

# Thyroid Function and the Risk of Atherosclerotic Cardiovascular Morbidity and Mortality: The Rotterdam Study

Arjola Bano<sup>1,2,3</sup>, Loyal Chaker<sup>1,2,3,4</sup>, Francesco U.S. Mattace-Raso<sup>1,5</sup>, Aad van der Lugt<sup>6</sup>, M. Arfan Ikram,<sup>3,6,7</sup> Oscar H. Franco<sup>3</sup>, Robin P. Peeters<sup>1,2,3</sup>, Maryam Kavousi<sup>3</sup>

<sup>1</sup>Department of Internal Medicine, Erasmus Medical Center, Rotterdam, The Netherlands; <sup>2</sup>Academic Center for Thyroid Diseases, Erasmus Medical Center, Rotterdam, The Netherlands; <sup>3</sup>Department of Epidemiology, Erasmus Medical Center, Rotterdam, The Netherlands; <sup>4</sup>Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, Massachusetts, USA; <sup>5</sup>Section of Geriatric Medicine, Erasmus Medical Center, Rotterdam, The Netherlands; <sup>6</sup>Department of Radiology, Erasmus Medical Center, Rotterdam, The Netherlands, and; <sup>7</sup>Department of Neurology, Erasmus Medical Center, Rotterdam, The Netherlands.

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### Address correspondence to:

Dr. Robin P. Peeters  
Department of Internal Medicine  
Room Ee 502a, Erasmus Medical Center  
Dr Molewaterplein 50  
Rotterdam 3015 GE  
The Netherlands  
r.peeters@erasmusmc.nl

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## ABSTRACT

**Rationale:** Thyroid hormones have been linked with various proatherogenic and antiatherogenic processes. However, the relationship of thyroid function with manifestations of atherosclerosis remains unclear.

**Objective:** To investigate the association of thyroid function with atherosclerosis throughout its spectrum; i.e. subclinical atherosclerosis, incident atherosclerotic cardiovascular (ASCV) events and ASCV mortality.

**Methods and Results:** 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 (CHD) mortality or stroke; 3. ASCV mortality, defined as death due to CHD, cerebrovascular or other atherosclerotic diseases. Associations of thyroid-stimulating hormone (TSH) and free thyroxine (FT4) with the outcomes were assessed through logistic regression and Cox proportional-hazard models, adjusted for potential confounders including cardiovascular risk factors. A total of 9420 community-dwelling participants (mean age±SD, 64.8±9.7 years) were included. During a median follow-up of 8.8 years (interquartile range:4.5-11.8 years), 934 incident ASCV events and 612 ASCV deaths occurred. FT4 levels were positively associated with high CAC score (odds ratio [OR]; 95% confidence interval [CI]: 2.28; 1.30-4.02) and incident ASCV events (hazard ratio [HR]; CI: 1.87; 1.34-2.59). The risk of ASCV mortality increased in a linear manner with higher FT4 levels (HR; CI: 2.41; 1.68-3.47 per 1 ng/dl) and lower TSH levels (HR; CI: 0.92; 0.84-1.00 per 1 logTSH). Results remained similar or became stronger among euthyroid participants.

**Conclusions:** FT4 levels in middle-aged and elderly subjects were positively associated with atherosclerosis throughout the whole disease spectrum, independently of cardiovascular risk factors.

### **Keywords:**

Thyroid function, atherosclerosis, population-based study.

### **Nonstandard Abbreviations and Acronyms:**

TH	thyroid hormone
CHD	coronary heart disease
CAC	coronary artery calcification
ASCV	atherosclerotic cardiovascular
TSH	thyroid-stimulating hormone
FT4	free thyroxine
AU	Agatston units
HR	hazard ratio
MI	myocardial infarction
BMI	body mass index
AF	atrial fibrillation
HF	heart failure
eGFR	estimated glomerular filtration rate
TPOAb	thyroid peroxidase antibodies
OR	odds ratio

## INTRODUCTION

Atherosclerosis progresses insidiously from a subclinical condition to the clinical onset of vascular events to death<sup>1</sup>. Despite advances in prevention and treatment, atherosclerotic disease remains a leading cause of death, with a considerable clinical and economic burden worldwide<sup>2</sup>. 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 (TH) concentrations have commonly been linked with systolic hypertension<sup>3, 4</sup> and hypercoagulation<sup>5</sup>; whereas lower circulating TH levels can instigate hyperlipidemia and inflammation<sup>6</sup>. Although atherosclerosis is a continuous process, prospective epidemiological studies so far have mainly focused on the relation between specific ranges of thyroid function and distinct atherosclerotic events, such as coronary heart disease (CHD) or stroke<sup>7-12</sup>. Results have been quite inconsistent, including studies that find no association between thyroid function and atherosclerotic outcomes<sup>8, 12</sup>, as well as other studies reporting an increased risk of atherosclerotic outcomes with either lower<sup>7, 10</sup> or higher thyroid function<sup>9, 11, 13</sup>. 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)<sup>14</sup>, 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<sup>15</sup>. 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 (RSII). In 2006, a third cohort of 3932 subjects aged 45 years or older was added (RSIII). 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. Complete data on thyroid function and incident events were available in 9420 participants from these visits. Detailed information on the selection of study participants is provided in Supplemental Methods and Online Figure I.

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 three study cohorts using the same method and assay. Measurements of thyroid-stimulating hormone (TSH), free thyroxine (FT4), 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 FT4 (0.86–1.94 ng/dL; equivalent to 11–25 pmol/L) were determined based on national guidelines and our previous studies <sup>16</sup>.

### ***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 tomography scans (C-150 Imatron GE) of the coronary arteries <sup>15</sup>. CAC score of 100 Agatston units (AU) or greater suggests clinically significant atherosclerotic plaque <sup>2</sup> and has been used in past consensus statements <sup>17</sup>. Therefore, we grouped participants into CAC score lower than 100 AU and CAC score of 100 AU or greater.

ASCV events were defined as fatal and nonfatal myocardial infarction (MI), other CHD mortality, or stroke, as previously described <sup>18</sup>. Prevalent ASCV disease was defined as history of MI, stroke, coronary or other arterial revascularization <sup>18, 19</sup>. Prevalent ASCV disease at baseline was assessed through interview and verified in medical records.

ASCV mortality was defined as death due to CHD, cerebrovascular disease or other atherosclerotic diseases, as previously described <sup>18, 20</sup>. Non-ASCV mortality was defined as death due to causes other than atherosclerotic disease. In the Supplemental Methods, we provide extensive information on CAC assessment, ASCV events ascertainment and evaluation of medical history, medication use, smoking, alcohol intake, glucose and lipid levels, body mass index (BMI), blood pressure, diabetes mellitus, atrial fibrillation (AF) and heart failure (HF).

### ***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 or greater, among participants that were free of atherosclerotic cardiovascular 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 FT4 with subclinical atherosclerosis, incident ASCV events and ASCV mortality, were additionally corrected for the false discovery rate using the Benjamini and Hochberg method <sup>21</sup>.

Analyses were stratified by sex (men vs 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 and use of antihypertensive and lipid-lowering medications. The analyses of ASCV mortality were additionally adjusted for presence of prevalent ASCV disease. Detailed descriptions of the research methods are provided in the Appendix.

### ***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 non-steroidal anti-inflammatory drugs, paracetamol and muscle relaxants), corticosteroids or amiodarone. Besides, we additionally adjusted our analyses for physical activity or eGFR; 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 that were free of atherosclerotic cardiovascular disease. Due to its skewed distribution, CAC score was log transformed after adding 1 ( $\ln[\text{CAC}+1]$ ); 7) To explore the role of thyroid autoimmunity on atherosclerotic outcomes, we investigated the association of thyroid peroxidase antibodies (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, 4.5-11.8 years). Baseline characteristics are presented in Table 1. The mean age of participants was 64.8 ( $\pm$  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<sup>22</sup>. In brief, the main determinants of FT4 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 FT4 levels were associated with higher odds of having CAC score of 100 AU or greater (odds ratio [OR], 2.28; CI, 1.30-4.02 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 of 100 AU or greater (OR, 0.94; CI, 0.84-1.05 per 1 logTSH) (Table 2). No evidence of non-linearity was observed (p for nonlinearity for FT4 and TSH 0.8 and 0.6, respectively) (Online Figure II). Restricting the analyses to participants with thyroid function within the reference ranges resulted in similar or increased point estimates, with wider CIs (OR,



2.43; CI, 1.14-5.16 per 1ng/dl FT4; OR, 0.91; CI, 0.72-1.16 per 1 logTSH) (Table 2). Results were consistent in the analyses with CAC score as a continuous variable ( $\beta$ , 0.54; CI, 0.01-1.08 per 1 ng/dl FT4;  $\beta$ , 0.01; CI, -0.10-0.12 per 1 log TSH) (Online Figure II). Furthermore, we found no association of TPOAb with the odds of having CAC score of 100 AU or greater (OR, 0.85; CI, 0.63-1.15) (Online Table I). In the analyses of CAC score, the interaction terms between the exposure (TSH or FT4) 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 FT4 with sex [men vs women], 0.96 and 0.88, respectively; p for interaction of TSH and FT4 with age [ $<65$  vs  $\geq 65$  years], 0.88 and 0.67, respectively) (Online Table IV).

#### *Thyroid function and incident ASCV events.*

There was a positive association of FT4 levels with the risk of incident ASCV events (HR, 1.87; CI, 1.34-2.59 per 1ng/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; CI, 0.89-1.03 per 1 logTSH) with the risk of incident ASCV events (Table 3). No evidence of non-linearity was observed (p for nonlinearity for FT4 and TSH 0.6 and 0.8, respectively) (Online Figure II). Restricting the analyses to participants with thyroid function within the reference ranges resulted in similar or increased point estimates, with wider CIs (HR, 2.50; CI, 1.58-3.94 per 1ng/dl; HR, 0.96; CI, 0.82-1.11 per 1 logTSH) (Table 3). This corresponds to a 2.70-times higher risk of incident ASCV events, for a participant with a FT4 in the higher limit of the reference range (1.94 ng/dl), compared with a participant with a FT4 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 (Online Table II). The associations remained similar after censoring the analyses at the time of incident HF; or after additionally adjusting for physical activity or eGFR. Results did not change substantially after excluding events that occurred during the first 2 years of follow-up (Online Table III). Furthermore, we found no association of TPOAb with the risk of incident ASCV events (HR, 0.95; CI, 0.78-1.16) (Online Table I). In the analyses of incident ASCV events, the interaction terms between the exposure (TSH or FT4) 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 FT4 with sex [men vs women], 0.92 and 0.62, respectively; p for interaction of TSH and FT4 with age [ $<65$  and  $\geq 65$  years], 0.30 and 0.34, respectively) (Online Table IV).

#### *Thyroid function and ASCV mortality.*

Higher FT4 levels were associated with a higher risk of ASCV mortality (HR, 2.41; CI, 1.68-3.47 per 1ng/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; CI, 0.84-1.00 per 1 logTSH), though the association was borderline significant (Table 4). No evidence of non-linearity was observed (p for nonlinearity for FT4 and TSH 0.8 and 0.8, respectively) (Online Figure II).

In the analysis of ASCV mortality, we found statistically significant differences by prevalent ASCV disease at baseline and by sex (p for interaction of FT4 with prevalent ASCV disease [present vs absent], 0.002; p for interaction of TSH and FT4 with sex [men vs women], 0.03 and 0.002, respectively), but no statistically significant age differences (p for interaction of TSH and FT4 with age [ $<65$  vs  $\geq 65$  years], 0.35 and 0.78, respectively) (Figure 1, Online Table IV). The association of FT4 with atherosclerotic mortality was more pronounced among participants with ASCV disease (HR, 5.59; 2.90; 10.80 per 1ng/dl) and among men (HR, 4.63; 2.70; 7.94 per 1ng/dl) (Figure 1, Online Table IV). Within the strata of prevalent ASCV disease (absent vs present), none of the interaction terms of TSH or FT4 concentrations with sex or age were statistically significant (p for interaction  $>0.05$ ) (Figure 1). Within the strata of sex (men vs women), none of the interaction terms of TSH or FT4 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 FT4) and prevalent ASCV disease (absent vs present), sex (men vs women) or age ( $<65$  vs  $\geq 65$  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; CI, 2.23-6.60 per 1 ng/dl FT4; HR, 0.80; CI, 0.67-0.96 per 1 logTSH) (Table 4, Figure 1). This corresponds to a 4.15–times higher risk of ASCV mortality, for a participant with a FT4 in the higher limit of the reference range (1.94 ng/dl), compared with a participant with a FT4 in the lower limit of the reference range (0.86 ng/dl). The associations remained similar after excluding participants using thyroid function-altering medications, after censoring the analyses at the time of incident HF, or after additionally adjusting for physical activity or eGFR. The associations slightly attenuated after censoring the analyses at the time of incident AF (Online Table II). Results did not change substantially after excluding events that occurred during the first 2 years of follow-up (Online Table III). 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; CI, 0.77-1.27) (Online Table I).



## DISCUSSION

In this large population-based cohort study, higher FT4 levels were associated with an increased risk of atherosclerosis, independently 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<sup>6</sup>, whereas high thyroid function has been associated with an increased prevalence of AF<sup>16</sup>. Our study suggests that the association of thyroid function with atherosclerosis is independent of these cardiovascular risk factors, as 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<sup>23,24</sup>. 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 TH concentrations can increase the production of reactive oxygen species that further induce the expression of adhesion molecules on endothelial cells<sup>25</sup>. Hence, hyperthyroidism has been commonly associated with early atherosclerosis and markers of endothelial dysfunction such as E-selectin, intracellular adhesion molecule-1 and vascular cell-adhesion molecule<sup>26</sup>. Second, TH regulates the synthesis of procoagulant proteins<sup>27</sup>. Excess FT4 levels have been linked with increased concentrations of various procoagulant proteins, namely von Willebrand factor, fibrinogen, factors VIII and IX, that can accelerate plaque vulnerability and rupture<sup>5</sup>. Third, high levels of TH can generate increased cardiac contractility and workload, augmenting myocardial oxygen demand that could eventually precipitate ischemic events and death<sup>3</sup>. These deleterious effects of high thyroid function may also be extended to the high-normal range of thyroid function<sup>11</sup>. 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<sup>28-31</sup>. In an attempt to identify potential subgroups at risk, prior research has suggested that the effect of thyroid function on mortality might be age<sup>28-31</sup> or sex dependent<sup>29, 30</sup>. Generally, studies performed in older participants have reported an increased risk of mortality with higher FT4 levels<sup>29, 30</sup>, whereas studies including younger participants have failed to show an association<sup>32, 33</sup>. Additional studies have reported an association of thyroid function with mortality risk exclusively in men<sup>34</sup> or women<sup>7</sup>. 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 TH 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 FT4 levels<sup>35</sup>. In contrast, we found an association of higher rather than lower FT4 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 FT4 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<sup>9</sup>. However, another analysis from the same collaboration failed to show an association between thyroid function within the reference range and the risk of CHD<sup>8</sup>, though this could be due to the relatively low proportion of CHD deaths (3.3%). We observed larger risk estimates after restricting the analyses to participants with TSH and FT4 levels within the reference ranges, though 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<sup>36</sup>. 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 re-evaluation of TSH and FT4 reference ranges, which are currently based on arbitrary statistical approaches (2.5<sup>th</sup> and 97.5<sup>th</sup> 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<sup>9, 11, 16</sup>. 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 FT4 levels and atherosclerotic outcomes. Although the association between TSH levels and atherosclerotic outcomes was in the expected opposite direction of FT4, it sometimes did not reach statistical significance. Similar observations have been also reported by studies investigating the relationship of thyroid function with various clinical endpoints<sup>9, 29</sup>. Serum FT4 levels are tightly regulated by the hypothalamic-pituitary-thyroid axis, with a different set point for each individual. This might explain why FT4 levels are associated with various clinical endpoints, especially within the euthyroid range which is generally defined by TSH. Alternatively, these results may reflect a slight shift in the TSH-FT4 set point, which may be due to ageing<sup>37</sup>.

To our knowledge, this is the first population-based cohort study which 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 prior to 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 regarding its fluctuations over time. Nevertheless, due to the intra-individual variability of TSH and FT4 levels, the lack of repeated measurements would tend to underestimate the association between thyroid function and atherosclerotic outcomes rather than generate spurious findings<sup>38</sup>. In addition, our results were consistent within the normal range of thyroid function, which is considered to be stable with very small intra-individual variability<sup>36</sup>. We lacked information on serum triiodothyronine levels. However, TSH and FT4 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 FT4 levels in middle-aged and elderly subjects were associated with an increased risk of atherosclerotic morbidity and mortality, independently of cardiovascular risk factors. These findings suggest that FT4 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 re-evaluation of the current reference ranges of TSH and FT4 tests, which are based on arbitrary statistical approaches rather than on clinical outcomes such as atherosclerotic morbidity and mortality.

## AUTHOR CONTRIBUTORS

AB, LC, RPP and MK conceived the study. AB, LC, FUSMR, AVDL, MAI, OHF, RPP and MK designed the study. AB, LC and MK collected the data. AB, LC, FUSMR, AVDL, MAI, OHF, RPP and MK drafted the final manuscript. All authors contributed to the critical revision of the manuscript and approved the final version.

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## DISCLOSURES

None.

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# Circulation Research

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## FIGURE LEGEND

**Figure 1.** Hazard ratios of atherosclerotic cardiovascular mortality, stratified by presence of ASCV disease at baseline, adjusting for age, sex, cohort, smoking, alcohol intake, body mass index, total cholesterol, triglycerides, systolic blood pressure, prevalent diabetes, use of antihypertensive and lipid-lowering medications. The *P* for interaction of TSH and FT4 with prevalent ASCV disease (absent vs present) were 0.09 and 0.002, respectively. Among participants without ASCV disease: the *P* for interaction of TSH and FT4 with sex (men vs women) were 0.18 and 0.07, respectively; the *P* for interaction of TSH and FT4 with age (<65 vs ≥65 years) were 0.38 and 0.29, respectively. Among participants with ASCV disease: the *P* for interaction of TSH and FT4 with sex (men vs women) were 0.34 and 0.44, respectively; the *P* for interaction of TSH and FT4 with age (<65 vs ≥65 years) were 0.61 and 0.13, respectively. Normal thyroid function was defined as serum TSH of 0.4-4.0 mIU/L and FT4 levels of 0.85-1.95 ng/dl, after excluding thyroid medication users and participants with history of thyroid disease. Error bars represent the 95% CI of hazard ratios (black dots). Within brackets: Number of atherosclerotic cardiovascular deaths/ Total number. Abbreviations: TN, Total number; ASCV, atherosclerotic cardiovascular; TSH, thyroid-stimulating hormone; FT4, free thyroxine; CI, confidence interval.



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## NOVELTY AND SIGNIFICANCE

### *What Is Known?*

- Atherosclerosis progresses from a subclinical condition to the clinical onset of vascular events to death.
- Previous studies have mainly investigated the association between thyroid function and distinct atherosclerotic events, with inconsistent results.

### *What New Information Does This Article Contribute?*

- This study investigated the relationship of thyroid function with atherosclerosis throughout its spectrum, from subclinical atherosclerosis to atherosclerotic events to atherosclerotic mortality.
- Higher circulating free thyroxine levels were associated with an increased risk of subclinical and clinical atherosclerosis, even within the euthyroid range.

Thyroid hormones have been linked with various proatherogenic and antiatherogenic processes. However, the relationship between thyroid function and atherogenesis remains unclear. In a large prospective population-based cohort study, we investigated the relationship between thyroid function and atherosclerosis at different stages of progression; ; from subclinical atherosclerosis to atherosclerotic events to atherosclerotic mortality. We found that higher FT4 levels are associated with an increased risk of subclinical and clinical manifestations of atherosclerosis, independent of cardiovascular risk factors. The association was consistent even among euthyroid subjects. Our study suggests the value of FT4 as a potential marker for predicting atherosclerotic morbidity and mortality. Moreover, our results 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.

**Table 1. Baseline characteristics of 9420 participants\***

Age, years	64.8 (9.7)
Female, n (%)	5342 (56.7)
Smoking, n (%)	
<i>current</i>	2001 (21.2)
<i>former</i>	4481 (47.6)
<i>never</i>	2938 (31.2)
TSH, mIU/L	1.9 (1.2-2.8)
FT4, 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)
BMI, kg/m <sup>2</sup>	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, 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)

\*Data are presented as mean (standard deviation) or median (25<sup>th</sup>-75<sup>th</sup> percentiles), unless otherwise specified.

Abbreviations: TSH, thyroid-stimulating hormone; FT4, free thyroxine; TPOAb, thyroid peroxidase antibodies (cutoff 35 kU/ml); BMI, body-mass index.

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

	High CAC score /TN (%)	Model 1 OR (95% CI)	p-value	Model 2 OR (95% CI)	p-value
TSH, mIU/L	817/1763 (46.3%)	0.98 (0.88; 1.10)	0.79	0.94 (0.84; 1.05)	0.29†
FT4, ng/dl	817/1763 (46.3%)	2.15 (1.22; 3.77)	0.008	2.28 (1.30; 4.02)	0.004§
<i>Thyroid function within the reference ranges†</i>					
TSH, mIU/L	626/1336 (46.8%)	1.00 (0.78; 1.27)	0.98	0.91 (0.72; 1.16)	0.48
FT4, ng/dl	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, use of antihypertensive and lipid-lowering medications.

\*CAC  $\geq 100$  AU was defined as high CAC score. All included participants were free of atherosclerotic cardiovascular disease.

†Normal reference ranges were defined as serum TSH of 0.4-4.0 mIU/L and FT4 levels of 0.85-1.95 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 one unit increase of natural log transformed TSH (mIU/L). ORs of FT4 are denoted per one unit increase in FT4 (ng/dl).

Abbreviations: CAC, coronary artery calcification; TN, total number; OR, odds ratio; CI, confidence interval; TSH, thyroid-stimulating hormone; FT4, free thyroxine.

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

Events/TN (%)		Model 1		Model 2	
		HR (95% CI)	p-value	HR (95% CI)	p-value
TSH, mIU/L	934/8498 (11.0%)	0.96 (0.89; 1.03)	0.34	0.96 (0.89; 1.03)	0.35†
FT4, ng/dl	934/8498 (11.0%)	1.89 (1.37; 2.61)	<0.0001	1.87 (1.34; 2.59)	0.0002§
<i>Thyroid function within the reference ranges†</i>					
TSH, mIU/L	736/6826 (10.8%)	0.95 (0.81; 1.10)	0.50	0.96 (0.82; 1.11)	0.59
FT4, ng/dl	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, use of antihypertensive and lipid-lowering medications.

\*All included participants were free of atherosclerotic cardiovascular disease at baseline.

†Normal reference ranges were defined as serum TSH of 0.4-4.0 mIU/L and FT4 levels of 0.85-1.95 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 one unit increase of natural log transformed TSH (mIU/L). HRs of FT4 are denoted per one unit increase in FT4 (ng/dl).

Abbreviations: TN, total number; HR, hazard ratio; CI, confidence interval; TSH, thyroid-stimulating hormone; FT4, free thyroxine.



**Table 4. Association of thyroid function with atherosclerotic cardiovascular mortality**

	Events/TN (%)	Model 1 HR (95% CI)	p-value	Model 2 HR (95% CI)	p-value
TSH, mIU/L	612/9420 (6.5%)	0.93 (0.86; 1.01)	0.12	0.92 (0.84; 1.00)	0.06†
FT4, ng/dl	612/9420 (6.5%)	2.23 (1.58; 3.14)	<0.0001	2.41 (1.68; 3.47)	<0.0001§
<i>Thyroid function within the reference ranges*</i>					
TSH, mIU/L	483/7575 (6.4%)	0.82 (0.68; 0.98)	0.02	0.80 (0.67; 0.96)	0.01
FT4, ng/dl	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, use of antihypertensive and lipid-lowering medications.

Both models are adjusted for presence of prevalent atherosclerotic cardiovascular disease at baseline.

\*Normal reference ranges were defined as serum TSH of 0.4-4.0 mIU/L and FT4 levels of 0.85-1.95 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 one unit increase of natural log transformed TSH (mIU/L). HRs of FT4 are denoted per one unit increase in FT4 (ng/dl).

Abbreviations: TN, total number; HR, hazard ratio; CI, confidence interval; TSH, thyroid-stimulating hormone; FT4, free thyroxine.

**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, mIU/L	612/9420 (6.5%)	0.92 (0.84; 1.00)	0.06	1476/9420 (15.7%)	0.94 (0.89; 0.99)	0.03
FT4, ng/dl	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, use of antihypertensive and lipid-lowering medications.

HRs of TSH are denoted per one unit increase of natural log transformed TSH (mIU/L). HRs of FT4 are denoted per one unit increase in FT4 (ng/dl).

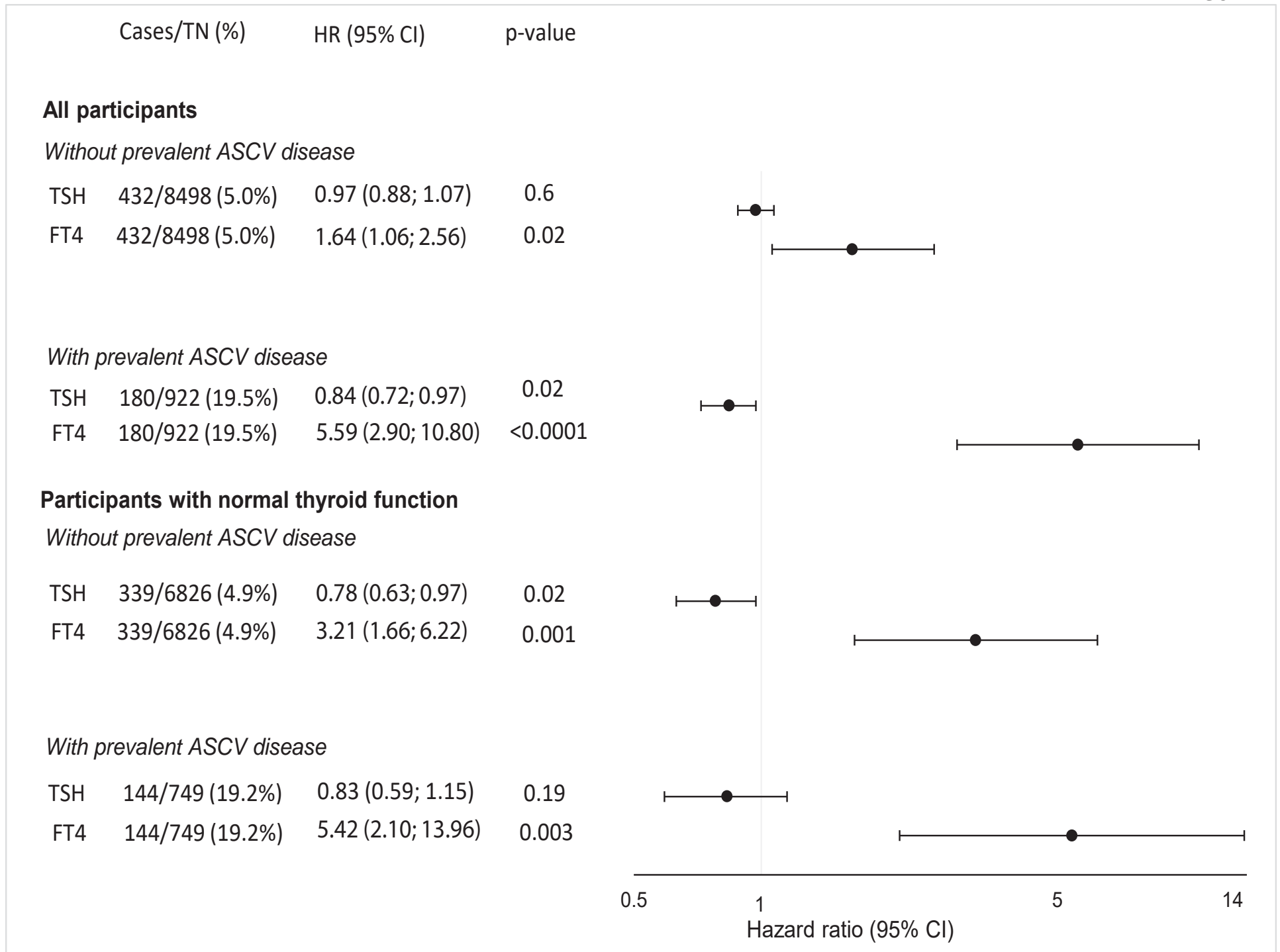
Abbreviations: ASCV, atherosclerotic cardiovascular; TN, total number; HR, hazard ratio; CI, confidence interval; TSH, thyroid-stimulating hormone; FT4, free thyroxine.



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FIGURE 1



## Thyroid Function and the Risk of Atherosclerotic Cardiovascular Morbidity and Mortality: The Rotterdam Study

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

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## **SUPPLEMENTAL MATERIAL**

### **Thyroid function and the risk of atherosclerotic cardiovascular morbidity and mortality: The Rotterdam Study**

Arjola Bano, MD, MSc, DSc<sup>1,2,3</sup>, Layal Chaker, MD, MSc<sup>1,2,3,4</sup>, Francesco U.S. Mattace-Raso, MD, PhD<sup>1,5</sup>, Aad van der Lugt, MD, PhD<sup>6</sup>, M. Arfan Ikram, MD, PhD<sup>3,6,7</sup>, Oscar H. Franco, MD, PhD<sup>3</sup>, Robin P. Peeters, MD, PhD<sup>1,2,3</sup>, Maryam Kavousi, MD, PhD<sup>3</sup>

<sup>1</sup>Department of Internal Medicine, Erasmus Medical Center, Rotterdam, The Netherlands

<sup>2</sup>Academic Center for Thyroid Diseases, Erasmus Medical Center, Rotterdam, The Netherlands

<sup>3</sup>Department of Epidemiology, Erasmus Medical Center, Rotterdam, The Netherlands

<sup>4</sup>Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, Massachusetts, USA

<sup>5</sup>Section of Geriatric Medicine, Erasmus Medical Center, Rotterdam, The Netherlands

<sup>6</sup>Department of Radiology, Erasmus Medical Center, Rotterdam, The Netherlands

<sup>7</sup>Department of Neurology, Erasmus Medical Center, Rotterdam, The Netherlands



## Appendix. Supplemental Methods

### *Study population*

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 (Online Figure I). 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 January 1, 2012, whichever came first. In the analysis of ASCV mortality, the end date of follow-up was considered the date of death or January 1, 2012, whichever came first.

### *Assessment of CAC and ascertainment of ASCV mortality*

Calcification of the coronary arteries was quantified through Acculmage software (Acculmage Diagnostics Corp), displaying all pixels with a density >130 Hounsfield units and using the Agatston's method<sup>1</sup>. Ascertainment of ASCV mortality in the Rotterdam Study has been described in detail previously<sup>2</sup>. 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 99.0%.

### *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 filtration rate (eGFR) was calculated according to the CKD Epidemiology Collaboration (CKD-EPI) formula<sup>3</sup>. 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  $\geq 7$  mmol/L, non-fasting plasma glucose level  $\geq 11.1$  mmol/L (when fasting samples were absent) or the use of antidiabetic medication<sup>4</sup>. 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<sup>4,5</sup>. Heart failure (HF) was defined as the presence of typical symptoms and signs (i.e. breathlessness at rest or during exertion, ankle edema, and pulmonary crepitations), confirmed by the objective evidence of cardiac dysfunction (i.e. chest X-ray, echocardiography) or a positive response to the initiated treatment<sup>4</sup>. The adjudication of HF was performed in accordance with the

guidelines of the European Society of Cardiology <sup>6</sup>. 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*

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 FT4 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 <sup>7</sup>.

### *STROBE Statement—checklist of items that should be included in reports of observational studies*

Item	No	Recommendation	Paragraph
<i>Title and abstract</i>	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
<i>Introduction</i>			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	1-3
Objectives	3	State specific objectives, including any prespecified hypotheses	3
<i>Methods</i>			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection-	4 Appendix
Participants	6	(a) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —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 <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants	4
		(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —For matched studies, give matching	NA

		criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5,6 Appendix
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	5-7
Bias	9	Describe any efforts to address potential sources of bias	7
Study size	10	Explain how the study size was arrived at	4, Appendix
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	7, Appendix
		(b) Describe any methods used to examine subgroups and interactions	7
		(c) Explain how missing data were addressed	7
		(d) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed <i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy	Appendix
		(e) Describe any sensitivity analyses	7
<i>Results</i>			
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 analysed	4,8 Appendix
		(b) Give reasons for non-participation at each stage	4
		(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	4-8, Appendix
		(b) Indicate number of participants with missing data for each variable of interest	Suppl
		(c) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	8
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time	8
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	NA
		<i>Cross-sectional study</i> —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	6

		categorized	
		(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	9-11
<i>Discussion</i>			
Key results	18	Summarise 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	21
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12-21
Generalisability	21	Discuss the generalisability (external validity) of the study results	21
<i>Other information</i>			
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](http://www.strobe-statement.org).

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**Online Table I. Association of TPOAb positivity with atherosclerotic outcomes**

	Events/TN (%)	Model 1 HR (95% CI)	p-value	Model 2 HR (95% CI)	p-value
<i>Association of TPOAb positivity with high CAC score*†</i>					
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
<i>Association of TPOAb positivity with incident atherosclerotic cardiovascular event†</i>					
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
<i>Association of TPOAb positivity with atherosclerotic cardiovascular mortality§</i>					
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, use of antihypertensive and lipid-lowering medications.

\*CAC  $\geq 100$  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 of 0.4-4.0 mIU/L and FT4 levels of 0.85-1.95 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.

**Online Table II. Sensitivity analyses for the association between thyroid function within the reference ranges\* and atherosclerotic outcomes**

	Events/TN (%)	Model 1 HR (95% CI)	p-value	Model 2 HR (95% CI)	p-value
<b>Incident atherosclerotic cardiovascular events†</b>					
<i>Thyroid function within the reference ranges*</i>					
TSH, mIU/L	736/6826 (10.8%)	0.95 (0.81; 1.10)	0.50	0.96 (0.82; 1.11)	0.59
FT4, ng/dl	736/6826 (10.8%)	2.67 (1.69; 4.20)	<0.0001	2.50 (1.58; 3.94)	<0.0001
<i>Excluding users of thyroid function-altering medications‡</i>					
TSH, mIU/L	638/5964 (10.7%)	0.92 (0.78; 1.09)	0.34	0.93 (0.79; 1.10)	0.41
FT4, ng/dl	638/5964 (10.7%)	3.05 (1.86; 4.98)	<0.0001	2.70 (1.64; 4.42)	<0.0001
<i>Additionally adjusted for physical activity</i>					
TSH, mIU/L	736/6826 (10.8%)	0.95 (0.82; 1.11)	0.56	0.96 (0.83; 1.12)	0.67
FT4, ng/dl	736/6826 (10.8%)	2.62 (1.67; 4.12)	<0.0001	2.47 (1.57; 3.90)	<0.0001
<i>Additionally adjusted for eGFR</i>					
TSH, mIU/L	736/6826 (10.8%)	0.93 (0.80; 1.08)	0.35	0.94 (0.81; 1.10)	0.48
FT4, ng/dl	736/6826 (10.8%)	2.56 (1.62; 4.03)	<0.0001	2.39 (1.51; 3.78)	<0.0001
<i>Excluding subjects with prevalent AF and censoring at the time of incident AF</i>					
TSH, mIU/L	574/6270 (9.2%)	0.94 (0.79; 1.12)	0.50	0.96 (0.80; 1.14)	0.63
FT4, ng/dl	574/6270 (9.2%)	2.25 (1.33; 3.82)	<0.003	2.05 (1.20; 3.48)	0.008
<i>Excluding subjects with prevalent HF and censoring at the time of incident HF</i>					
TSH, mIU/L	693/6640 (10.4%)	0.89 (0.77; 1.04)	0.17	0.91 (0.78; 1.06)	0.24
FT4, ng/dl	693/6640 (10.4%)	2.70 (1.68; 4.33)	<0.0001	2.35 (1.46; 3.78)	0.0004
<b>Atherosclerotic cardiovascular mortality§</b>					
<i>Thyroid function within the reference ranges*</i>					
TSH, mIU/L	483/7575 (6.4%)	0.82 (0.68; 0.98)	0.02	0.80 (0.67; 0.96)	0.01
FT4, ng/dl	483/7575 (6.4%)	3.92 (2.29; 6.71)	<0.0001	3.84 (2.23; 6.60)	<0.0001
<i>Excluding users of thyroid function-altering medications‡</i>					
TSH, mIU/L	389/6476 (6.0%)	0.74 (0.61; 0.90)	0.003	0.72 (0.58; 0.88)	0.001
FT4, ng/dl	389/6476 (6.0%)	3.82 (2.05; 7.14)	<0.0001	3.47 (1.84; 6.51)	<0.0001
<i>Additionally adjusted for physical activity</i>					
TSH, mIU/L	483/7575 (6.4%)	0.82 (0.69; 0.99)	0.04	0.81 (0.67; 0.97)	0.02
FT4, ng/dl	483/7575 (6.4%)	3.79 (2.22; 6.46)	<0.0001	3.74 (2.18; 6.43)	<0.0001
<i>Additionally adjusted for eGFR</i>					
TSH, mIU/L	483/7575 (6.4%)	0.79 (0.66; 0.95)	0.01	0.79 (0.65; 0.94)	0.01
FT4, ng/dl	483/7575 (6.4%)	3.65 (2.13; 6.26)	<0.0001	3.60 (2.09; 6.20)	<0.0001
<i>Excluding subjects with prevalent AF and censoring at the time of incident AF</i>					
TSH, mIU/L	357/6906 (5.2%)	0.81 (0.66; 0.99)	0.04	0.80 (0.65; 0.99)	0.04
FT4, ng/dl	357/6906 (5.2%)	2.90 (1.52; 5.56)	0.001	3.15 (1.64; 6.05)	0.001
<i>Excluding subjects with prevalent HF and censoring at the time of incident HF</i>					
TSH, mIU/L	356/7307 (4.9%)	0.75 (0.61; 0.92)	0.007	0.73 (0.59; 0.90)	0.004
FT4, ng/dl	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, use of antihypertensive and lipid-lowering medications.

\*Normal reference ranges were defined as serum TSH of 0.4-4.0 mIU/L and FT4 levels of 0.85-1.95 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 one unit increase of natural log transformed TSH (mIU/L). HRs of FT4 are denoted per one unit increase in FT4 (ng/dl). Abbreviations: TN, total number; HR, hazard ratio; CI, confidence interval; TSH, thyroid-stimulating hormone; FT4, free thyroxine; eGFR, estimated glomerular filtration rate; AF, atrial fibrillation; HF, heart failure.



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

	Events/TN (%)	Model 1 HR (95% CI)	p-value	Model 2 HR (95% CI)	p-value
<b>Incident atherosclerotic cardiovascular events*</b>					
TSH, mIU/L	775/8339 (9.3%)	0.93 (0.86; 1.01)	0.10	0.93 (0.86; 1.01)	0.09
FT4, ng/dl	775/8339 (9.3%)	1.91 (1.34; 2.72)	0.0003	1.87 (1.30; 2.69)	0.001
<i>Thyroid function within the reference ranges†</i>					
TSH, mIU/L	611/6701 (9.1%)	0.95 (0.80; 1.12)	0.57	0.96 (0.81; 1.14)	0.66
FT4, ng/dl	611/6701 (9.1%)	2.51 (1.52; 4.15)	0.0003	2.30 (1.39; 3.81)	<0.0001
<b>Atherosclerotic cardiovascular mortality‡</b>					
TSH, mIU/L	535/9343 (5.7%)	0.94 (0.86; 1.03)	0.23	0.93 (0.84; 1.02)	0.12
FT4, ng/dl	535/9343 (5.7%)	1.95 (1.34; 2.84)	0.0004	2.12 (1.42; 3.16)	0.0002
<i>Thyroid function within the reference ranges†</i>					
TSH, mIU/L	419/7511 (5.5%)	0.80 (0.66; 0.97)	0.02	0.78 (0.64; 0.95)	0.01
FT4, ng/dl	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, use of antihypertensive and lipid-lowering medications.

\*All included participants were free of atherosclerotic cardiovascular disease at baseline.

†Normal reference ranges were defined as serum TSH of 0.4-4.0 mIU/L and FT4 levels of 0.85-1.95 ng/dl, after excluding thyroid medication users and participants with history of thyroid disease.

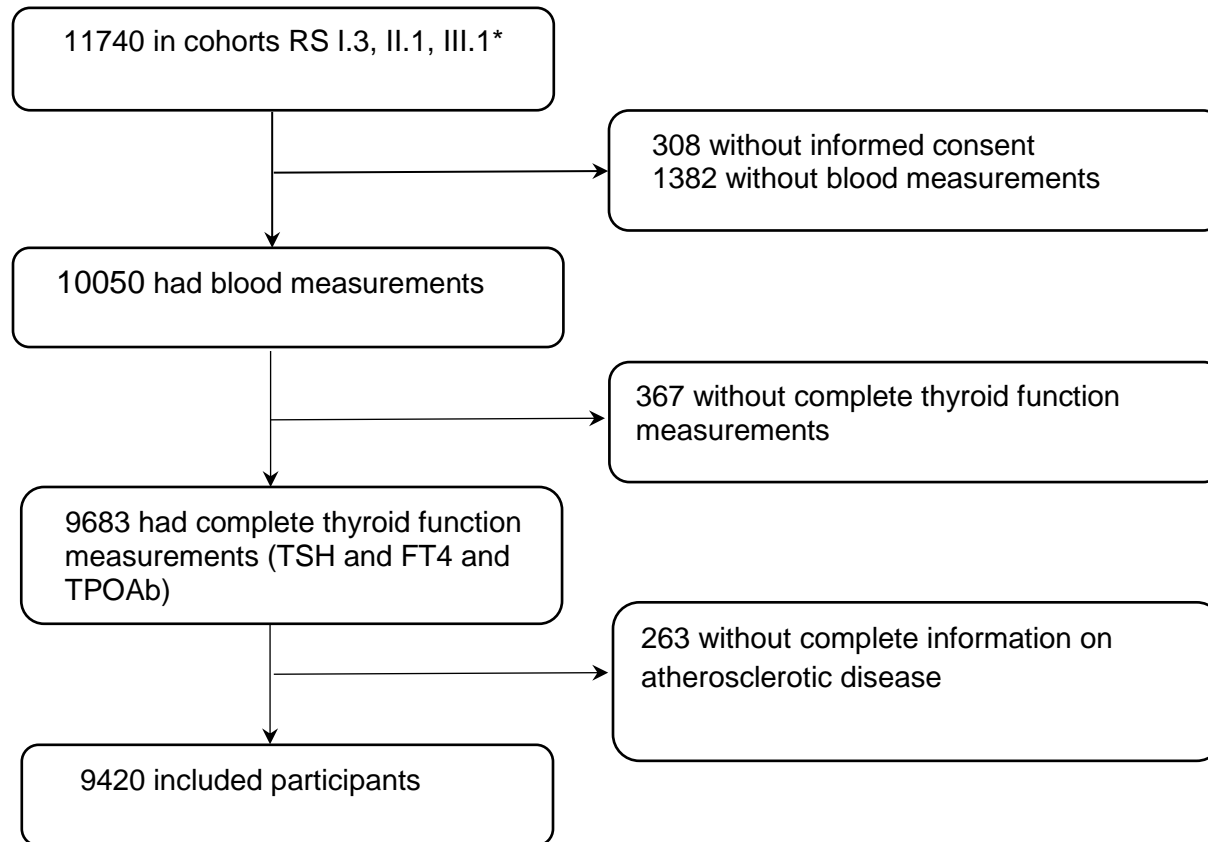
‡ Additionally adjusted for prevalent atherosclerotic cardiovascular disease.

HRs of TSH are denoted per one unit increase of natural log transformed TSH (mIU/L). HRs of FT4 are denoted per one unit increase in FT4 (ng/dl).

Abbreviations: TN, total number; HR, hazard ratio; CI, confidence interval; TSH, thyroid-stimulating hormone; FT4, free thyroxine.

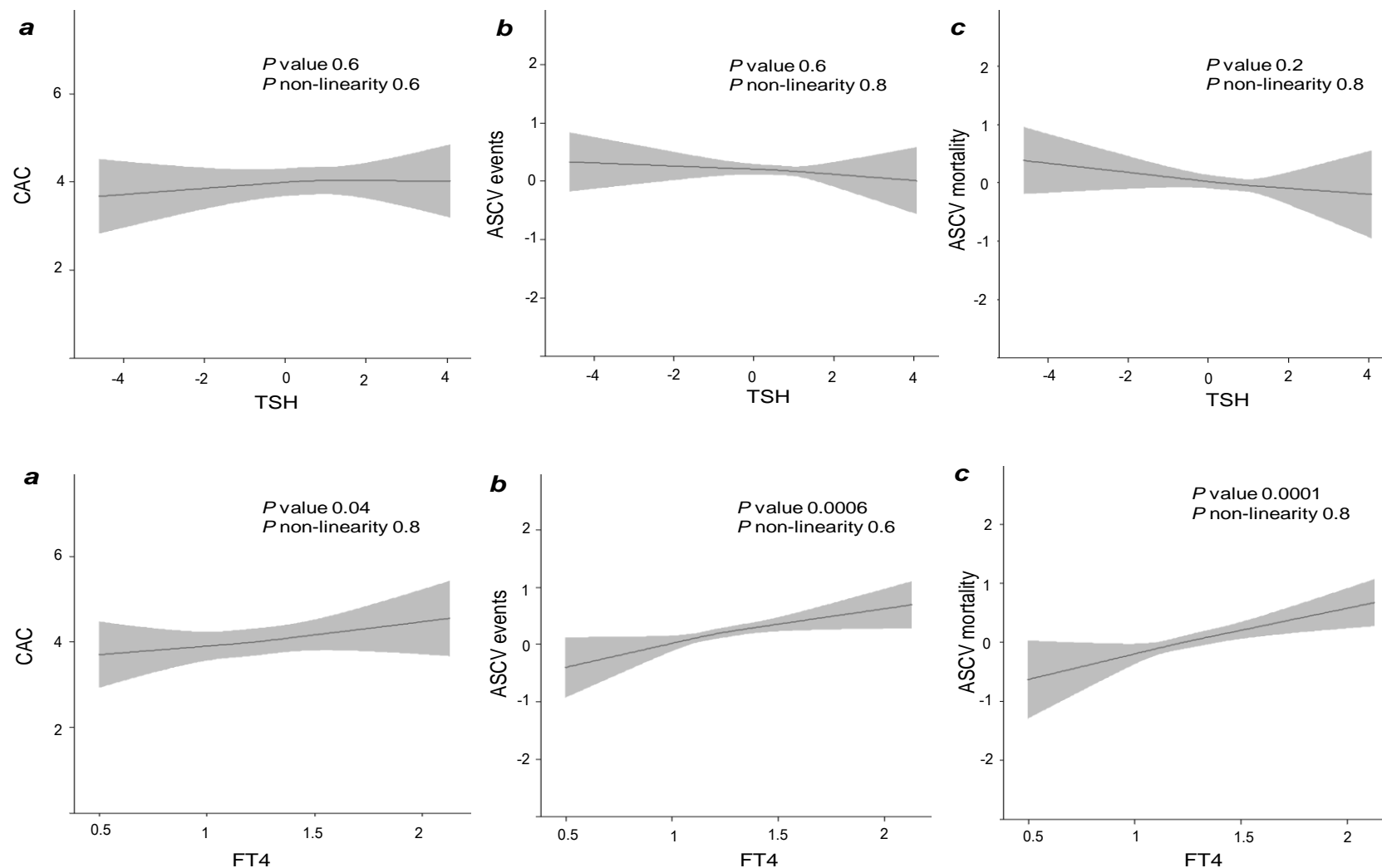
Online Table IV. Association of thyroid function with atherosclerotic outcomes, stratified by sex and age							
Strata	TSH/FT4	High CAC score		Incident ASCV events		ASCV mortality‡	
		Events/TN	OR (95% CI)	Events/TN	HR (95% CI)	Events/TN	HR (95% CI)
<b>Sex*</b>							
Men	TSH	444/738 (60.1%)	0.98 (0.80; 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)
<i>P for interaction</i>			0.96		0.92		0.03
Men	FT4	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)
<i>P for interaction</i>			0.88		0.62		0.002
<b>Age†</b>							
<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)
<i>P for interaction</i>			0.88		0.30		0.35
<65 years	FT4	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)
<i>P for interaction</i>			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; FT4, free thyroxine.							

**Online Figure I. 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

**Online Figure II. 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 FT4 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; FT4, free thyroxine; CAC, coronary artery calcification; ASCV, atherosclerotic cardiovascular.



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