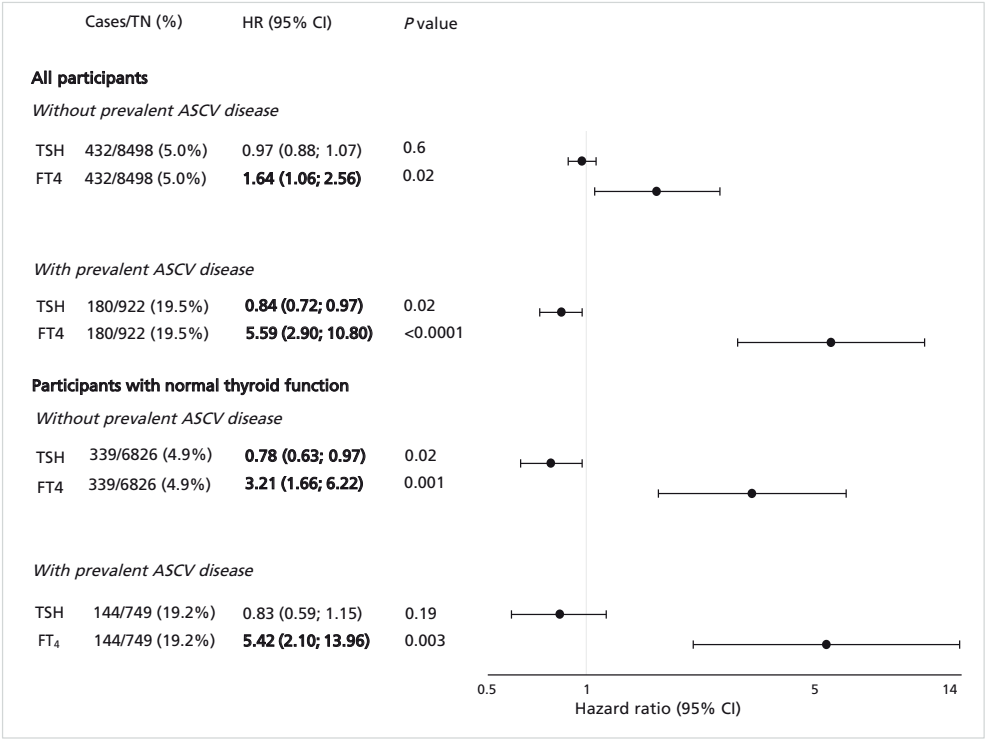
	Events/TN, %	HR (95% CI) Model 1	*P* value	HR (95% CI) Model 2	*P* value
All participants					
TSH	612/9420 (6.5%)	0.93 (0.86; 1.01)	0.12	0.92 (0.84; 1.00)	0.06†
FT_4	612/9420 (6.5%)	**2.23 (1.58; 3.14)**	<0.0001	**2.41 (1.68; 3.47)**	<0.0001‡
*Thyroid function within the reference ranges**					
TSH	483/7575 (6.4%)	0.82 (0.68; 0.98)	0.02	0.80 (0.67; 0.96)	0.01
FT_4	483/7575 (6.4%)	**3.92 (2.29; 6.71)**	<0.0001	**3.84 (2.23; 6.60)**	<0.0001

Model 1: age, sex, and cohort. Model 2: Model 1, smoking, alcohol intake, body mass index, total cholesterol, triglycerides, systolic blood pressure, prevalent diabetes mellitus, use of antihypertensive medications, and use of lipid-lowering medications. Both models are adjusted for presence of prevalent atherosclerotic cardiovascular disease at baseline. *Normal reference ranges of thyroid function were defined as serum TSH levels of 0.4 to 4.0 mIU/L and serum FT_4 levels of 0.85 to 1.95 ng/dL, after excluding thyroid medication users and participants with history of thyroid disease. †False discovery rate-corrected *P* value, 0.09. ‡False discovery rate-corrected *P* value, <0.0001. 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). Abbreviations: TN, total number; HR, hazard ratio; CI, confidence interval; TSH, thyroid-stimulating hormone; FT_4, free thyroxine.

significant age differences (*P* for interaction of TSH and FT_4 with age [<65 versus ≥65 years], 0.35 and 0.78, respectively; Figure 1, Supplemental Table 4). The association of FT_4 with atherosclerotic mortality was more pronounced among participants with ASCV disease (HR, 5.59; 95% CI, 2.90 to 10.80 per 1 ng/dL) and among men (HR, 4.63; 95% CI, 2.70 to 7.94 per 1 ng/dL) (Figure 1, Supplemental Table 4). Within the strata of prevalent ASCV disease (absent versus present), none of the interaction terms of TSH or FT_4 concentrations with sex or age were statistically significant (*P* for interaction, >0.05; Figure 1). Within the strata of sex (men versus women), none of the interaction terms of TSH or FT_4 concentrations with prevalent ASCV disease or age were statistically significant (*P* for interaction, >0.05). Among euthyroid participants, none of the interaction terms between the exposure (TSH or FT_4) and prevalent ASCV disease (absent versus present), sex (men versus women) or age (<65 versus ≥65 years) were statistically significant (*P* for interaction, >0.05).

The association of thyroid function with ASCV mortality became slightly stronger after restricting the analyses to participants with thyroid function within the reference ranges (HR, 3.84; 95% CI, 2.23 to 6.60 per 1 ng/dL FT_4; HR, 0.80; 95% CI, 0.67 to 0.96 per 1 logTSH; Table 4, Figure 1). This corresponds to a 4.15–times higher risk of ASCV mortality, for a participant with an FT_4 in the higher limit of the reference range (1.94 ng/dL), compared with a participant with an FT_4 in the lower limit of the reference range (0.86 ng/dL). The associations remained similar after excluding participants us-

Figure 1. Association of thyroid function with atherosclerotic cardiovascular mortality, stratified by presence of atherosclerotic cardiovascular disease at baseline.



Analyses are adjusted for age, sex, cohort, smoking, alcohol intake, body mass index, total cholesterol, triglycerides, systolic blood pressure, prevalent diabetes mellitus, use of antihypertensive medications, and use of lipid-lowering medications. The *P* for interactions of thyroid-stimulating hormone (TSH) and free thyroxine (FT_4) with prevalent atherosclerotic cardiovascular (ASCV) disease (absent vs present) were 0.09 and 0.002, respectively. Among participants without ASCV disease, the *P* for interactions of TSH and FT_4 with sex (men vs women) were 0.18 and 0.07, respectively; the *P* for interactions of TSH and FT_4 with age (<65 vs ≥65 years) were 0.38 and 0.29, respectively. Among participants with ASCV disease, the *P* for interactions of TSH and FT_4 with sex (men vs women) were 0.34 and 0.44, respectively; the *P* for interactions of TSH and FT_4 with age (<65 vs ≥65 years) were 0.61 and 0.13, respectively. Normal thyroid function was defined as serum TSH levels 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 history of thyroid disease. Error bars represent the 95% confidence interval (CI) of HRs (black dots). Within brackets: Number of ASCV deaths / Total number (TN).

ing thyroid function-altering medications, after censoring the analyses at the time of incident HF or after additionally adjusting for physical activity or estimated glomerular filtration rate. The associations slightly attenuated after censoring the analyses at the time of incident AF (Supplemental Table 2). Results did not change substantially after excluding events that occurred during the first 2 years of follow-up (Supplemental Table 3). The magnitude of association for ASCV mortality was larger than for non-ASCV mortality (Table 5). Furthermore, we found no association of TPOAb with the risk of ASCV mortality (HR, 0.99; 95% CI, 0.77 to 1.27; Supplemental Table 1).

Table 5. Comparison on the association of thyroid function with the risk of ASCV mortality and non-ASCV mortality

	ASCV deaths/TN (%)	ASCV mortality HR (95% CI)	*P* value	Non-ASCV deaths/TN (%)	Non-ASCV mortality HR (95% CI)	*P* value
TSH	612/9420 (6.5%)	0.92 (0.84; 1.00)	0.06	1476/9420 (15.7%)	**0.94 (0.89; 0.99)**	0.03
FT_4	612/9420 (6.5%)	**2.41 (1.68; 3.47)**	<0.0001	1476/9420 (15.7%)	**1.65 (1.29; 2.10)**	<0.0001

Adjusted for age, sex, cohort, prevalent atherosclerotic cardiovascular disease, smoking, alcohol intake, body mass index, total cholesterol, triglycerides, systolic blood pressure, prevalent diabetes mellitus, use of antihypertensive medications, and use of lipid-lowering medications. 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). Abbreviations: ASCV, atherosclerotic cardiovascular; TN, total number; HR, hazard ratio; CI, confidence interval; TSH, thyroid-stimulating hormone; FT_4, free thyroxine.

DISCUSSION

In this large population-based cohort study, higher FT_4 levels were associated with an increased risk of atherosclerosis, independent of cardiovascular risk factors. The association was consistent throughout the spectrum of atherosclerosis, from subclinical atherosclerosis to overt atherosclerosis to atherosclerotic mortality.

Various cardiovascular risk factors have been implicated in the pathways linking thyroid function to atherosclerosis. Low thyroid function has been associated with unfavorable levels of blood lipids and BMI,[6] whereas high thyroid function has been associated with an increased prevalence of AF.[16] Our study suggests that the association of thyroid function with atherosclerosis is independent of these cardiovascular risk factors because our results remained statistically significant after accounting for serum lipid levels, BMI, and AF. Thyroid autoimmunity has been suggested as another potential contributor to atherogenesis. Thus far, it has been speculated that thyroid autoantibodies may target the arterial wall and ultimately enhance the development of atherosclerotic plaque.[27,28] However, we found no association between TPOAb positivity and atherosclerotic outcomes. Taken together, these data suggest that the link between thyroid function and atherosclerosis could be explained by yet unexplored cardiovascular risk factors, alternative markers of thyroid autoimmunity (eg, TSH receptor antibodies) or other pathways.

Plausible mechanisms that can link high thyroid function to atherosclerosis include endothelial damage, hemostasis, thrombosis and hemodynamic changes. First, excess concentrations of thyroid hormones can increase the production of reactive oxygen species that further induce the expression of adhesion molecules on endothelial cells.[29] Hence, hyperthyroidism has been commonly associated with early atherosclerosis and markers of endothelial dysfunction such as E-selectin, intracel-

lular adhesion molecule-1, and vascular cell-adhesion molecule.[30] Second, thyroid hormones regulate the synthesis of procoagulant proteins.[31] Excess FT_4 levels have been linked with increased concentrations of various procoagulant proteins, namely von Willebrand factor, fibrinogen, and factors VIII and IX, that can accelerate plaque vulnerability and rupture.[5] Third, high levels of thyroid hormones can generate increased cardiac contractility and workload, augmenting myocardial oxygen demand that could eventually precipitate ischemic events and death.[3] These deleterious effects of high thyroid function may also be extended to the high-normal range of thyroid function.[11] Future research should pinpoint the exact mechanisms underlying the association of thyroid function with atherogenesis.

Our large cohort study sought to disentangle the association of thyroid function with atherosclerotic and non-atherosclerotic mortality. The effect of thyroid function on atherosclerotic mortality was greater compared with non-atherosclerotic mortality, indicating that atherosclerosis plays a major role in the pathways linking high thyroid function to increased mortality risk.

Previous cohort studies among middle-aged and elderly subjects have mainly reported an increased mortality risk with higher thyroid function.[32-35] In an attempt to identify potential subgroups at risk, prior research has suggested that the effect of thyroid function on mortality might be age [32-35] or sex dependent.[33,34] Generally, studies performed in older participants have reported an increased risk of mortality with higher FT_4 levels,[33,34] whereas studies including younger participants have failed to show an association.[36,37] Additional studies have reported an association of thyroid function with mortality risk exclusively in men [38] or women.[7] Our data on atherosclerotic mortality revealed stronger risk estimates among participants with preexisting atherosclerotic disease and among men. Prevalent atherosclerotic disease and sex might, therefore, be effect modifiers of the association between thyroid function and atherosclerotic mortality. However, these results should be interpreted with caution because the differences by atherosclerotic disease and sex were not statistically significant after restricting the analyses to the euthyroid participants.

Alternatively, one might argue that thyroid hormone is not a contributor but rather a marker of subclinical atherosclerosis or a marker of increased mortality in the setting of chronic atherosclerosis. In particular, it could be hypothesized that health problems underlying atherosclerotic disease can affect thyroid parameters. This condition, known as non-thyroidal illness, is typically characterized by normal

serum TSH levels combined with low serum triiodothyronine and FT_4 levels.[39] In contrast, we found an association of higher rather than lower FT_4 levels with an increased risk of atherosclerotic manifestations. Additionally, non-thyroidal illness occurs mainly in critically ill patients, whereas our population consists of relatively healthy community-dwelling adults. Furthermore, our study showed that higher FT_4 levels among participants without preexisting atherosclerotic disease were associated with higher risk of atherosclerotic events and atherosclerotic mortality. We took reverse causation into account by excluding events that occurred during the first 2 years of follow-up; and results remained similar. Overall, these data suggest that it is more likely that thyroid function affects atherosclerotic manifestations than vice versa.

Variations of thyroid function within the reference range markedly affected the risk of atherosclerotic morbidity and mortality in our participants. In line with these results, a recent individual participant data analysis reported a positive association between thyroid function within the reference range and the risk of stroke.[9] However, another analysis from the same collaboration failed to show an association between thyroid function within the reference range and the risk of CHD,[8] although this could be because of the relatively low proportion of CHD deaths (3.3%). We observed larger risk estimates after restricting the analyses to participants with TSH and FT_4 levels within the reference ranges, although one would expect a higher outcome risk within the full range of thyroid function. However, euthyroid participants are known to have a small intra-individual variation of thyroid function.[40] In contrast, participants with thyroid dysfunction are prone to treatment during follow-up, which could eventually reduce their risk for atherosclerotic morbidity and mortality over time. This can explain the increased atherosclerotic risk after we excluded the users of thyroid function-altering medications from the analyses. Future interventional studies, however, can provide more insight into the impact of thyroid function-altering medications on the risk of atherosclerosis. Finally, our data provide supporting evidence for a reevaluation of TSH and FT_4 reference ranges, which are currently based on arbitrary statistical approaches (2.5^{th} and 97.5^{th} percentiles) rather than on clinical outcomes. Previous prospective studies have also reported that variations in thyroid function within the reference range are associated with an increased risk of various adverse outcomes.[9,11,16] Thus, the challenge for future research will be to integrate the associated risk of relevant adverse outcomes, in order to eventually define the clinically relevant normal range of thyroid function.

In our study population, there was a positive association between FT_4 levels and atherosclerotic outcomes. Although the association between TSH levels and atherosclerotic outcomes was in the expected opposite direction of FT_4, it sometimes did not reach statistical significance. Similar observations have been also reported by studies investigating the relationship of thyroid function with various clinical end points.[9,33] Serum FT_4 levels are tightly regulated by the hypothalamic-pituitary-thyroid axis, with a different set point for each individual. This might explain why FT_4 levels are associated with various clinical end points, especially within the euthyroid range which is generally defined by TSH. Alternatively, these results may reflect a slight shift in the TSH-FT_4 set point, which may be because of ageing.[41]

To our knowledge, this is the first population-based cohort study that investigates the relationship of thyroid function with atherosclerosis throughout its spectrum, from subclinical atherosclerosis to overt atherosclerosis to atherosclerotic mortality. Thyroid function measurements were performed before the occurrence of atherosclerotic events. Another major strength is the long-term follow-up (maximum follow-up time was almost 15 years). Moreover, we included a large number of participants with extensive data on covariates and outcomes. Our large numbers further allowed us to perform multiple sensitivity analyses which provided consistent findings. Also, we were able to account for the main determinants of thyroid function in the Rotterdam Study population.

Several limitations should also be considered. Thyroid function was measured only at baseline and we had no information on its fluctuations over time. Nevertheless, because of the intra-individual variability of TSH and FT_4 levels, the lack of repeated measurements would tend to underestimate the association between thyroid function and atherosclerotic outcomes rather than generate spurious findings.[42] In addition, our results were consistent within the normal range of thyroid function, which is considered to be stable with small intra-individual variability.[40] We lacked information on serum triiodothyronine levels. However, TSH and FT_4 represent the most relevant measurements of thyroid function in clinical practice. Given that our study comprised mainly white middle-aged and older adults, the generalizability of our findings to nonwhite and younger populations remains to be investigated. Lastly, the possibility of residual confounding in an observational study design cannot be entirely ruled out.

Conclusions

Higher FT_4 levels in middle-aged and elderly subjects were associated with an increased risk of atherosclerotic morbidity and mortality, independent of cardiovascular risk factors. These findings suggest that FT_4 measurement can be a predictive marker of atherosclerotic mortality. Furthermore, our findings underscore the importance of identifying the modifiable mediators of the association between thyroid function and atherogenesis. Preventive strategies targeting thyroid function or certain mediators could further lead to a reduction in atherosclerotic events. Lastly, our findings provide supporting evidence for a reevaluation of the current reference ranges of TSH and FT_4 tests, which are based on arbitrary statistical approaches rather than on clinical outcomes such as atherosclerotic morbidity and mortality.

REFERENCES

1. Lusis AJ. Atherosclerosis. *Nature.* 2000;407(6801):233-241.
2. Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, de Ferranti S, Despres JP, Fullerton HJ, Howard VJ, et al. Heart disease and stroke statistics—2015 update: a report from the American Heart Association. *Circulation.* 2015;131(4):e29-322.
3. Klein I, Danzi S. Thyroid disease and the heart. *Circulation.* 2007;116(15):1725-1735.
4. Prisant LM, Gujral JS, Mulloy AL. Hyperthyroidism: a secondary cause of isolated systolic hypertension. *J Clin Hypertens (Greenwich).* 2006;8(8):596-599.
5. Stuijver DJ, van Zaane B, Romualdi E, Brandjes DP, Gerdes VE, Squizzato A. The effect of hyperthyroidism on procoagulant, anticoagulant and fibrinolytic factors: a systematic review and meta-analysis. *Thromb Haemost.* 2012;108(6):1077-1088.
6. Cappola AR, Ladenson PW. Hypothyroidism and atherosclerosis. *J Clin Endocrinol Metab.* 2003;88(6):2438-2444.
7. Asvold BO, Bjoro T, Nilsen TI, Gunnell D, Vatten LJ. Thyrotropin levels and risk of fatal coronary heart disease: the HUNT study. *Archives of internal medicine.* 2008;168(8):855-860.
8. Asvold BO, Vatten LJ, Bjoro T, Bauer DC, Bremner A, Cappola AR, Ceresini G, den Elzen WP, Ferrucci L, Franco OH, et al. Thyroid function within the normal range and risk of coronary heart disease: an individual participant data analysis of 14 cohorts. *JAMA Intern Med.* 2015; 175(6):1037-1047.
9. Chaker L, Baumgartner C, den Elzen WP, Collet TH, Ikram MA, Blum MR, Dehghan A, Drechsler C, Luben RN, Portegies ML, et al. Thyroid Function within the Reference Range and the Risk of Stroke: An Individual Participant Data Analysis. *J Clin Endocrinol Metab.* 2016:jc20162255.
10. Rodondi N, den Elzen WP, Bauer DC, Cappola AR, Razvi S, Walsh JP, Asvold BO, Iervasi G, Imaizumi M, Collet TH, et al. Subclinical hypothyroidism and the risk of coronary heart disease and mortality. *Jama.* 2010;304(12):1365-1374.
11. Cappola AR, Arnold AM, Wulczyn K, Carlson M, Robbins J, Psaty BM. Thyroid function in the euthyroid range and adverse outcomes in older adults. *J Clin Endocrinol Metab.* 2015;100(3): 1088-1096.
12. Cappola AR, Fried LP, Arnold AM, Danese MD, Kuller LH, Burke GL, Tracy RP, Ladenson PW. Thyroid status, cardiovascular risk, and mortality in older adults. *Jama.* 2006;295(9):1033-1041.
13. Collet TH, Gussekloo J, Bauer DC, den Elzen WP, Cappola AR, Balmer P, Iervasi G, Asvold BO, Sgarbi JA, Volzke H, et al. Subclinical hyperthyroidism and the risk of coronary heart disease and mortality. *Archives of internal medicine.* 2012;172(10):799-809.
14. Budoff MJ, Young R, Lopez VA, Kronmal RA, Nasir K, Blumenthal RS, Detrano RC, Bild DE, Guerci AD, Liu K, et al. Progression of coronary calcium and incident coronary heart disease events: MESA (Multi-Ethnic Study of Atherosclerosis). *J Am Coll Cardiol.* 2013;61(12):1231-1239.
15. Hofman A, Brusselle GG, Darwish Murad S, van Duijn CM, Franco OH, Goedegebure A, Ikram MA, Klaver CC, Nijsten TE, Peeters RP, et al. The Rotterdam Study: 2016 objectives and design update. *Eur J Epidemiol.* 2015;30(8):661-708.

16. Chaker L, Heeringa J, Dehghan A, Medici M, Visser WE, Baumgartner C, Hofman A, Rodondi N, Peeters RP, Franco OH. Normal Thyroid Function and the Risk of Atrial Fibrillation: the Rotterdam Study. *J Clin Endocrinol Metab.* 2015;100(10):3718-3724.

17. Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M, Jr., Detrano R. Quantification of coronary artery calcium using ultrafast computed tomography. *J Am Coll Cardiol.* 1990;15(4):827-832.

18. Greenland P, Bonow RO, Brundage BH, Budoff MJ, Eisenberg MJ, Grundy SM, Lauer MS, Post WS, Raggi P, Redberg RF, et al. ACCF/AHA 2007 clinical expert consensus document on coronary artery calcium scoring by computed tomography in global cardiovascular risk assessment and in evaluation of patients with chest pain: a report of the American College of Cardiology Foundation Clinical Expert Consensus Task Force (ACCF/AHA Writing Committee to Update the 2000 Expert Consensus Document on Electron Beam Computed Tomography) developed in collaboration with the Society of Atherosclerosis Imaging and Prevention and the Society of Cardiovascular Computed Tomography. *J Am Coll Cardiol.* 2007;49(3):378-402.

19. Kavousi M, Leening MJ, Nanchen D, Greenland P, Graham IM, Steyerberg EW, Ikram MA, Stricker BH, Hofman A, Franco OH. Comparison of application of the ACC/AHA guidelines, Adult Treatment Panel III guidelines, and European Society of Cardiology guidelines for cardiovascular disease prevention in a European cohort. *Jama.* 2014;311(14):1416-1423.

20. Wieberdink RG, Ikram MA, Hofman A, Koudstaal PJ, Breteler MM. Trends in stroke incidence rates and stroke risk factors in Rotterdam, the Netherlands from 1990 to 2008. *Eur J Epidemiol.* 2012;27(4):287-295.

21. Leening MJ, Kavousi M, Heeringa J, van Rooij FJ, Verkroost-van Heemst J, Deckers JW, Mattace-Raso FU, Ziere G, Hofman A, Stricker BH, et al. Methods of data collection and definitions of cardiac outcomes in the Rotterdam Study. *Eur J Epidemiol.* 2012;27(3):173-185.

22. Inker LA, Schmid CH, Tighiouart H, Eckfeldt JH, Feldman HI, Greene T, Kusek JW, Manzi J, Van Lente F, Zhang YL, et al. Estimating glomerular filtration rate from serum creatinine and cystatin C. *The New England journal of medicine.* 2012;367(1):20-29.

23. Swedberg K, Cleland J, Dargie H, Drexler H, Follath F, Komajda M, Tavazzi L, Smiseth OA, Gavazzi A, Haverich A, et al. Guidelines for the diagnosis and treatment of chronic heart failure: executive summary (update 2005): The Task Force for the Diagnosis and Treatment of Chronic Heart Failure of the European Society of Cardiology. *Eur Heart J.* 2005;26(11):1115-1140.

24. Benjamini Y HY. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc Series B.* 1995;57 (1):289-300.

25. von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, Vandenbroucke JP, Initiative S. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement: guidelines for reporting observational studies. *Int J Surg.* 2014;12(12):1495-1499.

26. Chaker L, Korevaar TI, Medici M, Uitterlinden AG, Hofman A, Dehghan A, Franco OH, Peeters RP. Thyroid Function Characteristics and Determinants: The Rotterdam Study. *Thyroid.* 2016; 26(9):1195-1204.

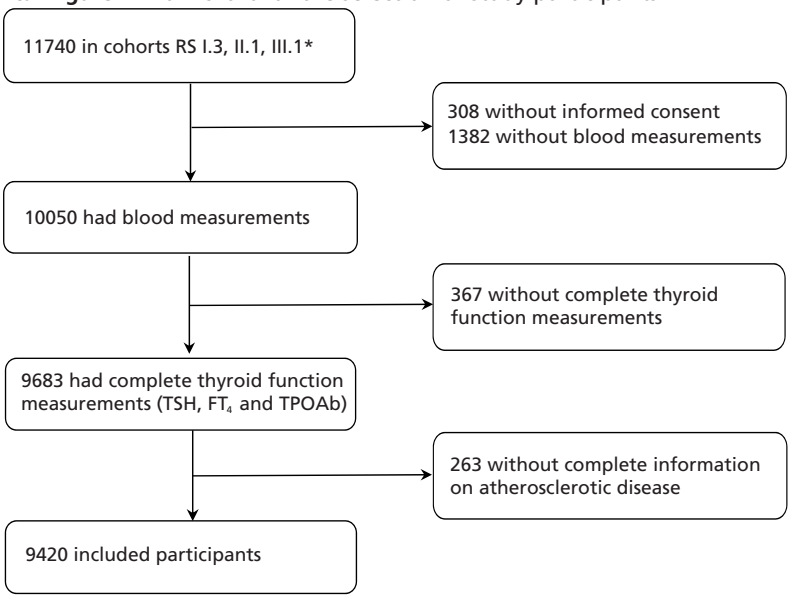
27. Tieche M, Lupi GA, Gutzwiller F, Grob PJ, Studer H, Burgi H. Borderline low thyroid function and thyroid autoimmunity. Risk factors for coronary heart disease? *Br Heart J.* 1981;46(2): 202-206.

28. Matsuura E, Atzeni F, Sarzi-Puttini P, Turiel M, Lopez LR, Nurmohamed MT. Is atherosclerosis an autoimmune disease? *BMC Med.* 2014;12:47.

29. De Sibio MT, Luvizotto RA, Olimpio RM, Correa CR, Marino J, de Oliveira M, Conde SJ, Ferreira AL, Padovani CR, Nogueira CR. A comparative genotoxicity study of a supraphysiological dose of triiodothyronine (T(3)) in obese rats subjected to either calorie-restricted diet or hyperthyroidism. *PLoS One.* 2013;8(2):e56913.
30. Wenisch C, Myskiw D, Gessl A, Graninger W. Circulating selectins, intercellular adhesion molecule-1, and vascular cell adhesion molecule-1 in hyperthyroidism. *J Clin Endocrinol Metab.* 1995;80(7):2122-2126.
31. Shih CH, Chen SL, Yen CC, Huang YH, Chen CD, Lee YS, Lin KH. Thyroid hormone receptor-dependent transcriptional regulation of fibrinogen and coagulation proteins. *Endocrinology.* 2004;145(6):2804-2814.
32. Parle JV, Maisonneuve P, Sheppard MC, Boyle P, Franklyn JA. Prediction of all-cause and cardiovascular mortality in elderly people from one low serum thyrotropin result: a 10-year cohort study. *Lancet (London, England).* 2001;358(9285):861-865.
33. Gussekloo J, van Exel E, de Craen AJ, Meinders AE, Frolich M, Westendorp RG. Thyroid status, disability and cognitive function, and survival in old age. *JAMA.* 2004;292(21):2591-2599.
34. van den Beld AW, Visser TJ, Feelders RA, Grobbee DE, Lamberts SW. Thyroid hormone concentrations, disease, physical function, and mortality in elderly men. *J Clin Endocrinol Metab.* 2005;90(12):6403-6409.
35. Ceresini G, Ceda GP, Lauretani F, Maggio M, Usberti E, Marina M, Bandinelli S, Guralnik JM, Valenti G, Ferrucci L. Thyroid status and 6-year mortality in elderly people living in a mildly iodine-deficient area: the aging in the Chianti Area Study. *Journal of the American Geriatrics Society.* 2013;61(6):868-874.
36. Ittermann T, Haring R, Sauer S, Wallaschofski H, Dorr M, Nauck M, Volzke H. Decreased serum TSH levels are not associated with mortality in the adult northeast German population. *Eur J Endocrinol.* 2010;162(3):579-585.
37. Boekholdt SM, Titan SM, Wiersinga WM, Chatterjee K, Basart DC, Luben R, Wareham NJ, Khaw KT. Initial thyroid status and cardiovascular risk factors: the EPIC-Norfolk prospective population study. *Clin Endocrinol (Oxf).* 2010;72(3):404-410.
38. Imaizumi M, Akahoshi M, Ichimaru S, Nakashima E, Hida A, Soda M, Usa T, Ashizawa K, Yokoyama N, Maeda R, et al. Risk for ischemic heart disease and all-cause mortality in subclinical hypothyroidism. *J Clin Endocrinol Metab.* 2004;89(7):3365-3370.
39. Fliers E, Bianco AC, Langouche L, Boelen A. Thyroid function in critically ill patients. *Lancet Diabetes Endocrinol.* 2015;3(10):816-825.
40. Andersen S, Pedersen KM, Bruun NH, Laurberg P. Narrow individual variations in serum T(4) and T(3) in normal subjects: a clue to the understanding of subclinical thyroid disease. *J Clin Endocrinol Metab.* 2002;87(3):1068-1072.
41. Mariotti S, Franceschi C, Cossarizza A, Pinchera A. The aging thyroid. *Endocr Rev.* 1995;16(6):686-715.
42. van de Ven AC, Netea-Maier RT, Medici M, Sweep FC, Ross HA, Hofman A, de Graaf J, Kiemeney LA, Hermus AR, Peeters RP, et al. Underestimation of effect of thyroid function parameters on morbidity and mortality due to intra-individual variation. *J Clin Endocrinol Metab.* 2011;96(12):E2014-2017.

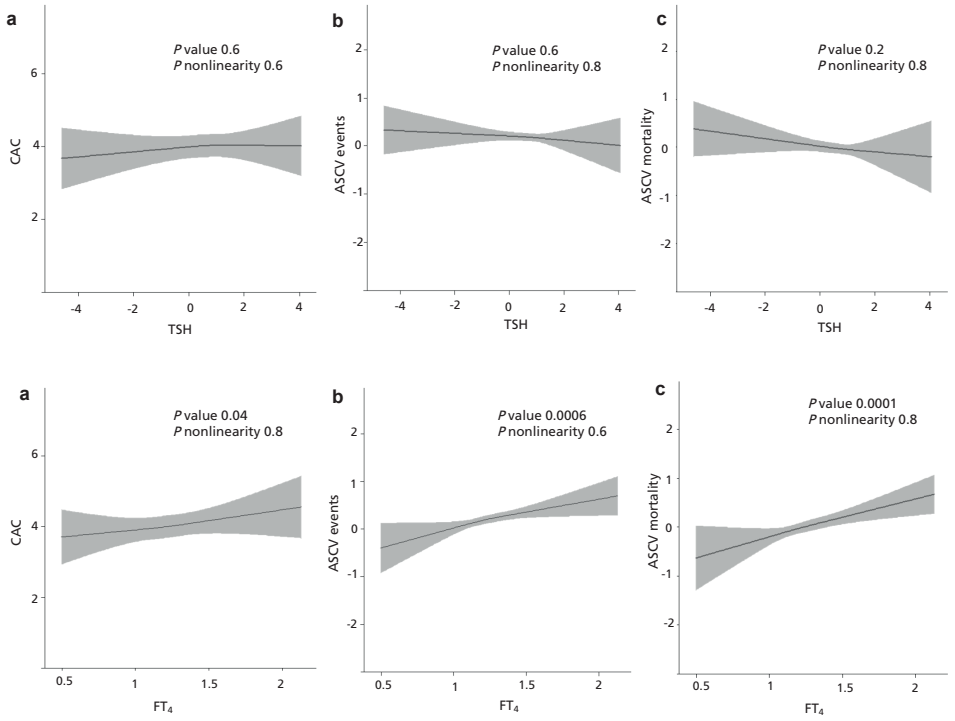
SUPPLEMENTAL MATERIAL

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



*A total of 11740 participants were enrolled during the third visit of the first cohort (n=4797) and the first visit of the second (n=3011) and third (n=3932) cohorts of the Rotterdam Study.

Supplemental Figure 2. Association of thyroid function with atherosclerotic risk.



Continuous CAC score, log relative hazard of incident ASCV events and ASCV mortality are plotted against TSH and FT_4 concentrations, by using restricted cubic splines with 3 knots. Adjusted for age, sex, and cohort. Analyses of ASCV mortality are additionally adjusted for prevalent ASCV disease at baseline. Abbreviations, TSH, thyroid-stimulating hormone; FT_4, free thyroxine; CAC, coronary artery calcification; ASCV, atherosclerotic cardiovascular.

STROBE Statement. Checklist of items that should be included in reports of observational studies

Item	No	Recommendation	Paragraph
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	Title
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	Abstract
Introduction			
Background/ rationale	2	Explain the scientific background and rationale for the investigation being reported	1
Objectives	3	State specific objectives, including any prespecified hypotheses	1
Methods			
Study design	4	Present key elements of study design early in the paper	2
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	2
Participants	6	(*a*) *Cohort study*: Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*: Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*: Give the eligibility criteria, and the sources and methods of selection of participants	4
		(*b*) *Cohort study*: For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*: For matched studies, give matching criteria and the number of controls per case	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	3-5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	3-5
Bias	9	Describe any efforts to address potential sources of bias	7
Study size	10	Explain how the study size was arrived at	2
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6
		(*b*) Describe any methods used to examine subgroups and interactions	6
		(*c*) Explain how missing data were addressed	6
		(*d*) *Cohort study*: If applicable, explain how loss to follow-up was addressed *Case-control study*: If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*: If applicable, describe analytical methods taking account of sampling strategy	7
		(*e*) Describe any sensitivity analyses	7

STROBE Statement. Checklist of items that should be included in reports of observational studies (continued)

Item	No	Recommendation	Paragraph
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study: (eg, numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analyzed)	2, Suppl
		(b) Give reasons for non-participation at each stage	2, Suppl
		(c) Consider use of a flow diagram	Suppl
Descriptive data	14*	(a) Give characteristics of study participants (eg, demographic, clinical, social) and information on exposures and potential confounders	2
		(b) Indicate number of participants with missing data for each variable of interest	Suppl
		(c) *Cohort study*: Summarize follow-up time (eg, average and total amount)	8
Outcome data	15*	*Cohort study:* Report numbers of outcome events or summary measures over time	8
		Case-control study: Report numbers in each exposure category, or summary measures of exposure	NA
		Cross-sectional study: Report numbers of outcome events or summary measures	NA
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9-11
		(*b*) Report category boundaries when continuous variables were categorized	6
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done: eg, analyses of subgroups and interactions, and sensitivity analyses	6,7
Discussion			
Key results	18	Summarize key results with reference to study objectives	12
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	12
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12
Generalizability	21	Discuss the generalizability (external validity) of the study results	12
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	†

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies. **Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org. †The Rotterdam Study is supported by the Erasmus Medical Center and Erasmus University of Rotterdam; the Netherlands Organization for Scientific Research; the Netherlands Organization for Health Research and Development; the Research Institute for Diseases in the Elderly; the Netherlands Genomics Initiative; the Ministry of Education, Culture and Science; the Ministry of Health Welfare and Sports; the European Commission (DG XII); and the Municipality of Rotterdam.

Supplemental Table 1. Association of TPOAb positivity with atherosclerotic outcomes

	Events/TN, %	HR (95% CI) Model 1	*P* value	HR (95% CI) Model 2	*P* value
TPOAb positivity and high CAC score†*					
All	817/1763 (46.3%)	1.01 (0.75; 1.37)	0.93	0.85 (0.63; 1.15)	0.28
Euthyroid participants‡	626/1336 (46.9%)	1.06 (0.69; 1.62)	0.80	0.93 (0.61; 1.41)	0.71
TPOAb positivity and incident atherosclerotic cardiovascular events†					
All	934/8498 (11.0%)	0.98 (0.80; 1.20)	0.87	0.95 (0.78; 1.16)	0.63
Euthyroid participants‡	736/6826 (10.8%)	1.18 (0.91; 1.52)	0.21	1.16 (0.90; 1.50)	0.25
TPOAb positivity and atherosclerotic cardiovascular mortality§					
All	612/9420 (6.5%)	1.03 (0.80; 1.32)	0.82	0.99 (0.77; 1.27)	0.94
Euthyroid participants‡	483/7575 (6.4%)	1.07 (0.77; 1.49)	0.67	1.09 (0.78; 1.51)	0.62

Model 1: age, sex, cohort, and lnTSH. Model 2: Model 1, smoking, alcohol intake, body mass index, total cholesterol, triglycerides, systolic blood pressure, prevalent diabetes mellitus, use of antihypertensive medications, and use of lipid-lowering medications. *CAC ≥100 AU was defined as high CAC score. †All included participants were free of atherosclerotic cardiovascular disease at baseline. ‡Normal reference ranges of thyroid function were defined as serum TSH levels of 0.4 to 4.0 mIU/L and serum FT_4 levels of 0.85 to 1.95 ng/dL, after excluding thyroid medication users and participants with personal history of thyroid disease. §Additionally adjusted for prevalent atherosclerotic cardiovascular disease. Abbreviations: TPOAb, thyroid peroxidase antibodies (cutoff 35 kU/ml); TN, total number; HR, hazard ratio; CI, confidence interval; TSH, thyroid-stimulating hormone.

Supplemental Table 2. Sensitivity analyses for the association between thyroid function within the reference ranges* and atherosclerotic outcomes

	Events/TN, %	HR (95% CI) Model 1	*P* value	HR (95% CI) Model 2	*P* value
Incident atherosclerotic cardiovascular events†					
*Thyroid function within the reference ranges**					
TSH	736/6826 (10.8%)	0.95 (0.81; 1.10)	0.50	0.96 (0.82; 1.11)	0.59
FT_4	736/6826 (10.8%)	**2.67 (1.69; 4.20)**	<0.0001	**2.50 (1.58; 3.94)**	<0.0001
Excluding users of thyroid function-altering medications‡					
TSH	638/5964 (10.7%)	0.92 (0.78; 1.09)	0.34	0.93 (0.79; 1.10)	0.41
FT_4	638/5964 (10.7%)	**3.05 (1.86; 4.98)**	<0.0001	**2.70 (1.64; 4.42)**	<0.0001
Additionally adjusted for physical activity					
TSH	736/6826 (10.8%)	0.95 (0.82; 1.11)	0.56	0.96 (0.83; 1.12)	0.67
FT_4	736/6826 (10.8%)	**2.62 (1.67; 4.12)**	<0.0001	**2.47 (1.57; 3.90)**	<0.0001
Additionally adjusted for eGFR					
TSH	736/6826 (10.8%)	0.93 (0.80; 1.08)	0.35	0.94 (0.81; 1.10)	0.48
FT_4	736/6826 (10.8%)	**2.56 (1.62; 4.03)**	<0.0001	**2.39 (1.51; 3.78)**	<0.0001
Excluding subjects with prevalent AF and censoring at the time of incident AF					
TSH	574/6270 (9.2%)	0.94 (0.79;1.12)	0.50	0.96 (0.80; 1.14)	0.63
FT_4	574/6270 (9.2%)	**2.25 (1.33; 3.82)**	<0.003	**2.05 (1.20; 3.48)**	0.008

Supplemental Table 2. Sensitivity analyses for the association between thyroid function within the reference ranges* and atherosclerotic outcomes (continued)

	Events/TN, %	HR (95% CI) Model 1	*P* value	HR (95% CI) Model 2	*P* value
Excluding subjects with prevalent HF and censoring at the time of incident HF					
TSH	693/6640 (10.4%)	0.89 (0.77; 1.04)	0.17	0.91 (0.78; 1.06)	0.24
FT_4	693/6640 (10.4%)	**2.70 (1.68; 4.33)**	<0.0001	**2.35 (1.46; 3.78)**	0.0004
Atherosclerotic cardiovascular mortality§					
*Thyroid function within the reference ranges**					
TSH	483/7575 (6.4%)	**0.82 (0.68; 0.98)**	0.02	**0.80 (0.67; 0.96)**	0.01
FT_4	483/7575 (6.4%)	**3.92 (2.29; 6.71)**	<0.0001	**3.84 (2.23; 6.60)**	<0.0001
Excluding users of thyroid function-altering medications‡					
TSH	389/6476 (6.0%)	**0.74 (0.61; 0.90)**	0.003	**0.72 (0.58; 0.88)**	0.001
FT_4	389/6476 (6.0%)	**3.82 (2.05; 7.14)**	<0.0001	**3.47 (1.84; 6.51)**	<0.0001
Additionally adjusted for physical activity					
TSH	483/7575 (6.4%)	**0.82 (0.69; 0.99)**	0.04	**0.81 (0.67; 0.97)**	0.02
FT_4	483/7575 (6.4%)	**3.79 (2.22; 6.46)**	<0.0001	**3.74 (2.18; 6.43)**	<0.0001
Additionally adjusted for eGFR					
TSH	483/7575 (6.4%)	**0.79 (0.66; 0.95)**	0.01	**0.79 (0.65; 0.94)**	0.01
FT_4	483/7575 (6.4%)	**3.65 (2.13; 6.26)**	<0.0001	**3.60 (2.09; 6.20)**	<0.0001
Excluding subjects with prevalent AF and censoring at the time of incident AF					
TSH	357/6906 (5.2%)	**0.81 (0.66; 0.99)**	0.04	**0.80 (0.65; 0.99)**	0.04
FT_4	357/6906 (5.2%)	**2.90 (1.52; 5.56)**	0.001	**3.15 (1.64; 6.05)**	0.001
Excluding subjects with prevalent HF and censoring at the time of incident HF					
TSH	356/7307 (4.9%)	**0.75 (0.61; 0.92)**	0.007	**0.73 (0.59; 0.90)**	0.004
FT_4	356/7307 (4.9%)	**4.94 (2.61; 9.34)**	<0.0001	**4.23 (2.22; 8.09)**	<0.0001

Model 1: age, sex, and cohort. Model 2: Model 1, smoking, alcohol intake, body mass index, total cholesterol, triglycerides, systolic blood pressure, prevalent diabetes mellitus, use of antihypertensive medications, and use of lipid-lowering medications. *Normal reference ranges of thyroid function were defined as serum TSH levels of 0.4 to 4.0 mIU/L and serum FT_4 levels of 0.85 to 1.95 ng/dL, after excluding thyroid medication users and participants with personal history of thyroid disease.†All included participants were free of atherosclerotic cardiovascular disease at baseline.‡Thyroid function-altering medications included thyroid medications, analgesics, corticosteroids and amiodarone. §Additionally adjusted for prevalent atherosclerotic cardiovascular disease. 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). Abbreviations: TN, total number; HR, hazard ratio; CI, confidence interval; TSH, thyroid-stimulating hormone; FT_4, free thyroxine; eGFR, estimated glomerular filtration rate; AF, atrial fibrillation; HF, heart failure.

Supplemental Table 3. Association of thyroid function with atherosclerotic events, after excluding events that occurred during first 2 years of follow-up

	Events/TN, %	HR (95% CI) Model 1	*P* value	HR (95% CI) Model 2	*P* value
Incident atherosclerotic cardiovascular events*					
All participants					
TSH	775/8339 (9.3%)	0.93 (0.86; 1.01)	0.10	0.93 (0.86; 1.01)	0.09
FT_4	775/8339 (9.3%)	**1.91 (1.34; 2.72)**	0.0003	**1.87 (1.30; 2.69)**	0.001
Thyroid function within the reference ranges†					
TSH	611/6701 (9.1%)	0.95 (0.80; 1.12)	0.57	0.96 (0.81; 1.14)	0.66
FT_4	611/6701 (9.1%)	**2.51 (1.52; 4.15)**	0.0003	**2.30 (1.39; 3.81)**	<0.0001
Atherosclerotic cardiovascular mortality‡					
All participants					
TSH	535/9343 (5.7%)	0.94 (0.86; 1.03)	0.23	0.93 (0.84; 1.02)	0.12
FT_4	535/9343 (5.7%)	**1.95 (1.34; 2.84)**	0.0004	**2.12 (1.42; 3.16)**	0.0002
Thyroid function within the reference ranges†					
TSH	419/7511 (5.5%)	**0.80 (0.66; 0.97)**	0.02	**0.78 (0.64; 0.95)**	0.01
FT_4	419/7511 (5.5%)	**3.57 (1.99; 6.39)**	<0.0001	**3.49 (1.93; 6.29)**	<0.0001

Model 1: age, sex, and cohort. Model 2: Model 1, smoking, alcohol intake, body mass index, total cholesterol, triglycerides, systolic blood pressure, prevalent diabetes mellitus, use of antihypertensive medications, and use of lipid-lowering medications. *All included participants were free of atherosclerotic cardiovascular disease at baseline. †Normal reference ranges of thyroid function were defined as serum TSH levels of 0.4 to 4.0 mIU/L and serum FT_4 levels of 0.85 to 1.95 ng/dL, after excluding thyroid medication users and participants with history of thyroid disease. ‡Additionally adjusted for prevalent atherosclerotic cardiovascular disease. 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). Abbreviations: TN, total number; HR, hazard ratio; CI, confidence interval; TSH, thyroid-stimulating hormone; FT_4, free thyroxine.

Supplemental Table 4. Association of thyroid function with atherosclerotic outcomes, stratified by sex and age

		High CAC score		Incident ASCV events		ASCV mortality‡	
Strata	**TSH/FT_4**	**Events/TN**	**OR (95% CI)**	**Events/TN**	**HR (95% CI)**	**Events/TN**	**HR (95% CI)**
*Sex**							
Men	TSH	444/738 (60.1%)	0.98 (0.80; 1.20)	432/3454 (12.5%)	0.95 (0.83; 1.09)	316/4078 (7.7%)	0.83 (0.73; 0.94)
Women		373/1025 (36.4%)	0.98 (0.86; 1.13)	502/5044 (10.0%)	0.97 (0.89; 1.06)	296/5342 (5.5%)	1.00 (0.90; 1.13)
P for interaction			*0.96*		*0.92*		*0.03*
Men	FT_4	444/738 (60.1%)	2.08 (0.82; 5.26)	432/3454 (12.5%)	1.71 (0.98; 2.98)	316/4078 (7.7%)	**4.63 (2.70; 7.94)**
Women		373/1025 (36.4%)	**2.25 (1.10; 4.58)**	502/5044 (10.0%)	**1.89 (1.29; 2.79)**	296/5342 (5.5%)	1.35 (0.83; 2.18)
P for interaction			*0.88*		*0.62*		*0.002*
Age†							
<65 years	TSH	151/479 (31.5%)	1.00 (0.79; 1.31)	210/5043 (4.2%)	0.89 (0.74; 1.05)	68/5331 (1.3%)	0.80 (0.60; 1.06)
≥65 years		666/1284 (51.9%)	0.97 (0.86; 1.10)	724/3455 (21.1%)	0.98 (0.91; 1.06)	544/4089 (13.3%)	0.94 (0.86; 1.04)
P for interaction			*0.88*		*0.30*		*0.35*
<65 years	FT_4	151/479 (31.5%)	1.90 (0.63; 5.77)	210/5043 (4.2%)	1.52 (0.71; 3.26)	68/5331 (1.3%)	2.65 (0.72; 9.80)
≥65 years		666/1284 (51.9%)	**2.26 (1.18; 4.37)**	724/3455 (21.1%)	**1.97 (1.38; 2.82)**	544/4089 (13.3%)	**2.19 (1.53; 3.13)**
P for interaction			*0.67*		*0.34*		*0.78*

*Adjusted for age and cohort. †Adjusted for age, sex, and cohort. ‡Additionally adjusted for prevalent atherosclerotic cardiovascular disease. Abbreviations: ASCV, atherosclerotic cardiovascular; HR, hazard ratio; CI, confidence interval; TSH, thyroid-stimulating hormone; FT_4, free thyroxine.