THYROID FUNCTION, CARDIOMETABOLIC HEALTH AND GENERAL HEALTH

In middle-aged and older adults

Arjola Bano

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THYROID FUNCTION, CARDIOMETABOLIC HEALTH AND GENERAL HEALTH

In middle-aged and older adults

Schildklierfunctie, cardiometabole gezondheid en algemene gezondheid van middelbare en oudere volwassenen

Thesis

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Let all that you do be done in love
1 Corinthians 16:14

In loving memory of Liri Bano, my grandmother,
the kindest person I have ever known

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MANUSCRIPTS BASED ON THIS THESIS

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Bano A*, Chaker L*, Plompen EP, Hofman A, Dehghan A, Franco OH, Janssen HL, Darwish Murad S, Peeters RP. Thyroid function and the risk of nonalcoholic fatty liver disease: The Rotterdam Study. *J Clin Endocrinol Metab.* 2016;101(8):3204-3211.

Bano A, et al. Thyroid function and the risk of fibrosis of the liver, lung, and heart: A systematic review of human studies. *Manuscript in preparation*

Bano A, Chaker L, Mattace-Raso FUS, van der Lugt A, Ikram MA, Franco OH, Peeters RP, Kavousi M. Thyroid function and the risk of atherosclerotic cardiovascular morbidity and mortality: The Rotterdam Study. *Circ Res.* 2017;121(12):1392-1400.

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Chapter 3

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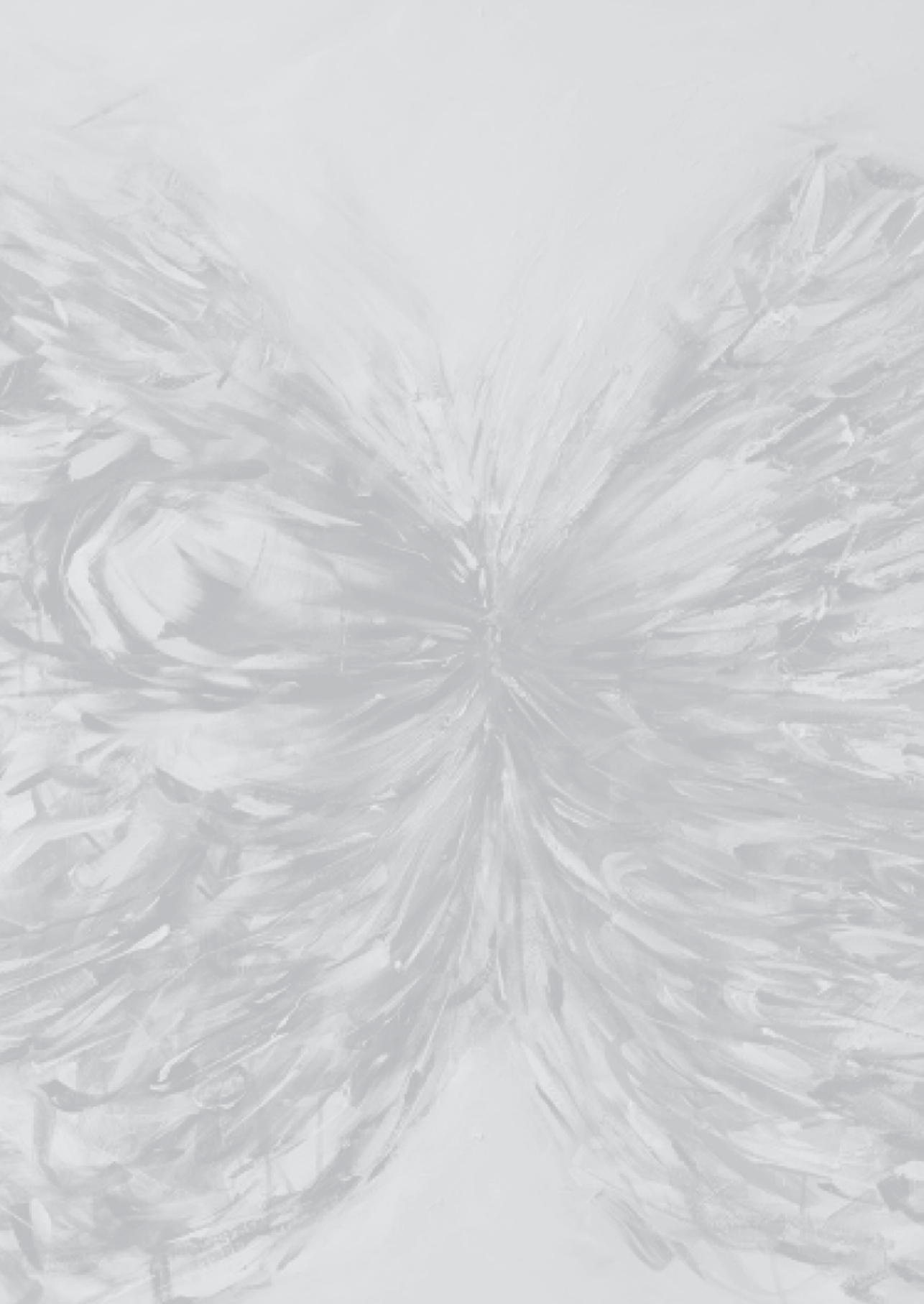
** Denotes equal contribution within a manuscript*



CHAPTER 1

GENERAL INTRODUCTION

THYROID FUNCTION

The thyroid gland synthetizes and secretes thyroid hormones T_4 (3, 5, 3', 5'-tetraiodothyronine, also known as thyroxine) and T_3 (3, 5, 3'-triiodothyronine).[1] Thyroid hormones are produced in response to the thyroid–stimulating hormone (TSH). TSH is secreted from the anterior pituitary gland in response to the thyrotropin-releasing hormone (TRH), which is secreted from the hypothalamus (Figure 1). The production of TSH and TRH is downregulated by thyroid hormones (Figure 1).[2] T_3, which is mainly derived from the local metabolism of circulating T_4, inhibits the synthesis and secretion of TRH and TSH, via binding to the thyroid hormone receptors in the hypothalamus and pituitary.[2] This negative feedback mechanism ensures the stability of circulating thyroid hormone levels, which is crucial for the biological functioning of all organs.

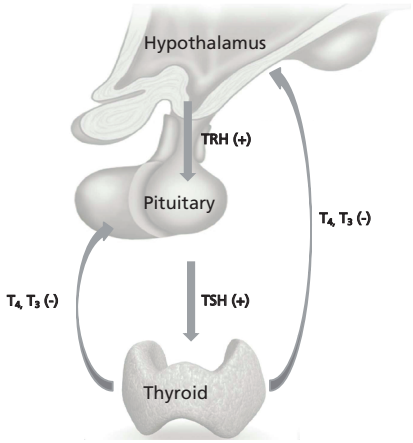


Figure 1. The hypothalamic-pituitary-thyroid axis.
Abbreviations: T_4, thyroxine; T_3, triiodothyronine, TSH, thyroid–stimulating hormone, TRH, thyrotropin-releasing hormone.

Thyroid function is clinically defined by the measurements of TSH and free thyroxine (FT_4) levels. Clinical hypothyroidism is characterized by TSH above the reference range and FT_4 levels below the reference range, whereas clinical hyperthyroidism is characterized by TSH below the reference range and FT_4 levels above the reference range. Subclinical hypothyroidism is defined by FT_4 within the reference range combined with elevated TSH levels. Subclinical hyperthyroidism is defined by FT_4 within the reference range combined with reduced TSH levels. In the adult population, the prevalence of clinical and subclinical hypothyroidism ranges from 0.2 to 5.3% and from 4 to 15%, respectively.[3-9] The prevalence of overt and subclinical hyperthyroid-

ism ranges from 0.8 to 1.3% and from 0.6 to 12.4%, respectively.[5,10-17] This variability may be explained by the different characteristics of the studied populations (eg, different iodine status) and the different assays of thyroid function used.

TSH levels needed to achieve the same thyroid hormone levels vary significantly among individuals,[18] indicating that each individual has a unique pituitary-thyroid set point. Several genetic loci have been linked to the pituitary-thyroid set point, suggesting that the set point is to some extent, genetically determined.[19,20] In addition, the relationship between TSH and FT_4 concentrations can be modulated throughout ageing.[21] Several studies have suggested that increasing age can reduce the sensitivity of the pituitary gland to thyroid hormones.[22-24] As a result, TSH levels needed to maintain the same FT_4 levels are different in younger and older adults.[24]

The reference ranges of TSH and FT_4 levels provide the basis for the diagnosis and treatment of thyroid disease. At present, the reference ranges of thyroid function are determined by a statistical approach, which is based on the 2.5^{th} and 97.5^{th} percentiles of the TSH and FT_4 distributions in an apparently healthy population. That is, TSH (or FT_4) levels above the 2.5^{th} and below the 97.5^{th} percentiles are considered as normal, whereas TSH (or FT_4) levels below the 2.5^{th} and above the 97.5^{th} percentiles are considered as abnormal. However, many studies have reported that the clinical consequences of abnormal thyroid function are extended even within the current reference ranges of TSH and FT_4,[25-28] thus indicating that the statistically defined reference ranges do not properly reflect the risk of developing clinical outcomes. Therefore, over the past years, there has been an ongoing debate on whether the reference ranges of TSH and FT_4 should be reevaluated. While some researchers support a reevaluation of TSH and FT_4 reference ranges, suggested measures are inconsistent varying from a lowering of the upper TSH reference limit (eg, from approximately 4 to 2.5 mIU/L) to an increase of the upper TSH reference limit or a downward shift of the FT_4 reference limit.[25,29-31] Others do not support a reevaluation, suggesting that more robust evidence needs to illustrate the risk of clinical outcomes within the reference ranges of TSH and FT_4.[32-35]

THE ROLE OF THYROID FUNCTION ON CARDIOMETABOLIC HEALTH

Thyroid hormones play a critical role in maintaining cardiometabolic homeostasis, via regulating cardiac and vascular physiology, as well as lipid, glucose and protein metabolism.[36] Besides, thyroid hormones influence energy expenditure by

accelerating basal metabolic rate, mitochondrial oxygen consumption and thermogenesis.[37] In the heart, thyroid hormones exert genomic effects via binding to the thyroid hormone receptors that are located in the nucleus of cardiomyocytes, further promoting the expression of target genes.[38] Thyroid hormones also exert non-genomic effects on various ion channels in the membranes of cardiomyocytes.[38] These genomic and non-genomic effects are translated into inotropic, chronotropic and bathmotropic cardiac effects of thyroid hormones.[38] Previous studies have extensively explored the association of thyroid function with various cardiometabolic conditions, including atrial fibrillation (AF), coronary heart disease (CHD), stroke, heart failure, hypertension, diabetes mellitus, dyslipidemia, and obesity.[25,28,32,39-48] Interestingly, even minimal fluctuations in TSH and FT_4 concentrations have been associated with remarkable alterations in cardiometabolic health.[27,49-53]

Atrial fibrillation: High and high-normal thyroid function constitute an increased risk of AF. An individual participant data (IPD) meta-analysis from the Thyroid Studies Collaboration showed that subclinical hyperthyroidism is associated with a 1.68 times higher risk of AF compared with euthyroidism.[39] Prospective studies focusing on the normal range of thyroid function have also consistently reported an association between high-normal thyroid function and increased AF risk.[28,40]

Coronary heart disease: Three large IPD meta-analyses from the Thyroid Studies Collaboration have focused on the risk of CHD and CHD mortality in subclinical hypothyroidism, subclinical hyperthyroidism and euthyroidism, respectively.[32,39,41] The first reported that subclinical hypothyroidism with TSH levels above 10 mIU/L is associated with a 1.89 and 1.58 times higher risk of CHD events and CHD mortality than euthyroidism, respectively.[41] The second reported that subclinical hyperthyroidism is associated with a 1.21 and 1.29 times higher risk of CHD events and CHD mortality than euthyroidism, respectively.[39] The third IPD meta-analysis, performed among euthyroid subjects, showed no association between thyroid function within the reference range and CHD risk.[32]

Stroke: The association of thyroid function with stroke has been investigated in two IPD meta-analyses from the Thyroid Studies Collaboration.[43] One of them found no overall effect of subclinical hypothyroidism on the risk of stroke events or fatal stroke.[43] However, age-stratified analyses (younger versus older than 65 years) revealed that in

younger participants, subclinical hypothyroidism was associated with a higher risk of stroke than euthyroidism. The other IPD meta-analysis, which included only euthyroid participants, showed that low-normal TSH levels and high-normal FT_4 levels are associated with an increased risk of stroke.[42]

Hypertension: Overt and subclinical hyperthyroidism often lead to systolic hypertension via increasing cardiac output.[45,54] Overt and subclinical hypothyroidism, on the other hand, promote diastolic hypertension via increasing systemic vascular resistance.[55,56] Even in euthyroid subjects, higher TSH levels have been associated with both systolic and diastolic hypertension.[51,52]

Heart failure: In an IPD meta-analysis from the Thyroid Studies Collaboration, both higher and lower TSH levels showed a significant trend for an increased risk of heart failure.[44] Participants with TSH levels ≥10 and <0.1 mIU/L had a 1.86 and 1.94 times higher risk of heart failure than euthyroid participants, respectively. Several mechanisms can explain the role of thyroid function on heart failure. Subclinical thyroid dysfunction can increase the risk of CHD, which is a common cause of heart failure. Moreover, alterations in thyroid function affect heart rate, cardiac contractility, cardiac output and vascular resistance, that can all contribute to the development of heart failure.[57] On the other hand, a potential influence of heart failure on the metabolism of thyroid hormones is also likely. Heart failure-related hypoxia increases the gene expression of type 3 deiodinase, which promotes the degradation of thyroid hormone, eventually reducing the local availability of T_3 in cardiomyocytes.[58,59] Low levels of T_3 further contribute to a progressive deterioration of cardiac function in heart failure and have been proposed as an independent predictor of New York Heart Association functional class.[60]

Diabetes mellitus: Hypothyroidism is associated with an increased risk of diabetes, most likely due to a decreased insulin sensitivity and glucose tolerance.[46,61] Accordingly, restauration of euthyroidism after treatment of hypothyroidism has been shown to improve insulin sensitivity.[62,63] These negative consequences of hypothyroidism on glucose metabolism can also be extended within the reference range of thyroid function. In a large prospective population-based cohort study, even low-normal thyroid function was associated with an increased risk of type 2 diabetes and progression from prediabetes to diabetes.[27]

Dyslipidemia: Overt hypothyroidism commonly leads to hypercholesterolemia and hypertriglyceridemia,[36] via decreasing the expression of hepatic LDL receptors, reducing cholesterol clearance and modulating fatty acid metabolism.[47] The role of subclinical hypothyroidism on dyslipidemia is less clear. Some studies have suggested that thyroid hormone replacement may improve the lipid parameters.[64-66] This, however, was not confirmed in a meta-analysis of randomized clinical trials, that showed no overall effects of thyroid hormone replacement in the lipid profiles of patients with subclinical hypothyroidism.[67] Levothyroxine treatment did not result in a reduction of total cholesterol, HDL cholesterol, triglycerides, apolipoprotein A and B, and lipoprotein A, though there was a trend towards reducing LDL cholesterol >155 mg/dl.[67]

Obesity: In some,[48,53,68] but not all[69] population-based studies, high and high-normal TSH levels have been associated with an increased body weight. The association between thyroid function and body weight is likely bidirectional. On one hand, low thyroid function is typically characterized by decreased energy expenditure and low metabolic rate, resulting in weight gain.[70] On the other hand, adipose tissue has been recognized as an endocrine organ because it secretes leptin,[70-72] which is known to stimulate TSH release.[70]

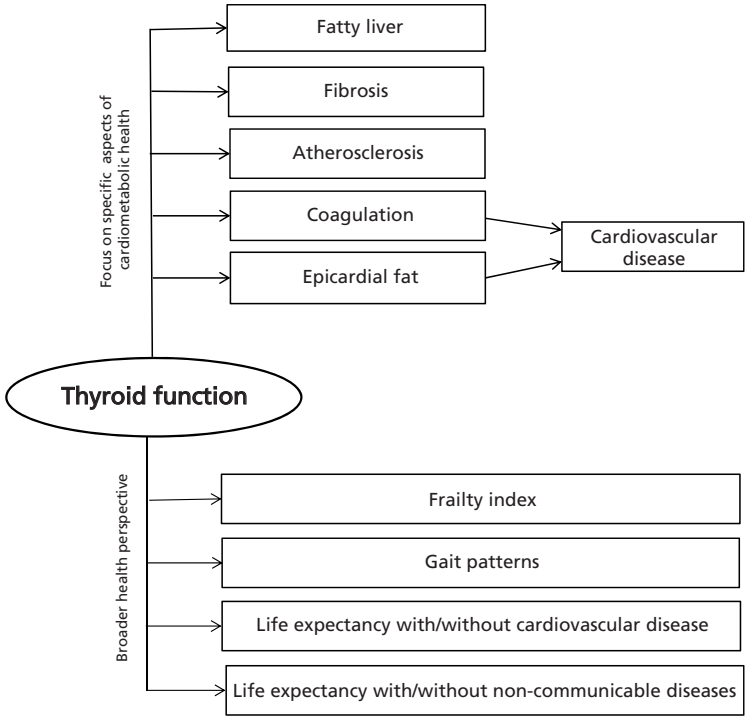
THE PLEIOTROPIC EFFECTS OF THYROID HORMONES

Thyroid hormones have complex pleiotropic effects in nearly all tissues and organs.[73,74] Clinical, epidemiological and experimental evidence suggests that even subtle changes in circulating thyroid hormone levels can adversely affect cardiovascular, musculoskeletal and neurocognitive functioning.[26,28,73,74] The effects of thyroid hormones vary in character, some being stimulatory and others inhibitory. This is illustrated by several prospective studies, showing that circulating thyroid hormones are negatively associated with the risk of diabetes or dyslipidemia[27,49] and are positively associated with the risk of cognitive decline or atrial fibrillation.[26,28] The effects of thyroid hormones also vary in magnitude, depending on the targeted tissues and organs. For example, in middle-aged and older adults, increasing FT_4 levels have been prospectively associated with an increased risk of dementia, and even higher risk of AF.[26,28]

AIMS OF THIS THESIS

This thesis has two main aims. The first aim is to extend the current knowledge on the specific effects of thyroid function on cardiometabolic health. In view of the inconsistent results of previous studies,[32,42,75-78] we investigate the association of thyroid function with cardiometabolic diseases, such as nonalcoholic fatty liver disease, fibrotic disease, and atherosclerosis. Furthermore, we focus on the association of thyroid function with some aspects of cardiometabolic health that have been studied less extensively so far, such as coagulation and epicardial adipose tissue. To provide some mechanistic evidence, we also investigate whether and to what extent coagulation factors and epicardial adipose tissue can explain certain cardiovascular effects of thyroid hormones. The second aim is to yield novel insights about the qualitative and quantitative impact of thyroid function on general health. We thus adopt a broader perspective, using multidimensional measures

Figure 2. Implications of thyroid function among middle-aged and older adults: Focused versus broader perspective.



that can reflect the pleiotropic effects of thyroid hormones, such as frailty index, global gait, and measurements of life expectancy with and without diseases. The conceptual framework of this thesis is presented in Figure 2.

RATIONALE OF THIS THESIS

Beyond thyroid status categories

The classification of thyroid status in categories of euthyroidism, clinical and subclinical hypothyroidism, clinical and subclinical hyperthyroidism, is useful in clinical decision making. However, thyroid status categories are based on arbitrary cutoffs of TSH and FT_4 levels. As a result, cohort studies exclusively investigating thyroid status categories or arbitrary cutoffs of thyroid function may not properly account for potential nonlinear effects of thyroid function. Hypothetically, variations throughout the full spectrum of TSH and FT_4 levels may be associated with the risk of adverse outcomes. Therefore, our investigations were mainly focused on the risk of adverse outcomes throughout the continuous range of TSH and FT_4 levels, beyond the above described thyroid status categories.

Thyroid function, a potential risk factor for cardiometabolic conditions

The burden of diseases that affect cardiometabolic health can be reduced by identifying and modifying their determinants. High thyroid function, for example, is a well-established risk factor for AF, and thyroid function measurements are routinely performed in patients diagnosed with newly-onset AF.[79] Yet, the association of thyroid function with some other aspects of cardiometabolic health is less established. Previous studies focusing on the role of thyroid function on fatty liver, fibrosis or atherosclerosis have yielded inconsistent results.[32,42,75-78] Moreover, current data on the role of thyroid function on coagulation or epicardial adipose tissue are scarce. Therefore, we aimed to extend the current knowledge about the role of thyroid function on several aspects of cardiometabolic health, including fatty liver, fibrosis, atherosclerosis, coagulation, and epicardial adipose tissue.

Potential mediators linking thyroid function to cardiovascular disease

The influence of thyroid function on cardiovascular events, such as AF, CHD, and stroke, seems to be independent of hypertension, dyslipidemia, obesity, and diabetes.[25] This suggests that alternative factors beyond traditional cardiovascular risk

factors can mediate the effects of thyroid function on the cardiovascular system. The elucidation of these mediators is important, not only for a better pathophysiological understanding of cardiovascular diseases, but also for establishing novel preventive and treatment strategies. Therefore, we hypothesized that coagulation factors and epicardial adipose tissue can partially explain the effects of thyroid function on cardiovascular disease and AF, respectively.

The need for a broader health perspective

Thyroid hormones have stimulatory or inhibitory, major or minor effects, depending on the targeted tissues and organs. The resultant of all the specific effects of thyroid hormones is likely reflected in general health. However, the role of thyroid hormones on general health remains unclear. This information could help improve the prevention and possible prediction of health deterioration, and would also be relevant in view of the ongoing debate on the optimal reference ranges of thyroid function. Therefore, we sought to provide novel insights regarding the qualitative and quantitative impact of thyroid function on general health. Given that a "golden standard" measure of general health is lacking, we used several multidimensional measures that can reflect the pleiotropic effects of thyroid hormones, such as frailty index (measure of general health and frailty), global gait (measure of general health and functional mobility), and measurements of life expectancy with and without diseases.

SETTING

The study presented in Chapter 2.2 is a systematic review of the literature. Two reviewers independently screened the titles and abstracts, further selecting the eligible studies. The Newcastle-Ottawa Scale for non-randomized studies was used to assess the quality of the included studies based on 3 predefined domains, namely selection of participants, comparability of study groups, and ascertainment of the outcomes of interest.

The other studies presented in Chapters 2 and 3 of this thesis were performed within the framework of the Rotterdam Study. The Rotterdam Study is an ongoing prospective population-based cohort study that investigates the determinants, occurrence, and progression of chronic diseases among middle-aged and older adults.[80] In 1989, the Rotterdam Study enrolled participants into its first cohort (RS

cohort I), which was further extended in 2000 (RS cohort II) and 2006 (RS cohort III). Study participants are followed-up for the occurrence of chronic diseases. Extensive medical examinations are performed every 3 to 5 years. Thyroid function tests were measured in the three Rotterdam Study cohorts using the same method and assay.

OUTLINE OF THIS THESIS

Chapter 1 provides a general background on the pleiotropic effects of thyroid hormones, with a particular focus on cardiometabolic health. The objectives, rationale and outline of the thesis are further described.

Chapter 2 aims to extend the knowledge on the association of thyroid function with specific aspects of cardiometabolic health, including fatty liver, fibrosis, and atherosclerosis. Most studies examining the role of thyroid function on fatty liver are characterized by inconsistent results, that can be explained by cross-sectional designs and small sample sizes.[75,76] Therefore, Chapter 2.1 prospectively investigates the association of thyroid function with the risk of nonalcoholic fatty liver disease, in a large population-based cohort.

Furthermore, it has been suggested that variations in thyroid function may affect the occurrence and progression of fibrosis, but the data are fragmented and inconclusive.[77,78,81,82] In this context, Chapter 2.2 systematically appraises the evidence regarding the role of thyroid function on fibrosis of the liver, lung, and heart.

Thyroid hormones have been linked to both proatherogenic[83,84] and antiatherogenic[36] processes, but the role of thyroid function on the different stages of atherosclerosis progression has not been investigated. The cohort study presented in Chapter 2.3 examines the association of thyroid function with different stages of atherosclerosis, from subclinical atherosclerosis to atherosclerotic cardiovascular events to atherosclerotic cardiovascular mortality.

The effects of thyroid hormones on the cardiovascular system seem to be independent of traditional cardiovascular risk factors, such as hypertension or dyslipidemia.[25] In Chapters 2.4 and 2.5, we aim to identify potential mediators linking thyroid function to cardiovascular events. In Chapter 2.4, we hypothesize that blood coagulation can be one of the underlying mechanisms through which thyroid hormones affect cardiovascular health. Using a four-way decomposition approach,

Chapter 2.5 explores whether epicardial adipose tissue mediates the association of thyroid function with atrial fibrillation.

Thyroid hormones exert specific effects on nearly all tissues and organs, the resultant of which can be reflected in general health. In ***Chapter 3***, we aim to provide novel insights on the qualitative and quantitative impact of thyroid function on general health. We therefore evaluate conditions that can reflect the pleiotropic effects of thyroid hormones, including general health, vulnerability to adverse outcomes, functional mobility, and life expectancy. In Chapter 3.1, we cross-sectionally and longitudinally investigate the association of thyroid function with frailty index, a well-established measure of frailty and general health. Chapter 3.2 seeks to identify the spatiotemporal gait aspects that are related to thyroid function. Comprehensive measurements of gait patterns, including global gait, rhythm, variability, phases, pace, base of support, tandem, turning, and velocity, are used. In view of the current debate on the reference ranges of TSH and FT_4 levels, Chapters 3.3 and 3.4 investigate whether there are meaningful differences in total life expectancy and disease-specific life expectancy within the reference range of thyroid function. Given the important role of thyroid hormones on cardiovascular health, Chapter 3.3 focuses on the association between thyroid function within the reference range and life expectancy with and without cardiovascular disease. Meanwhile, Chapter 3.4 provides a broader perspective by investigating the association between thyroid function within the reference range and life expectancy with and without non-communicable diseases.

Chapter 4 summarizes the principal findings of this thesis, elaborates on the main methodological considerations, and further discusses the clinical implications and potential directions for future research.

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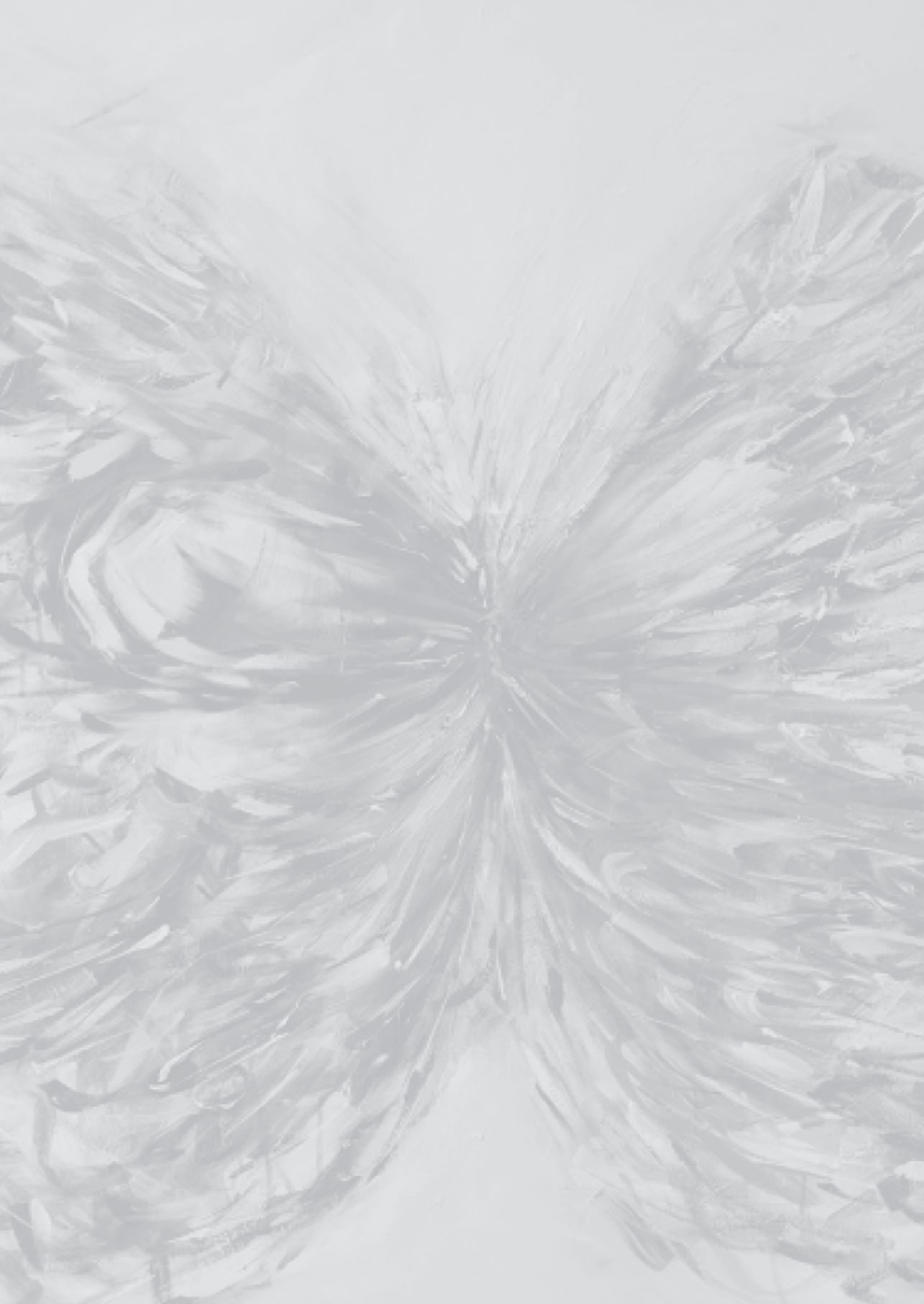

CHAPTER 2

THYROID FUNCTION AND SPECIFIC ASPECTS OF CARDIOMETABOLIC HEALTH

CHAPTER 2.1

THYROID FUNCTION AND THE RISK OF NONALCOHOLIC FATTY LIVER DISEASE

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ABSTRACT

Background Although thyroid function is associated with several risk factors of nonalcoholic fatty liver disease (NAFLD), its role in NAFLD development remains unclear. We therefore aimed to prospectively investigate the association between variations in thyroid function and NAFLD, in a large population-based, prospective cohort study.

Methods Participants from the Rotterdam Study with thyroid function measurements at baseline and NAFLD data (ie, at baseline fatty liver index, at follow-up ultrasound) were eligible. Transient elastography was performed to assess the presence of fibrosis in patients with NAFLD, using the liver stiffness measurements ≥8 kilopascals as cutoff for clinically relevant fibrosis. The association between thyroid parameters and incident NAFLD was explored by using logistic regression models.

Results A total of 9419 participants (mean age, 64.75 years) were included. The median follow-up time was 10.04 years (interquartile range, 5.70 to 10.88 years). After adjusting for age, sex, cohort, follow-up time, use of lipid-lowering medications, and cardiovascular risk factors, higher free thyroxine levels were associated with a decreased risk of NAFLD (odds ratio [OR], 0.42; 95% confidence interval [95% CI], 0.28 to 0.63). In line, higher thyroid-stimulating hormone levels were associated with an increased risk of having clinically relevant fibrosis in NAFLD (OR, 1.49; 95% CI, 1.04 to 2.15). Compared to euthyroidism, hypothyroidism was associated with a 1.24 times higher NAFLD risk (95% CI, 1.01 to 1.53). Moreover, NAFLD risk decreased gradually from hypothyroidism to hyperthyroidism (*P* for trend, 0.003).

Conclusions Lower thyroid function is associated with an increased NAFLD risk. These findings may lead to new avenues regarding NAFLD prevention and treatment.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver condition worldwide.[1] It comprises a broad spectrum ranging from simple steatosis to nonalcoholic steatohepatitis with fibrosis, which can eventually progress to cirrhosis and hepatocellular carcinoma.[2,3] Nonalcoholic steatohepatitis-related cirrhosis is anticipated to become the leading indication for liver transplantation by 2030.[4] Moreover, accumulating evidence has shown that NAFLD, either independently or in combination with other metabolic risk factors, is associated with extrahepatic complications such as cardiovascular disease, type 2 diabetes, chronic kidney disease, malignancy, and all-cause mortality.[5] Despite improved understanding and treatment of its risk factors (eg, diabetes mellitus and dyslipidemia), prevalence of NAFLD has rapidly increased.[6] Hence, investigation of additional modifiable risk factors is urgently needed.

Thyroid hormone is the major regulator of metabolic rate. Although hypothyroidism has been implicated in the etiology of NAFLD,[7] prior studies regarding the association between thyroid function and NAFLD risk have yielded controversial results, varying from a strong [8,9] to no association.[10,11] Studies confined to euthyroid subjects have been inconsistent as well, reporting that free thyroxine (FT_4) alone,[12] thyroid-stimulating hormone (TSH) alone,[13] both,[8] or neither of them[14] are linked with NAFLD. These discrepancies are mainly due to small sample sizes and cross-sectional design of previous studies.

The only prospective study to date focused exclusively on the risk of NAFLD in subclinical hypothyroidism.[15] As a consequence, the risk of NAFLD has not been explored prospectively in the remaining categories of thyroid function, other than subclinical hypothyroidism. A recent review has also highlighted the need for prospective research on the association between normal thyroid function and NAFLD risk.[16] Moreover, it remains unclear whether and to what extent thyroid function affects fibrosis risk in NAFLD patients. Therefore, we prospectively investigated the association between variations in thyroid function and NAFLD spectrum, in a large population-based cohort study.

METHODS

Study population

The Rotterdam Study (RS) is a large, prospective, population-based cohort study, conducted among middle-aged and elderly inhabitants of the Ommoord district in Rotterdam, the Netherlands. The complete rationale and study design have been described in detail previously.[17] In brief, all residents of Ommoord aged 55 years or older were invited to participate. Firstly, 7983 participants were enrolled between 1990 and 1993 (RS I). 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 and over was added (RS III), and thereafter the study population comprised a total of 14926 subjects.

Participants from study cohorts RS I visit 3 (RS I.3), RS II visit 1 (RS II.1) and RS III visit 1 (RS III.1) were eligible for the study if they had thyroid function measurements and data available on ultrasound-diagnosed NAFLD at follow-up or fatty liver index (FLI) at baseline. We considered the date of baseline laboratory testing, which comprised the assessment of thyroid function and FLI components, the start date of follow-up. The end date of follow-up was considered the date of the ultrasound measurement (Supplemental Figure 1).

The Medical Ethics Committee of the Erasmus University and the Ministry of Health, Welfare and Sport of the Netherlands approved the study protocols, implementing the "Wet Bevolkingsonderzoek: ERGO (Population Studies Act: Rotterdam Study)". All included participants provided a written informed consent in accordance with the Declaration of Helsinki to participate in the study and to obtain information from their family physicians.

Assessment of thyroid function

We performed thyroid function tests in the 3 independent Rotterdam Study cohorts using the same method and assay. Thyroid function assessment was performed for TSH, FT_4, and thyroid peroxidase antibodies (TPOAbs) in baseline serum samples stored at -80°C (The electrochemiluminescence immunoassay ECLIA, Roche). We determined the reference range of TSH (0.4 to 4.0 mIU/L) and FT_4 (0.85 to 1.95 ng/dL [to convert to picomoles per liter, multiply by 12.871]), according to national guidelines and previous reports from the Rotterdam Study.[18] Thyroid function was defined as euthyroid if serum TSH was within the reference range. Subclinical hypothyroidism was defined as serum TSH >4.0 mIU/L and FT_4 levels within the reference range.

Overt hypothyroidism was defined as serum TSH >4.0 mIU/L and FT_4 levels <0.85 ng/dL. Subclinical hyperthyroidism was defined as serum TSH <0.4 mIU/L and FT_4 levels within the reference range. Overt hyperthyroidism was defined as serum TSH <0.4 mIU/L and FT_4 levels >1.95 ng/dL. Levels of TPOAb >35 kU/ml were regarded as positive, as recommended by the assay manufacturer.

Assessment of NAFLD

Assessment of NAFLD comprised abdominal ultrasonographies at follow-up and FLI measurements at baseline. To assess incident NAFLD during follow-up, abdominal ultrasonography was performed by a single trained technician and subsequently images were reevaluated by an experienced hepatologist.[17] NAFLD was defined by the presence of liver steatosis on abdominal ultrasound, in the absence of secondary causes as excessive alcohol consumption (>14 alcoholic beverages weekly), hepatitis B surface antigen, and/or hepatitis C virus positivity, and use of fatty liver inducing pharmacological agents (ie, amiodarone, tamoxifen, corticosteroids, and methotrexate).

At baseline, ultrasound measurements were not available and instead, we utilized FLI measurements. FLI, an algorithm based on levels of triglycerides, gamma-glutamyl transferase, body mass index (BMI) and waist circumference, was calculated by the formula previously described by Bedogni et al.[19] The accuracy of FLI in the detection of NAFLD has been demonstrated in various studies, including the Rotterdam Study.[20-22] FLI ≥60 has a probability of 82.3% to identify the presence of NAFLD.[22] Therefore, we used a cutoff of 60 to classify participants into low and high probability of NAFLD, after primarily excluding subjects with a secondary cause of hepatic steatosis.

Liver stiffness (LS) was examined using transient elastography (Fibroscan; Echosens). LS measurements were performed by a single operator, on the right lobe of the liver, through the intercostal spaces, with the participant lying flat on his back with the right arm laying in maximal abduction. Either M- or XL-probe was applied, based on the manufacturer's instructions. Reliability of LS measurements was defined according to the criteria by Boursier et al.[23] LS measurements were considered poorly reliable if interquartile range /median LS >0.30 with median LS ≥7.1 kilopascals (kPa). A total of 48 participants with NAFLD diagnosis had unreliable LS measurements and were therefore excluded from the analyses involving LS. LS

≥8.0 kPa was used as a cutoff suggesting clinically relevant fibrosis. A high positive predictive value of this cutoff has been previously reported.[24,25]

Additional measurements

Information was obtained from each participant through a home questionnaire concerning demographics, medical history, alcohol intake, tobacco smoking, and medication use. Blood lipids, glucose, gamma-glutamyl transferase, were measured using automatic enzymatic procedures (Roche Diagnostics GmbH). BMI was calculated as weight in kilograms divided by height in meters squared. Waist circumference was measured in centimeters, at the level midway between the lower rib margin and the iliac crest with participants in standing position without heavy outer garments and with emptied pockets, breathing out gently. Blood pressure was calculated as the average of two consecutive measurements, realized in the sitting position at the right upper arm with a random-zero-sphygmomanometer. Hypertension was defined as a systolic blood pressure ≥140 mmHg or a diastolic blood pressure ≥90 mmHg or the use of blood pressure-lowering drugs prescribed for hypertension. Diabetes mellitus was defined as fasting plasma 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.

Statistical analysis

We prospectively assessed the association between thyroid parameters (TSH, FT_4, and TPOAb) and incident NAFLD, by using logistic regression models. Subsequently, we restricted the analyses to those with baseline FLI values <60, to minimize the possibility of misclassification of cases with incident NAFLD.

We explored differences in the risk of NAFLD throughout tertiles of FT_4, taking the highest tertile as reference. After our primary analyses, we performed sensitivity analyses, restricting to subjects with TSH and FT_4 within the reference ranges, excluding thyroid medication users and participants with previous thyroid surgery.

Next, we evaluated the risk of NAFLD throughout thyroid status categories of participants, taking euthyroid subjects as reference group. After excluding thyroid medication users and participants with previous thyroid surgery, we investigated the association between thyroid function/status and the risk of having a combination of NAFLD and LS ≥8.0 kPa.

After excluding thyroid medication users and participants with previous thyroid surgery, we cross-sectionally assessed the association between thyroid function and NAFLD, performing logistic regression analysis. Here, NAFLD was defined on basis of categorized FLI, in the absence of secondary causes of hepatic steatosis.

In longitudinal analyses, we first adjusted for age, sex, cohort, alcohol intake, smoking, and follow-up time (Model 1). Further adjustments were made for the use of lipid-lowering medications, total cholesterol, triglycerides, BMI, hypertension, and diabetes mellitus (Model 2). Lipids, BMI, hypertension, and diabetes mellitus could act as confounders as well as possible mediators depending on the presumed pathway through which thyroid function is related to NAFLD and therefore included in the multivariable model (Model 2). In mediation analyses, we calculated the percentage of excess risk mediated $((\text{odds ratio } [OR]_{\text{con adj}} - OR_{\text{con+med adj}})/(OR_{\text{con adj}} - 1)) \times 100\%$, where $OR_{\text{con adj}}$ is the confounder-adjusted OR and $OR_{\text{con+med adj}}$ is the confounder and mediator–adjusted OR.

In cross-sectional analyses, we adjusted for the aforementioned covariates, excluding lipids and BMI, as these variables are used to calculate FLI. High-density lipoprotein cholesterol and waist circumference were not included as covariates in the multivariable model to avoid multicollinearity. TSH was naturally log transformed in the continuous analyses in order to approximate a normal distribution. We checked for risk modification by adding an interaction term of the exposure (TSH or FT_4) with covariates of the multivariable model, but none of the interaction terms were significant. There was no departure from linearity for the TSH and FT_4 analyses, assessed by adding quadratic terms of covariates in the multivariable model. Multiple imputations were performed in case of missing covariates (<2% for all covariates). Statistical analyses were conducted using IBM SPSS version 21 (IBM Corp) and R statistical software (R-project, Institute for Statistics and Mathematics, R Core Team [2013], version 3.0.2). Reporting is done according to the Strengthening of the Reporting of Observational Studies in Epidemiology Statement.

RESULTS

We included a total of 9419 eligible participants with thyroid function measurements at baseline and data available on ultrasound-diagnosed NAFLD at follow-up or FLI at baseline.

Table 1 and Supplemental Table 1 summarize the baseline characteristics of included participants. The mean age was 64.7 years and 56.5% were females. Amongst 5324 participants in whom follow-up data were available, we documented 1763 cases of incident hepatic steatosis, of which 1217 cases of incident NAFLD (median follow-up time, 10.0 years; interquartile range, 5.7 to 10.9 years). A total of 546 subjects with hepatic steatosis had secondary causes, comprising 460 subjects with excessive alcohol consumption, 54 subjects with known steatosis-inducing drugs, 15 subjects with viral hepatitis, and 17 with combinations of the above. After excluding thyroid medication users and participants with previous thyroid surgery, reliable LS measurements were available in 805 participants with ultrasound-diagnosed NAFLD, of which 69 (8.6%) had LS ≥8.0 kPa.

Table 1. Baseline characteristics of 9419 participants*

Age, years	64.7 (9.7)
Women, n (%)	5321 (56.5)
Smoking, n (%)	
Current	1989 (21.1)
Past	4490 (47.7)
Never	2940 (31.2)
Use of lipid-lowering medications, n (%)	1508 (16.0)
Use of thyroid medication, n (%)	296 (3.1)
Total cholesterol, mmol/l	5.7 (1.0)
High-density lipoprotein cholesterol, mmol/l	1.4 (0.4)
Triglycerides, mmol/l	1.5 (0.8)
Body mass index, kg/m^2	27.2 (4.2)
Waist circumference, cm	93.7 (12.1)
Hypertension, n (%)	5881 (62.4)
Diabetes mellitus, n (%)	1073 (11.4)
TSH, mIU/L, median (IQR)	1.9 (1.3-2.8)
FT_4, ng/dL	1.2 (0.1)
TPOAb positive, n (%)	1240 (13.2)

*Data are mean (standard deviation), unless otherwise specified. Abbreviations: TSH, thyroid-stimulating hormone; IQR, interquartile range; FT_4, free thyroxine; TPOAb, thyroid peroxidase antibodies.

Thyroid function and the risk of NAFLD

The risk of NAFLD decreased gradually with higher FT_4 levels (OR, 0.33; 95% confidence interval [95% CI], 0.22 to 0.48 per 1ng/dL) (Table 2). These results remained similar after further adjustments for cardiovascular risk factors (OR, 0.42; 95% CI, 0.28 to 0.63), and also after restricting the analyses to participants with baseline FLI <60 (OR, 0.42; 95% CI, 0.24 to 0.74). In the multivariable-adjusted model, participants in the lowest FT_4 tertile had a 1.31 times higher risk of NAFLD, compared with those in the highest tertile (95% CI, 1.11 to 1.56; Supplemental Table 2). There was a positive linear association between TSH levels and NAFLD risk (OR, 1.09; 95% CI, 1.01 to 1.19 per 1 logTSH), which was attenuated after additional adjustment for cardiovascular risk factors (OR, 1.07; 95% CI, 0.98 to 1.17; Table 2). After separate and simultaneous additions of cardiovascular risk factors to Model 1, BMI and triglycerides were held accountable for the attenuation (Supplemental Table 3). The percentage of excess risk mediated by BMI and triglycerides was 22.2% in the association of TSH with

Table 2. Longitudinal association between thyroid function and NAFLD risk

	Events/TN	OR (95% CI) Model 1	OR (95% CI) Model 2
All participants			
TSH	1216/5321	**1.09 (1.01; 1.19)**	1.07 (0.98; 1.17)
FT_4	1217/5320	**0.33 (0.22; 0.48)**	**0.42 (0.28; 0.63)**
Baseline FLI < 60			
TSH	553/3379	**1.13 (1.00; 1.27)**	1.08 (0.95; 1.23)
FT_4	553/3376	**0.42 (0.24; 0.74)**	**0.52 (0.29; 0.92)**

Model 1: age, sex, cohort, alcohol intake, smoking, and follow-up time. Model 2: Model 1, use of lipid-lowering medications, total cholesterol, triglycerides, body mass index, hypertension, and diabetes mellitus. 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: NAFLD, nonalcoholic fatty liver disease; TN, total number; OR, odds ratio; CI, confidence interval; TSH, thyroid-stimulating hormone; FT_4, free thyroxine; FLI, fatty liver index.

Table 3. Longitudinal association of thyroid status with NAFLD risk

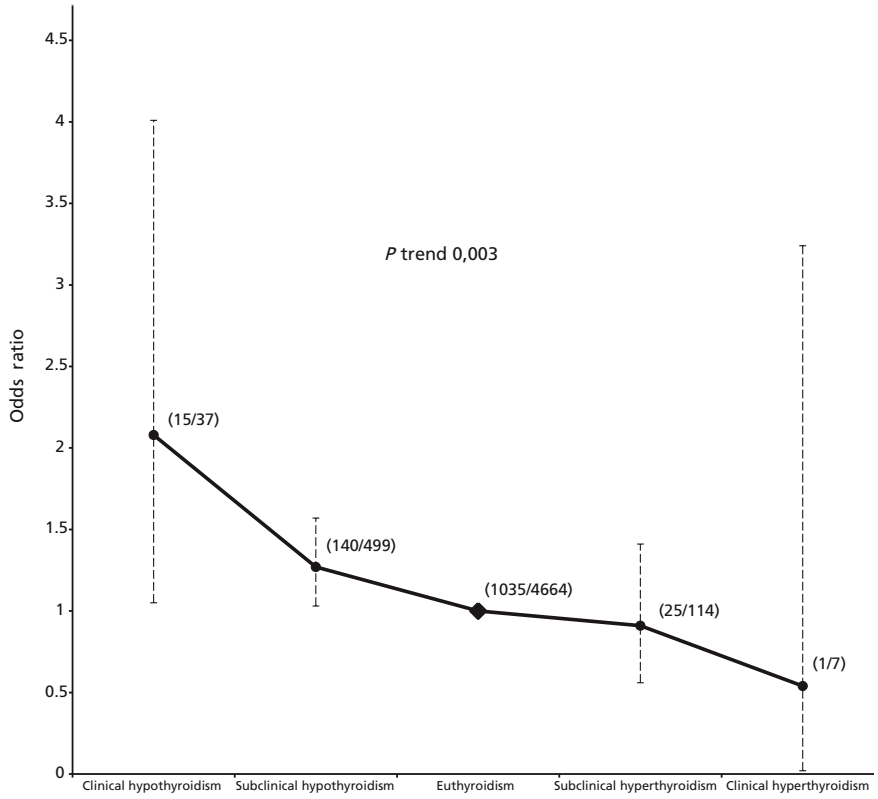
	Events/TN	OR (95% CI) Model 1	OR (95% CI) Model 2
Hypothyroidism*	155/536	**1.32 (1.08; 1.62)**	**1.24 (1.01; 1.53)**
Euthyroidism	1035/4664	1 (Reference)	1 (Reference)
Hyperthyroidism*	26/121	0.88 (0.56; 1.36)	0.88 (0.54; 1.37)

Model 1: age, sex, cohort, alcohol intake, smoking, and follow-up time. Model 2: Model 1, use of lipid-lowering medications, total cholesterol, triglycerides, body mass index, hypertension, and diabetes mellitus. * includes subclinical and clinical range. Abbreviations: NAFLD, nonalcoholic fatty liver disease; TN, total number; OR, odds ratio; CI, confidence interval.

NAFLD and 13.4% in the association of FT_4 with NAFLD; that is 22.2% and 13.4% of the respective associated effect size of TSH and FT_4 on NAFLD is explained by BMI and triglycerides. No significant association was observed for TPOAb and NAFLD risk (OR, 1.09; 95% CI, 0.89 to 1.32; Supplemental Table 2).

There was a significant trend (*P* for trend, 0.003) in the decrease of NAFLD risk, across categories of thyroid function from clinical hypothyroidism to clinical hyperthyroidism (OR from 2.08 to 0.54; Figure 1, Supplemental Table 4). Compared to euthyroidism, hypothyroidism was associated with a 1.24 times higher risk of NAFLD (95% CI, 1.01 to 1.53; Table 3).

Figure 1. Longitudinal association between thyroid status and NAFLD.



Point estimates for NAFLD (nonalcoholic fatty liver disease) were plotted against thyroid status of participants, taking euthyroid subjects as reference, after adjusting for age, sex, cohort, alcohol intake, smoking, follow-up time. Euthyroidism was defined as TSH (thyroid-stimulating hormone) within reference range (0.4 to 4.0 mIU/L); overt hypothyroidism as TSH >4.0 mU/L and FT_4 (free thyroxine) <0.85 ng/dL; subclinical hypothyroidism as TSH >4.0 mU/L and FT_4 0.85 to 1.95 ng/dL; overt hyperthyroidism as TSH <0.4 mU/L and FT_4 >1.95 ng/dL; subclinical hyperthyroidism as TSH <0.4 mU/L and FT_4 0.85 to 1.95 ng/dL. Dashed lines represent confidence intervals. Within brackets: NAFLD events/Total number.

Cross-sectional analyses, based on categorized FLI, demonstrated a significant association of TSH (OR, 1.11; 95% CI, 1.04 to 1.18) and FT_4 (OR, 0.45; 95% CI, 0.34 to 0.60) with NAFLD (Supplemental Table 5). We found similar results in sensitivity analyses conducted only among euthyroid subjects, after excluding thyroid medication users and participants with previous thyroid surgery (Supplemental Table 2 and Supplemental Table 5).

Thyroid function and the risk of having a combination of NAFLD and LS ≥8 kPa

There was a positive association between TSH levels and the risk of having a combination of NAFLD and LS ≥8.0 kPa (OR, 1.55; 95% CI, 1.09 to 2.20). In line, higher FT_4 levels were associated with a lower risk of having a combination of NAFLD and LS ≥8.0 kPa, but not significantly (OR, 0.41; 95% CI, 0.09 to 1.73) (Table 4). The risk of having a combination of NAFLD and LS ≥8.0 kPa decreased gradually from hypothyroidism to hyperthyroidism (*P* for trend, 0.002) (Table 4).

Compared with euthyroidism, subclinical hypothyroidism was associated with a 2.30 times higher risk of having a combination of NAFLD and LS ≥8.0 kPa (95% CI, 1.12 to 4.31; Table 4). Results remained similar after further adjustments for

Table 4. Longitudinal association of thyroid function and status with the risk of having a combination of NAFLD and LS ≥8 kPa*†

	NAFLD with LS ≥8.0 kPa/ TN	OR (95% CI) Model 1	OR (95% CI) Model 2
Thyroid function and the risk of having combined NAFLD & LS ≥8.0 kPa			
TSH	69/4762	**1.55 (1.09; 2.20)**	**1.49 (1.04; 2.15)**
FT_4	69/4762	0.41 (0.09; 1.73)	0.59 (0.13; 2.59)
Thyroid status and the risk of having combined NAFLD & LS ≥8.0 kPa			
Clinical hypothyroidism	2/31	5.93 (0.93; 20.85)	**6.64 (1.04; 23.98)**
Subclinical hypothyroidism	11/408	**2.30 (1.12; 4.31)**	**2.14 (1.04; 4.07)**
Euthyroidism	55/4240	1 [Reference]	1 [Reference]
Subclinical hyperthyroidism	1/81	0.87 (0.04; 4.11)	0.80 (0.04; 3.91)
Clinical hyperthyroidism	NA	NA	NA
P value for trend		0.002	0.004

Model 1: age, sex, cohort, alcohol intake, smoking, and follow-up time. Model 2: Model 1, use of lipid-lowering medications, total cholesterol, triglycerides, body mass index, hypertension, and diabetes mellitus. *LS ≥8.0 kilopascals suggests clinically relevant fibrosis. †For this analysis, we excluded thyroid medication users and participants with previous thyroid surgery. 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: NAFLD, nonalcoholic fatty liver disease; LS, liver stiffness; kPa, kilopascals, TN, total number; OR, odds ratio; CI, confidence interval; TSH, thyroid-stimulating hormone; FT_4, free thyroxine; NA, not applicable.

cardiovascular risk factors (Table 4). In euthyroid subjects, higher TSH and lower FT_4 concentrations were associated with an increased risk of having a combination of NAFLD and LS ≥8.0 kPa, but not significantly (OR, 1.13; 95% CI, 0.63 to 2.03 for TSH; OR, 0.81; 95% CI, 0.11 to 5.75 for FT_4).

DISCUSSION

The current study is the first prospective population-based study to evaluate the relation between the whole spectrum of thyroid function and subsequent risk of NAFLD. We demonstrated a negative linear association between FT_4 levels and incident NAFLD, even among euthyroid subjects, as well as a positive linear association for TSH levels. Moreover, the risk of NAFLD progressively decreased from a hypothyroid to a hyperthyroid state. Hypothyroidism was associated with a higher NAFLD risk compared to euthyroidism. Lower thyroid function was also associated with an increased risk of having NAFLD with fibrosis. We demonstrate for the first time that subclinical hypothyroidism is associated with an increased risk of having NAFLD with fibrosis in the general population.

There are various pathways via which the beneficial effects of thyroid hormone on NAFLD risk can be mediated. Thyroid dysfunction is related to several cardiovascular risk factors that are in turn associated with an increased NAFLD risk (eg, higher BMI and dyslipidemia). When we add BMI and triglycerides into the model, the risk estimates of the association between thyroid function and NAFLD attenuate, indeed suggesting a mediating role of these factors.

Studies in rodents have demonstrated a regression of hepatic steatosis after treatment with liver-targeted thyroid hormone receptor agonists.[26-28] Thyroid hormone induces intrahepatic lipolysis through lipophagy, that involves the sequestration and degradation of lipid droplets within hepatic lysosomes.[29] Moreover, thyroid hormone receptor-mediated lipophagy enhances fatty acid oxidation, which may accelerate the clearance of liver lipids and reduce hepatosteatosis.[29]

Conversely, the decreased activity of hepatic lipases that occurs under hypothyroid conditions can promote NAFLD via decreased triglyceride clearance and hepatic triglyceride accumulation.[30] In addition, the insulin resistance state associated with hypothyroidism [31] can contribute to NAFLD by concomitantly inducing "de novo" lipogenesis and generating a flux of free fatty acids from adipose tissue to the liver.[32] Furthermore, decreased thyroid hormones might affect circulating levels of

adipocytokines, such as tumor necrosis factor-α, leptin and adiponectin.[32,33] Altered adipocytokines may then contribute to hepatic inflammation and fibrosis, by exerting direct hepatotoxic effects or promoting oxygen radicals.[34]

A putative role of thyroid autoimmunity has also been suggested in NAFLD pathogenesis, because various autoantibodies such as antinuclear antibodies and antismooth muscle antibodies, have been reported in patients with NAFLD.[35] However, our findings do not support this hypothesis, as there was no association between TPOAb and NAFLD.

Our findings consistently demonstrate that low thyroid function is associated with an increased risk of developing NAFLD, as well as higher risk of having NAFLD with fibrosis. Therefore, it can be hypothesized that a hypothyroid state might accelerate the progression of liver steatosis to fibrosis. Alternatively, low thyroid function might contribute on the development of liver fibrosis, independently of steatosis. Additional prospective research is needed to address these underlying mechanisms and possible mediating role of cardiovascular risk factors.

The results of the present study confirm a negative linear association between FT_4 levels and the risk of NAFLD. Based on the negative feedback regulation of hypothalamus-pituitary-thyroid axis, we would expect an analogous opposite association for TSH. Although there was a positive linear relationship between TSH levels and NAFLD risk, it attenuated among euthyroid subjects and after adjustment for cardiovascular risk factors. Several comparable studies exploring the association between thyroid function and different clinical end points have shown that FT_4, rather than TSH, is significantly related to the outcome risk,[18,36,37] particularly within the euthyroid range.[18,36] This may be ascribed to the distinct central and peripheral effects of thyroid hormone, as pituitary gland and liver differ in thyroid hormone transporters, receptors and deiodinases.[38] Also, genetic determinants and ageing can modify the TSH-FT_4 set point of the feedback mechanism, accounting for the weaker TSH-FT_4 association predominantly among euthyroid subjects.[39,40]

Our study has several important strengths. To our knowledge, it represents the first population-based prospective study to assess the effect of the whole spectrum of thyroid function on NAFLD and presence of clinically relevant fibrosis. The large sample size allowed us to conduct multiple sensitivity analyses. Other strengths include the extensive data on potential confounding factors and the laboratory assessment of thyroid parameters. In addition, we minimized the possibility of misclassification of cases with incident NAFLD, by excluding individuals with baseline

FLI ≥60 (thus highest probability of already having NAFLD), which however did not affect our results.

One limitation of our study is that we could not restrict the analysis to participants with baseline FLI values <30, due to a large sample size reduction (over 70% of the total population and over 80% of the NAFLD cases). Moreover, the diagnosis of NAFLD was based on ultrasonographic examination, whereas liver biopsy is considered the gold standard for the detection of mild steatosis or liver fibrosis. However, liver biopsies are not conducted routinely in NAFLD diagnosis and are considered unethical in population-based studies, because of invasiveness and potential complications. Also, abdominal ultrasonography has a sensitivity of 80-90% for detecting liver steatosis compared with histology, and its accuracy for diagnosing steatosis meets other imaging modalities.[41] In addition, transient elastography is considered reproducible and effective in liver fibrosis assessment.[24,25] Thyroid parameters were tested only at baseline and we lacked information regarding their variations over time. However, this would generate an underestimation of the association strength, rather than a spurious finding. Serum triiodothyronine measurements were not available in our study. Nevertheless, thyroid function is clinically defined by the combined TSH and FT_4 measurement. Furthermore, the generalizability of our findings to non-Caucasian populations remains uncertain. Finally, we cannot dismiss the possibility of residual confounding in an observational study design, even though we accounted for a large number of covariates.

Conclusions

Individuals with hypothyroidism are at increased risk of NAFLD compared with euthyroid subjects. The current study also reveals a negative linear association between FT_4 levels and the subsequent risk of NAFLD, even within the euthyroid reference range. Lower thyroid function is associated with an increased risk of fibrosis in NAFLD patients. Our findings highlight the need for future investigations on preventive measures (eg, screening of thyroid function in NAFLD patients) and possible therapeutic interventions (eg, decision of treatment in subclinical thyroid dysfunction).

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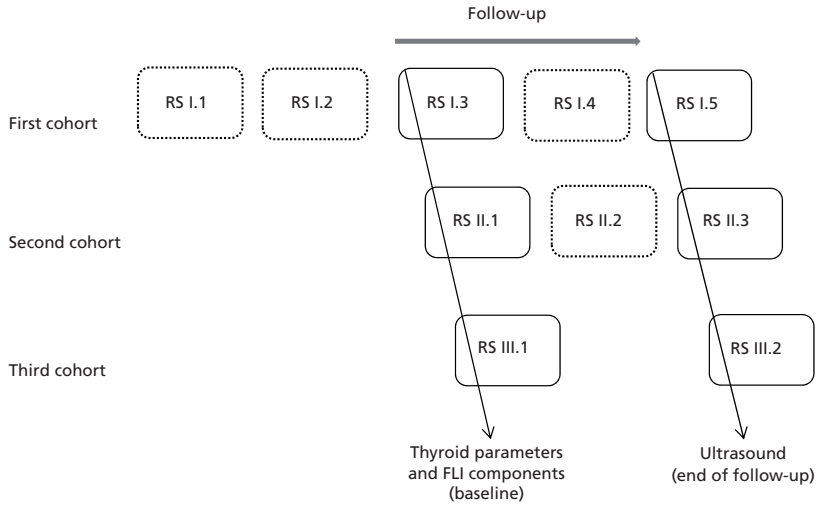
SUPPLEMENTAL MATERIAL

Supplemental Figure 1. Assessment of thyroid function and NAFLD in the Rotterdam Study.



Supplemental Table 1. Baseline characteristics of 9419 participants in three RS cohorts

	RS I	RS II	RS III
Total number	3694	2355	3370
Age, years	72.2 (6.8)	64.3 (7.7)	56.8 (6.7)
Women, n (%)	2139 (57.9)	1281 (54.4)	1901 (56.4)
Smoking, n (%)			
Current	628 (17.0)	468 (19.9)	893 (26.5)
Past	1849 (50.0)	1173 (49.8)	1468 (43.6)
Never	1217 (33.0)	714 (30.3)	1009 (29.9)
Use of lipid-lowering medications, n (%)	459 (12.4)	308 (13.1)	741 (22.0)
Use of thyroid medications, n (%)	104 (2.8)	70 (3.0)	122 (3.6)
Total cholesterol, mmol/l	5.8 (0.9)	5.7 (0.9)	5.5 (1.6)
HDL-C, mmol/l	1.4 (0.4)	1.4 (0.4)	1.4 (0.4)
Triglycerides, mmol/l	1.5 (0.7)	1.5 (0.8)	1.5 (0.9)
Body mass index, kg/m^2	26.8 (3.9)	27.1 (3.9)	27.7 (4.5)
Waist circumference, cm	93.4 (11.4)	93.7 (11.8)	93.8 (12.9)
Hypertension, n (%)	2816 (76.2)	1423 (60.4)	1642 (48.7)
Diabetes mellitus, n (%)	532 (14.4)	265 (11.3)	276 (8.2)
TSH, mIU/L, median (IQR)	1.8 (1.2-2.7)	1.8 (1.2-2.7)	2.02 (1.4-2.8)
FT_4, ng/dL	1.2 (0.1)	1.2 (0.1)	1.2 (0.1)
TPOAb positive, n (%)	473 (12.8)	326 (13.8)	441 (13.1)

*Data are mean (standard deviation), unless otherwise specified. Abbreviations: RS, Rotterdam Study; HDL-C, high density lipoprotein cholesterol; TSH, thyroid-stimulating hormone; IQR, interquartile range; FT_4, free thyroxine; TPOAb, thyroid peroxidase antibodies.

Supplemental Table 2. Longitudinal association of thyroid parameters with NAFLD risk

	Events/TN	OR (95% CI) Model 1	OR (95% CI) Model 2
FT_4 in tertiles and NAFLD risk			
All participants			
FT_4 (ng/dL) tertiles			
0.10-1.13	466/1774	**1.47 (1.25; 1.73)**	**1.31 (1.11; 1.56)**
1.14-1.27	420/1776	**1.31 (1.12; 1.55)**	**1.27 (1.07; 1.51)**
1.27-2.37	331/1770	1 [Reference]	1 [Reference]
P value for trend		<0.001	0.003
*Thyroid function within the reference ranges**			
FT_4 (ng/dL) tertiles			
0.85-1.14	385/1503	**1.54 (1.29; 1.84)**	**1.36 (1.12; 1.64)**
1.14-1.27	339/1500	**1.33 (1.11; 1.60)**	**1.25 (1.05; 1.53)**
1.28-1.89	264/1501	1 [Reference]	1 [Reference]
P value for trend		<0.001	0.003
Thyroid function within the reference ranges* and NAFLD risk			
TSH	988/4504	0.97 (0.83; 1.13)	0.92 (0.78; 1.08)
FT_4	988/4504	**0.25 (0.15; 0.41)**	**0.37 (0.22; 0.63)**
TPOAb and NAFLD risk			
TPOAb	1217/5316	1.10 (0.91; 1.31)	1.09 (0.89; 1.32)†

Model 1: age, sex, cohort, alcohol intake, smoking, and follow-up time. Model 2: Model 1, use of lipid-lowering medications, total cholesterol, triglycerides, body mass index, hypertension, and diabetes mellitus. *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. For this analysis, we excluded thyroid medication users and participants with previous thyroid surgery. †Additionally adjusted for lnTSH. 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: NAFLD, nonalcoholic fatty liver disease; TN, total number; OR, odds ratio; CI, confidence interval; TSH, thyroid-stimulating hormone; FT_4, free thyroxine; TPOAb, thyroid peroxidase antibodies.

Supplemental Table 3. Longitudinal association of thyroid function with NAFLD risk

	OR (95% CI) Model 1	OR (95% CI) Model 2[a]	OR (95% CI) Model 2[b]
TSH	**1.09 (1.01; 1.19)**	**1.09 (1.01; 1.19)**	1.07 (0.98; 1.17)
FT_4	**0.33 (0.22; 0.48)**	**0.32 (0.22; 0.48)**	**0.42 (0.28; 0.63)**

Model 1: age, sex, cohort, alcohol intake, smoking, and follow-up time. Model 2[a]: Model 1, use of lipid-lowering medications, total cholesterol, hypertension, and diabetes mellitus; Model 2[b]: Model 2[a], triglycerides, and body mass index. 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: NAFLD, nonalcoholic fatty liver disease; OR, odds ratio; CI, confidence interval; TSH, thyroid-stimulating hormone; FT_4, free thyroxine.

Supplemental Table 4. Longitudinal association of thyroid status with NAFLD risk

	NAFLD/TN	Median TSH (IQR)	Mean FT_4 (Extreme values)	OR (95% CI)
Clinical hypothyroidism	15/37	17.19 (8.54-40.75)	0.64 (0.10-0.84)	**2.08 (1.05; 4.01)**
Subclinical hypothyroidism	140/499	5.15 (4.44-6.43)	1.13 (0.86-1.67)	**1.27 (1.03; 1.57)**
Euthyroidism	1035/4664	1.86 (1.34-2.54)	1.21 (0.74-2.07)	1 [Reference]
Subclinical hyperthyroidism	25/114	0.17 (0.06-0.31)	1.36 (0.90-1.91)	0.91 (0.56; 1.41)
Clinical hyperthyroidism	1/7	0.10 (0.01-0.16)	2.09 (1.97-2.37)	0.54 (0.02; 3.24)
				P for trend 0.003

Adjusted for age, sex, cohort, alcohol intake, smoking, and follow-up time. Abbreviations: NAFLD, nonalcoholic fatty liver disease; TN, total number; TSH, thyroid-stimulating hormone; IQR, interquartile range; FT_4, free thyroxine; OR, odds ratio; CI, confidence interval.

Supplemental Table 5. Cross-sectional association between thyroid function and NAFLD defined by FLI*

	Total number	OR (95% CI) Model 1	OR (95% CI) Model 2
All participants			
TSH	8777	**1.11 (1.05; 1.19)**	**1.11 (1.04; 1.18)**
FT_4	8779	**0.45 (0.34; 0.59)**	**0.45 (0.34; 0.60)**
Thyroid function within the reference ranges†			
TSH	7668	**1.12 (1.01; 1.24)**	1.11 (0.99; 1.24)
FT_4	7668	**0.41 (0.29; 0.57)**	**0.41 (0.29; 0.58)**

Model 1: age, sex, cohort, alcohol intake, and smoking. Model 2: Model 1, hypertension, and diabetes mellitus. *For this analysis, we excluded thyroid medication users and participants with previous thyroid surgery. NAFLD was based on categorized baseline FLI, with 60 as a cutoff, in the absence of secondary causes of hepatic steatosis (>14 alcoholic beverages weekly, viral hepatitis, use of fatty liver inducing pharmacological agents). †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. 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: NAFLD, nonalcoholic fatty liver disease; FLI, fatty liver index; OR, odds ratio; CI, confidence interval; TSH, thyroid-stimulating hormone; FT_4, free thyroxine.

CHAPTER 2.2

THYROID FUNCTION AND THE RISK OF FIBROSIS OF THE LIVER, LUNG AND HEART
A SYSTEMATIC REVIEW OF HUMAN STUDIES

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Manuscript in preparation

ABSTRACT

Background Variations in thyroid function may affect the occurrence and progression of liver, pulmonary and myocardial fibrosis. However, evidence is fragmented and inconclusive.

Objective We aimed to systematically appraise the evidence regarding the role of thyroid function on fibrosis of the liver, lung, and heart.

Data Sources Pubmed Publisher, Web-of-Science, Embase and Medline Ovid were searched for studies published from inception to 12.07.2018.

Study Selection Two independent reviewers evaluated and selected observational studies that investigated the association of thyroid function with fibrosis of the liver, lung or heart, in humans.

Data Extraction and Synthesis Data were extracted independently by two reviewers, with disagreement resolved by consensus. PRISMA guidelines were followed. Study quality and risk of bias were evaluated based on the Newcastle-Ottawa Quality Assessment Scale.

Main Outcomes and Measures Fibrotic diseases of the liver, lung and heart, evaluated via noninvasive or invasive measures.

Results After screening 1764 titles and abstracts, we identified 10 studies meeting the inclusion criteria. Of the included studies, 6 studies reported on liver fibrosis, 2 on pulmonary fibrosis, and 2 on myocardial fibrosis. The population sample size ranged from 53 to 4761 subjects. The median mean age was 54 years (range, 36-69), and the median percentage of women was 48 (range, 17-100). The general quality of the data was moderate. Overall, low thyroid function was associated with an increased risk of fibrosis of the liver, lung, and heart. Compared with euthyroidism, hypothyroidism was associated with a higher risk of liver fibrosis (n=2 studies), pulmonary fibrosis (n=2 studies), and myocardial fibrosis (n=1 study). The results were not combined in a quantitative meta-analysis, due to the heterogeneity in the population characteristics, differences in methodology, or differences in fibrotic outcomes of the included studies.

Conclusions and Relevance This systematic review suggests that low thyroid function is associated with an increased risk of chronic fibrotic diseases of the liver, lung, and heart. The evidence, however, is mainly based on cross-sectional data. Therefore, future prospective studies are needed to investigate the long-term effects of thyroid hormones on the occurrence and progression of fibrosis.

INTRODUCTION

Various chronic diseases, including liver cirrhosis, idiopathic pulmonary fibrosis and hypertrophic cardiomyopathy, are characterized by fibrosis.[1] The development of fibrosis is attributable to the accumulation of extracellular matrix proteins such as collagen and fibronectin.[2] Fibrotic elements progressively remodel and destroy the normal tissue architecture, ultimately resulting in organ failure. Hence, nonalcoholic steatohepatitis can progress to decompensated cirrhosis;[3] pulmonary fibrosis contributes to a decline in the lung function;[4] whereas myocardial fibrosis leads to ventricular diastolic dysfunction.[5]

Current research is focused on the identification of novel determinants of fibrosis, which could be further translated into the development of effective antifibrotic drugs.[6-8] Among other factors, hypothyroidism has been implicated in the aetiology of fibrosis. Pronounced hypothyroidism is typically characterized by an increased production of mucopolysaccharides, resulting in interstitial fibrosis and extracellular water retention, also known as myxedema. Yet, the exact role of thyroid hormones on the development of fibrosis remains controversial. Many animal studies have reported profibrotic effects of hypothyroidism.[9-15] Additionally, beneficial effects of thyroid hormone supplementation have been reported on the disease course of the liver,[16-18] lung,[19] or heart[20-23] fibrosis. In contrast, other animal studies have observed an attenuation of fibrosis in experimental hypothyroidism,[24,25] and have shown profibrotic effects of thyroid hormone administration.[26-30] Similar to these animal studies, the results of epidemiological studies are also inconsistent. Some studies report an association of thyroid function with certain fibrotic processes,[31,32] whereas others report no association.[33]

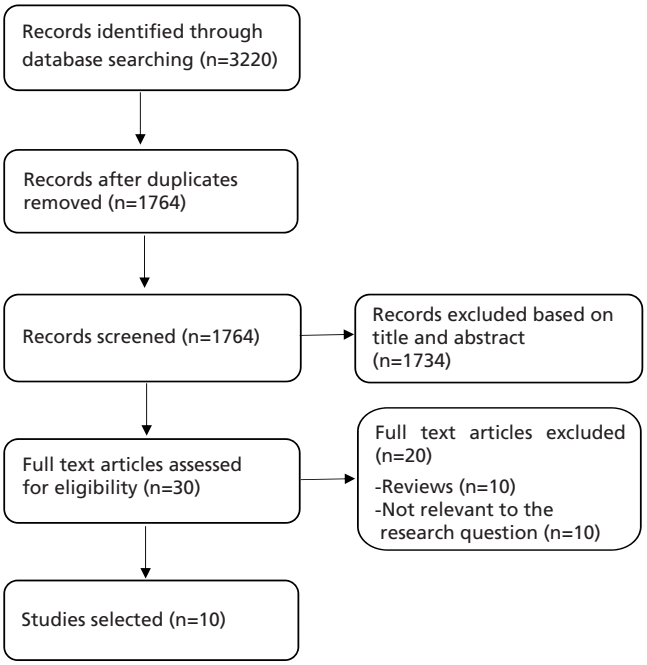
To date, there is a critical lack of literature synthesis concerning the impact of thyroid function on the occurrence and progression of fibrosis. In this context, the present review aims to summarize the current observational evidence regarding the association of thyroid function with fibrosis of the liver, lung and heart in humans.

METHODS

Data sources and search strategy

This systematic review was conducted in accordance with the PRISMA guidelines for transparent reporting.[34] The checklist is provided in Appendix 1. Four electronic databases, including Pubmed Publisher, Web-of-Science, Embase and Medline Ovid, were searched from inception to 12.07.2018, with the help of librarians. The computer-based searches combined terms related to thyroid function and fibrosis of the liver, heart, or lung. An outline of the step-wise inclusion and exclusion procedure is shown in Figure 1. Details of the search strategy are provided in Appendix 2.

Figure 1. Flowchart for study inclusion, adapted from the PRISMA statement.



Example of electronic search strategy (EMBASE): ((exp thyroid gland/ or exp thyroid disease/ or exp thyroxine/ or exp thyroid function/ or thyroid hormone/ or thyrotropin/ or thyroxine/ or exp thyroid hormone blood level/ or exp thyroid gland examination/) or (thyroid* or hyperthyro* or hypothyro* or free thyroxine or Graves or thyrotropin or thyroxin or deiodinase or hashimoto or triiodothyronine or thyronine* or myxedema or thyrotoxicosis or hyperthyroxinemia).ab,ti.) and ((fibrotic or

fibrosis or nonalcoholic steatohepatitis or fibroblast).ab,ti. or (exp fibrosing alveolitis/ or exp fibrosing interstitial pneumonia/ or exp heart muscle fibrosis/ or exp liver fibrosis/ or exp lung fibrosis/)) and ((heart or cardiac or endocardium or myocardium or pericardium or lung or pulmonary or liver or hepatic or cardiovascular).ab,ti.)

Study selection

The titles and abstracts of the citations were screened for: (i) Observational studies (ii) that investigated the role of thyroid function on fibrosis of the liver, lung, or heart, in humans; (iii) reporting effect estimates with 95% confidence intervals, or mean differences with standard deviations (*P* values), or prevalence differences (*P* values). Case-reports, letters to the editor, proceedings, reviews, systematic reviews, meta-analyses, and animal studies were excluded. There were no restrictions on publication year or language. Two independent reviewers screened the titles and abstracts, and selected the eligible studies. Any disagreement regarding inclusion was resolved through consensus. Full texts and reference lists of the selected articles were hand searched to identify additional studies.

A predesigned data collection form was used to extract relevant information from the selected studies, including article source, sample size, demographics of study participants, methods of assessing thyroid function and fibrosis, study results and conclusions. The results were not combined in a quantitative meta-analysis, due to the heterogeneity in the population characteristics, differences in methodology, or differences in fibrotic outcomes of the included studies. The quality of the included studies was assessed by using the Newcastle–Ottawa Scale (NOS) for nonrandomized studies in meta-analyses (Appendix 3).[35] The quality of cross-sectional studies was assessed by using an adapted NOS scale (Appendix 4). The NOS scale evaluates the study quality based on 3 domains, namely selection of participants, comparability of study groups, and ascertainment of the outcomes of interest. Each study could have a maximum of 9 stars.

RESULTS

Literature search

The results of the search strategy are presented in Figure 1. After excluding duplicates, we identified 1764 relevant citations. The citations were screened based on the abovementioned predefined selection criteria. As a result, 30 potentially

relevant articles were identified. After examining the full text of these articles, 10 eligible studies were selected.[31-33,36-42]

Thyroid function and fibrosis of the liver, lung and heart

Table 1A summarizes the main characteristics of the 10 included studies reporting on the association of thyroid function with fibrosis. The population sample size ranged from 53 to 4762 subjects (Table 1A). The median mean age was 54 years (range, 36-69), and the median percentage of women was 48 (range, 17-100) (Table 1A). Of the 10 included studies, 5 studies were performed in the United States,[33,36,37,39,40] 3 in Asia,[32,41,42] and 2 in Europe (Table 1A).[31,38] All the studies recruited patients from hospital units, except for one study which was performed in a population-based cohort (Table 1A).[31] Though 2 studies reported unadjusted estimates,[33,42] most of the included studies controlled for potential confounders (Table 1A).[31,32,36-41]

The included studies reported on blood measurements of thyroid function (TSH, FT_4, FT_3),[31,33,38,41] overt hypothyroidism,[31,36,37,39,40,42] and subclinical hypothyroidism.[31,32] Several definitions of hypothyroidism were used. In 4 studies, the diagnosis of hypothyroidism was based on a self-reported disease history and use of thyroid hormone replacement therapy.[36,37,39,40] Two studies diagnosed overt and subclinical hypothyroidism based on the serum TSH and FT_4 measurements, after excluding the thyroid medication users.[31,32] In another study, overt hypothyroidism was caused by chronic lymphocytic thyroiditis, in the absence of concomitant diseases or medical treatments.[42]

The outcomes were liver fibrosis, fibrotic pulmonary diseases, and myocardial fibrosis. Of the 6 studies assessing liver fibrosis, 5 used liver biopsy,[32,33,36-38] and one study used liver elastography.[31] The fibrotic pulmonary diseases included IPF (idiopathic pulmonary fibrosis) (n=1 study)[40] and chronic hypersensitivity pneumonitis (CHPP) (n=1 study).[39] IPF was diagnosed based on lung biopsy or computed tomography scan,[40] whereas the diagnosis of CHPP was based on the American Thoracic Society criteria.[39] Myocardial fibrosis was assessed by cardiac magnetic resonance imaging, using measurements of myocardial longitudinal relaxation time (T1) mapping (n=1 study),[42] or measurements of late gadolinium enhancement (n=1 study).[41]

Liver fibrosis: We identified 6 studies investigating the association of overt hypothyroidism (n=3 studies),[31,36,37] subclinical hypothyroidism (n=2 studies),[31,32] and thyroid function measurements (TSH, FT_4) (n=3 studies)[31,33,38] with liver fibrosis. Overt hy-

Table 1A. Description of included studies on the association of thyroid function with fibrosis*

First author, year (Reference)	Country	N	Age (mean)	% Women	Study design	Population	Additional	Covariates adjusted for*
Thyroid function and liver fibrosis								
Liangpunsakul, 2003[36]	United States	616	49	59	Case-control	General medicine unit	174 NASH cases; 442 non-NASH controls matched for age, sex, race, body weight	Diabetes, hyperlipidemia, hypertension
Pagadala, 2012[37]	United States	246	54	56	Cross-sectional	NAFLD patients	168 events	Diabetes, dyslipidemia, hypertension†
Carulli, 2013[38]	Italy	69	44.4	24	Cross-sectional	Euthyroid NAFLD patients	44 events	Age, sex, chol, BMI, HOMAIR†
Bano, 2016[31]	Netherlands	4762	65	57	Prospective	General population	69 events	Age, sex, cohort, alcohol, smoking, hypolipidemic drugs, chol, Tg, BMI, hypertension, diabetes
Bril, 2016[33]	United States	NR	57	17	Cross-sectional	Euthyroid diabetic NAFLD patients	-	-
Kim, 2017[32]	South Korea	425	53	48	Cross-sectional	NAFLD patients	180 events	Age, sex, BMI, smoking, diabetes, hypertension, Tg, chol, ratio of visceral and subcutaneous tissue area
Thyroid function and pulmonary fibrosis								
Oldham, 2015[40]	United States	392	69	25.5	Case-control	IPF patients and COPD patients	196 IPF cases, 196 COPD controls matched for age, sex, race	BMI, smoking, diabetes, gastroesophageal reflux, CS use
Adegunsoye, 2017[39]	United States	484	65	58	Case-control	CHPP patients and asthma patients	121 CHPP cases, 363 asthma controls matched for age, sex, race	BMI, smoking, diabetes, CS use
Thyroid function and myocardial fibrosis								
Gao, 2016[42]	China	53	36	100	Cross-sectional	Hypothyroid patients and healthy subjects	-	-
Wang, 2016[41]	China	71	54	33.2	Cross-sectional	Patients with IDCM	-	Age, diabetes, renal dysfunction, hypertension†

*Information is related to the analyses of interest for our particular research question. †Stepwise strategy: Statistically significant predictors were kept in the model. Abbreviations: N, total number; NASH, nonalcoholic steatohepatitis; NAFLD, nonalcoholic fatty liver disease; chol, total cholesterol; Tg, triglycerides; BMI, body mass index; HOMAIR, insulin resistance; NR, not reported; IPF, idiopathic pulmonary fibrosis; COPD, chronic obstructive pulmonary disease; CS, corticosteroid; CHPP, chronic hypersensitivity pneumonitis; IDCM, idiopathic dilated cardiomyopathy.

pothyroidism was associated with a higher risk of liver fibrosis than euthyroidism, with odds ratios (ORs) ranging from 2.3 to 6.64.[31,36,37] Subclinical hypothyroidism was associated with a higher risk of liver fibrosis than euthyroidism, with ORs ranging from 2.17 to 2.3.[31,32] Increasing TSH levels were associated with higher odds of liver fibrosis (OR, 1.49 per 1 log TSH).[31] In euthyroid subjects, results varied from a positive association[38] to no association[31,33] of thyroid function with liver fibrosis.

Pulmonary fibrosis: Two case-control studies investigated the association of overt hypothyroidism with fibrotic diseases of the lung, providing similar results.[39,40] Overt hypothyroidism was associated with a 2.39 and a 2.70 times higher risk of CHPP and IPF, respectively (Table 1B).[39,40]

Myocardial fibrosis: Two studies investigated the association of hypothyroidism and thyroid function measurements with myocardial fibrosis (Table 1A).[41,42] One of the studies showed that hypothyroidism is associated with a higher degree of diffuse fibrosis than euthyroidism (Table 2B).[42] The other study showed a negative linear association between FT_3 levels and the risk of myocardial fibrosis among patients with idiopathic dilated cardiomyopathy (Table 1B). TSH and FT_4 levels, on the other hand, were not associated with myocardial fibrosis (Table 1B).[41]

Quality assessment

Study bias assessment scores are shown in Table 4. The general quality of the included studies was moderate. 2 studies scored 7/9 stars, 4 studies scored 6/9 stars, 3 studies scored 5/9 stars, and 1 study scored 4/9 (Table 4).

DISCUSSION

This systematic review summarizes the current evidence regarding the role of thyroid function on fibrosis of the liver, lung and heart in humans. Of the 10 identified studies, 6 reported on liver fibrosis, 2 on pulmonary fibrosis, and 2 on myocardial fibrosis. The general quality of the data was moderate. Overall, low thyroid function was associated with increased odds of liver fibrosis, pulmonary fibrosis, and myocardial fibrosis. Results were consistent, despite the diversity of the study populations, the different methodologies of the included studies and the different locations of

Table 1B. Description of included studies on the association of thyroid function with fibrosis

First author, year	Outcome	Assessment	TSH/FT_4/FT_3	Overt hypothyroidism (unless otherwise specified)
Liver fibrosis				
Liangpunsakul, 2003 [a]	NASH	85% liver biopsy 25% radiologic	NA	**OR (CI), 2.3 (1.2-4)**
Pagadala, 2012 [b]	NASH	Liver biopsy	NA	**OR (CI), 3.8 (2-6.9)**
Carulli, 2013	NASH	Liver biopsy	TSH: **OR (CI), 2.74 (1.15-6.53)**	NA
Bano, 2016 [b]	Combined NAFLD and fibrosis	Ultrasound and elastography	*All participants:* LogTSH: **OR (CI), 1.49 (1.04-2.15)** FT_4 (ng/dL): OR (CI), 0.59 (0.13-2.59) *Euthyroid participants:* LogTSH: OR (CI), 1.13 (0.63-2.03) FT_4 (ng/dL): OR (CI), 0.81 (0.11-5.75)	**OR (CI), 6.64 (1.04-23.98)** Subclinical hypothyroidism: **OR (CI), 2.30 (1.12-4.31)** Subclinical hyperthyroidism: OR (CI), 0.80 (0.04; 3.91)
Bril, 2016	NASH	Liver biopsy	Prevalence of NASH was not different among the FT_4 quintiles (71%, 59%, 61%, 76%, 80%; *P* value, 0.28)	NA
Kim, 2017 [c]	NASH	Liver biopsy	NA	Subclinical hypothyroidism: **OR (CI), 2.17 (1.17-4.01)**
Pulmonary fibrosis				
Oldham, 2015 [a]	IPF	Lung biopsy or CT	NA	**OR (CI), 2.70 (1.31-5.54)**
Adegunsoye, 2017 [a]	CHPP	ATS criteria	NA	**OR (CI), 2.39 (1.36-4.2)**
Myocardial fibrosis				
Gao, 2016 [d]	T1-Mapping [e]	Cardiac MRI	NA	Hypothyroid cases had higher T1-values than controls[f]
Wang, 2016	↑ LGE	Cardiac MRI	TSH: OR (CI), 0.98 (0.93-1.04) FT_4: OR (CI), 0.37 (0.05-2.75) FT_3: **OR (CI), 0.14 (0.04-0.57)**	NA

[a]The diagnosis of hypothyroidism was based on self-reported history of hypothyroidism and/or use of thyroid hormone replacement. [b]Thyroid status categories were defined based on FT_4 and TSH measurements. Euthyroidism as reference. [c]Thyroid function was categorized into strict-normal thyroid function (reference), low-normal thyroid function, and subclinical hypothyroidism. Patients with past history of overt thyroid dysfunction, and/or using thyroid medications were excluded. [d]Hypothyroid patients had overt hypothyroidism caused by chronic lymphocytic thyroiditis, free from concomitant disease and without medical treatment. [e]T1-mapping of the myocardium assesses diffuse myocardial fibrosis. Increased T1-values reflect a longer relaxation time and a more advanced stage of diffuse fibrosis. [f]*T1-left ventricular anterior wall (ms):* controls, 1083±51.28; hypothyroid, 1220±75.85 (*P* value<0.001). *T1-interventricular septum (ms):* controls, 1048±66.29; hypothyroid, 1175±81.87 (*P* value<0.001). *T1-left ventricular inferior wall (ms):* controls, 1062±56.56; hypothyroid, 1179±80.21 (*P* value<0.001). *T1-left ventricular lateral wall (ms):* controls, 1066±47.69; hypothyroid, 1185±81.79 (*P* value<0.001). Abbreviations: TSH, thyroid-stimulating hormone; FT_4, free thyroxine; FT_3, free triiodothyronine; NASH, nonalcoholic steatohepatitis; NA, not applicable; OR, odds ratio; CI, confidence interval; NAFLD, nonalcoholic fatty liver disease; IPF, idiopathic pulmonary fibrosis; CT, computed tomography; CHPP, chronic hypersensitivity pneumonitis; ATS, American Thoracic Society; T1, myocardial longitudinal relaxation time; MRI, cardiac magnetic resonance; LGE, late gadolinium enhancement. NB: Lighter grey highlight indicates a negative association between thyroid function and the risk of fibrosis. Darker grey highlight indicates no association.

Table 2. Quality Assessment Scale

First author, year (Reference)	Selection	Comparability	Exposure/Outcome	Total
Liangpunsakul, 2003[36]	3/4	2/2	1/3	6/9
Pagadala, 2012[37]	1/4	2/2	3/3	6/9
Carulli, 2013[38]	2/4	2/2	3/3	7/9
Bano, 2016[31]	3/4	2/2	2/3	7/9
Bril, 2016[33]	2/4	0/2	3/3	5/9
Kim, 2017[32]	1/4	2/2	3/3	6/9
Oldham, 2015[40]	2/4	2/2	1/3	5/9
Adegunsoye, 2017[39]	1/4	2/2	2/3	5/9
Gao, 2016[42]	1/4	0/2	3/3	4/9
Wang, 2016[41]	1/4	2/2	3/3	6/9

fibrosis. However, the number of identified studies and the amount of prospective evidence were limited.

Hypothyroidism was consistently associated with an increased risk of fibrosis. The magnitudes of the associations differed across studies, which may be partly attributable to the different definitions of hypothyroidism used across studies. In general, the studies that excluded the thyroid medication users[31,32] reported larger effect estimates compared with the studies that included hypothyroid patients under thyroid medications.[36,37,39,40] In the latter group, the levothyroxine treatment may have reduced the risk of fibrosis, further resulting in an underestimation of the observed associations. In addition, the different magnitudes of the associations can be derived from the heterogeneity of fibrotic outcomes and the different population characteristics across the included studies.

We identified several studies investigating the association of thyroid function parameters with the risk of fibrosis.[31,33,38,41] Overall, there was a trend towards a positive association of circulating TSH levels with the risk of fibrosis.[31,38] FT_4 levels, on the other hand, tended to be negatively associated with the risk of fibrosis, but not significantly.[31,41] These results could be explained by the lack of sufficient statistical power to detect an association between serum FT_4 levels and the risk of fibrosis. Alternatively, it is also likely that the risk of fibrosis may be more sensitive to fluctuations in serum TSH rather than FT_4 levels. Furthermore, we identified a limited number of studies investigating the association between thyroid function within the reference range and the risk of fibrosis. Results varied from a negative association[38] to no association,[31,33] and the inconsistencies are likely due to the insuf-

ficient sample sizes. Future larger studies are therefore needed to clarify whether the risk of fibrosis is affected by the fluctuations within the reference ranges of TSH or thyroid hormone levels.

The extracellular matrix, which represents one of the targets of thyroid hormone action, is a likely candidate to be involved in the pathways linking hypothyroidism to fibrosis. Animal studies have shown that experimental hypothyroidism can lead to fibrosis via upregulation of the collagen gene expression.[12-14] Accordingly, the administration of thyroid hormones is shown to reduce the collagen gene expression in the liver[16,17] and myocardium.[20-22] Thyroid hormones enhance the matrix metalloproteinase activity, further resulting in a collagen breakdown. Another factor that may play a mediating role in the pathways linking hypothyroidism to fibrosis is the transforming growth factor beta (TGFβ), which represents one of the most potent fibrogenic cytokines.[43] Animal data have shown that thyroid hormones antagonize hepatic and pulmonary fibrosis through inhibiting the TGFβ/SMAD-dependent transcriptional activation.[18] Besides, the expression of deiodinases is likely altered in fibrotic tissues. In humans, the fibrotic lung has been characterized by an increased expression of DIO2 gene, which further increases the local conversion of T_4 to T_3.[19,44] Moreover, research in animal models of experimental hypothyroidism has shown that DIO2 plays a protective role against lung injury.[45] Overall, these data suggest that the increased expression of DIO2 gene may represent a compensatory mechanism which tends to improve the stressed environment of the fibrotic lung. Still, the expression of DIO2 in other fibrotic organs, such as the heart, remain to be clarified. Future studies may also examine the expression of deiodinases type 1 and type 3 in fibrotic tissues.

To the best of our knowledge, this is the first systematic review which combines the literature regarding the role of thyroid function on fibrosis of the liver, lung and heart, in humans. In accordance with the NOS scale, we used strict criteria for the quality assessment of the risk of bias. Most of the included studies adjusted for potential confounders, including age and sex. Another strength is the consistency of the results in the setting of diverse study populations. The wide range of ages (mean age ranging from 36 to 69) and ethnicities of participants may increase the generalizability of our conclusions.

Several limitations of this systematic review warrant consideration. The limited number of identified studies illustrates the scarcity of evidence in this topic. Most of the included studies were characterized by a cross-sectional design, which does

not provide evidence with regard to the temporality of the associations. The large heterogeneity across studies, including different definitions of thyroid dysfunction and various measures of fibrotic outcomes, did not allow us to perform summary statistics. Nevertheless, the results of the included studies were overall consistent.

Conclusions

This systematic review of human studies suggests that low thyroid function is associated with an increased risk of chronic fibrotic diseases of the liver, lung, and heart. Results were consistent in the setting of diverse study populations. However, most of the current evidence on this topic is based on cross-sectional data. In the future, adequately powered studies are needed to prospectively investigate the long-term effects of thyroid hormones on the occurrence and progression of fibrosis. Furthermore, future interventional studies in humans are needed to explore whether thyroid hormones or thyroid hormone analogues can prevent the progression of fibrosis. These investigations could eventually lead to new avenues regarding the development of therapies against fibrotic diseases. Lastly, future research is needed to elucidate the exact underlying mechanisms linking low thyroid function to fibrosis.

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SUPPLEMENTAL MATERIAL

Appendix 1. PRISMA 2009 checklist.

Section/Topic		Checklist item	Page
Title			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	54
Abstract			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	55-56
Introduction			
Rationale	3	Describe the rationale for the review in the context of what is already known.	57
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	57
Methods			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (eg, Web address), and, if available, provide registration information including registration number.	x
Eligibility criteria	6	Specify study characteristics (eg, PICOS, length of follow-up) and report characteristics (eg, years considered, language, publication status) used as criteria for eligibility, giving rationale.	59
Information sources	7	Describe all information sources (eg, databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	58
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	58-59
Study selection	9	State the process for selecting studies (ie, screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	59
Data collection process	10	Describe method of data extraction from reports (eg, piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	59
Data items	11	List and define all variables for which data were sought (eg, PICOS, funding sources) and any assumptions and simplifications made.	58, Appendix 2
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	58-59, Appendix 3, Appendix 4
Summary measures	13	State the principal summary measures (eg, risk ratio, difference in means).	x
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (eg, I^2) for each meta-analysis.	x

Appendix 1. PRISMA 2009 checklist. (continued)

Section/Topic		Checklist item	Page
Methods			
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (eg, publication bias, selective reporting within studies).	x
Additional analyses	16	Describe methods of additional analyses (eg, sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	x
Results			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	59, Figure 1
Study characteristics	18	For each study, present characteristics for which data were extracted (eg, study size, PICOS, follow-up period) and provide the citations.	58, Table 1A
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	60, Table 2
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Table 1B
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	x
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	x
Additional analysis	23	Give results of additional analyses, if done (eg, sensitivity or subgroup analyses, meta-regression [see Item 16]).	x
Discussion			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (eg, healthcare providers, users, and policy makers).	62
Limitations	25	Discuss limitations at study and outcome level (eg, risk of bias), and at review-level (eg, incomplete retrieval of identified research, reporting bias).	64-65
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	65-66
Funding			
Funding	27	Describe sources of funding for the systematic review and other support (eg, supply of data); role of funders for the systematic review.	66

Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

Appendix 2. Supplemental information on Search strategy

Search in Pubmed

(((Thyroid*[Title/Abstract] or hyperthyro* [Title/Abstract] or hypothyro* [Title/Abstract] or thyronine* [Title/Abstract] or Hashimoto[Title/Abstract] or thyroid-stimulating hormone [Title/Abstract] or "free thyroxine"[Title/Abstract] or Graves[Title/Abstract] or thyrotropin[Title/Abstract] or deiodinase[Title/Abstract] or triiodothyronine[Title/Abstract] or myxedema [Title/Abstract] or thyrotoxicosis[Title/Abstract] or hyperthyroxinemia [Title/Abstract])) AND (fibrosis[Title/Abstract] or fibrotic[Title/Abstract] or fibrosing[Title/Abstract] or fibroblast[Title/Abstract] or "nonalcoholic steatohepatitis"[Title/Abstract])) AND (heart [Title/Abstract] or cardiac [Title/Abstract] or cardiovascular[Title/Abstract] or lung [Title/Abstract] or pulmonary [Title/Abstract] or liver [Title/Abstract] or hepatic[Title/Abstract] or endocardium [Title/Abstract] or myocardium [Title/Abstract] or pericardium[Title/Abstract])

Search in Medline

((exp thyroid gland/ or hyperthyroxinemia/ or hyperthyroidism/ or hypothyroidism/ or thyroid function tests/ or thyroxine/ or thyroid hormones/ or thyrotropin/ or thyroxine/) or (thyroid* or hyperthyro* or hypothyro* or free thyroxine or thyroid-stimulating hormone or Graves or thyrotropin or thyroxin or deiodinase or Hashimoto or triiodothyronine or thyronine* or myxedema or thyrotoxicosis or hyperthyroxinemia).ab,ti.) AND ((fibrotic or fibrosis or nonalcoholic steatohepatitis or fibrosing).ab,ti. or fibroblast.mp or fibrosis/ or exp pulmonary fibrosis/ or exp endomyocardial fibrosis/ or idiopathic pulmonary fibrosis/) AND (heart or cardiac or endocardium or myocardium or pericardium or lung or pulmonary or liver or hepatic or cardiovascular).ab,ti.

Search in Embase

((exp thyroid gland/ or exp thyroid disease/ or exp thyroxine/ or exp thyroid function/ or thyroid hormone/ or thyrotropin/ or thyroxine/ or exp thyroid hormone blood level/ or exp thyroid gland examination/) or (thyroid* or hyperthyro* or hypothyro* or free thyroxine or Graves or thyrotropin or thyroxin or deiodinase or Hashimoto or triiodothyronine or thyronine* or myxedema or thyrotoxicosis or hyperthyroxinemia).ab,ti.) AND ((fibrotic or fibrosis or nonalcoholic steatohepatitis or fibroblast).ab,ti. or (exp fibrosing alveolitis/ or exp fibrosing interstitial pneumonia/ or exp heart muscle fibrosis/ or exp liver fibrosis/ or exp lung fibrosis/)) AND ((heart or cardiac or endocardium or myocardium or pericardium or lung or pulmonary or liver or hepatic or cardiovascular).ab,ti.)

Search in Web-of-Science

(TS=(thyroid* or hyperthyro* or hypothyro* or "free thyroxine" or thyroid-stimulating hormone or Graves or thyrotropin or deiodinase or triiodothyronine or myxedema or thyrotoxicosis or hyperthyroxinemia or thyronine* or Hashimoto)) AND (TS=(fibrosis or fibrotic or fibroblast or nonalcoholic steatohepatitis or fibrosing)) AND (TS=(heart or cardiovascular or cardiac or endocardium or myocardium or pericardium or lung or pulmonary or liver or hepatic))

Appendix 3. Newcastle-Ottawa Quality Assessment Scale for case-control studies

Selection (max 4 stars)

1) *Is the case definition adequate?*
   a. yes, with independent validation*
   b. yes, eg, record linkage or based on self-reports
   c. no description

2) *Representativeness of the cases*
   a. consecutive or obviously representative series of cases*
   b. potential for selection biases or not stated

3) *Selection of controls*
   a. community controls*
   b hospital controls
   c. no description

4) *Definition of controls*
   a. no history of disease (end point)*
   b. no description of source

Comparability (max 2 stars)

1) *Comparability of cases and controls on the basis of the design or analysis*
   a. study controls for the most important factors*
   b. study controls for any additional factor**

Exposure (max 3 stars)

1) *Ascertainment of the exposure*
   a. secure record (eg, surgical records)*
   b. structured interview where blind to case/control status*
   c. interview not blinded to case/control status
   d. written self-report or medical record only
   e. no description

2) *Same method of ascertainment for cases and controls*
   a. yes*
   b. no

3) *Non-response rate*
   a. same rate for both groups*
   b. non-respondents described
   c. rate different and no designation

Appendix 4. Adapted Scale from the Newcastle-Ottawa Quality Assessment Scale for cohort studies

Selection (max 4 stars)

1) Representativeness of the exposed cohort
   a. Truly representative of the average in the target population (all subjects or random sampling)*
   b. Somewhat representative of the average in the target population (non-random sampling)*
   c. Selected group of users
   d. No description of the derivation of the cohort

2) Sample size
   a. Justified and satisfactory*
   b. Not satisfied

3) Ascertainment of the exposure
   a. Secure record (*eg*, medical records)*
   b. Structured interview *
   c. Written self-report
   d. No description of the measurement tool

4) Non-respondents
   a. Comparability between respondents and non-respondents characteristics is established, and the response rate is satisfactory*
   b. The response rate is unsatisfactory, or the comparability between respondents and non-respondents is unsatisfactory
   c. No description of the response rate or the characteristics of the respondents and the non-respondents

Comparability (max 2 stars)

1) The subjects in the different outcome groups are comparable, based on the study design or analysis. Confounding factors are controlled.
   a. Study controls for the most important factors (age, sex)*
   b. Study controls for additional relevant factors**
   c. Inadequate degree of control

Outcome (max 3 stars)

1) Assessment of the outcome
   a. Independent or blind assessment stated in the paper, or confirmation of the outcome by reference to secure records (eg, X-rays, medical records)**
   b. Record linkage (eg, identified through ICD codes on database records)**
   c. Self-report (ie, no reference to original medical records or X-rays to confirm the outcome)*
   d. No description

2) Statistical test
   a. The statistical test used to analyze the data is clearly described and appropriate, and the measurement of the association is presented, including the probability level (*P* value)*
   b. The statistical test is not appropriate, not described or incomplete.

CHAPTER 2.3

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

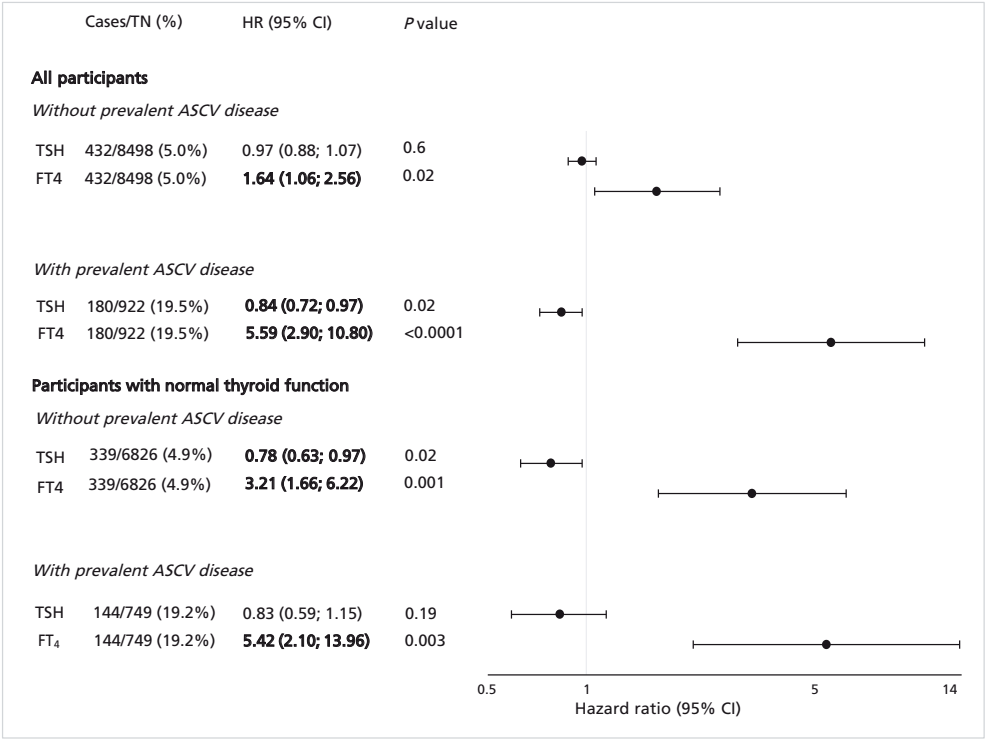
	Events/TN, %	HR (95% CI) Model 1	*P* value	HR (95% CI) Model 2	*P* value
All participants					
TSH	612/9420 (6.5%)	0.93 (0.86; 1.01)	0.12	0.92 (0.84; 1.00)	0.06†
FT_4	612/9420 (6.5%)	**2.23 (1.58; 3.14)**	<0.0001	**2.41 (1.68; 3.47)**	<0.0001‡
*Thyroid function within the reference ranges**					
TSH	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.

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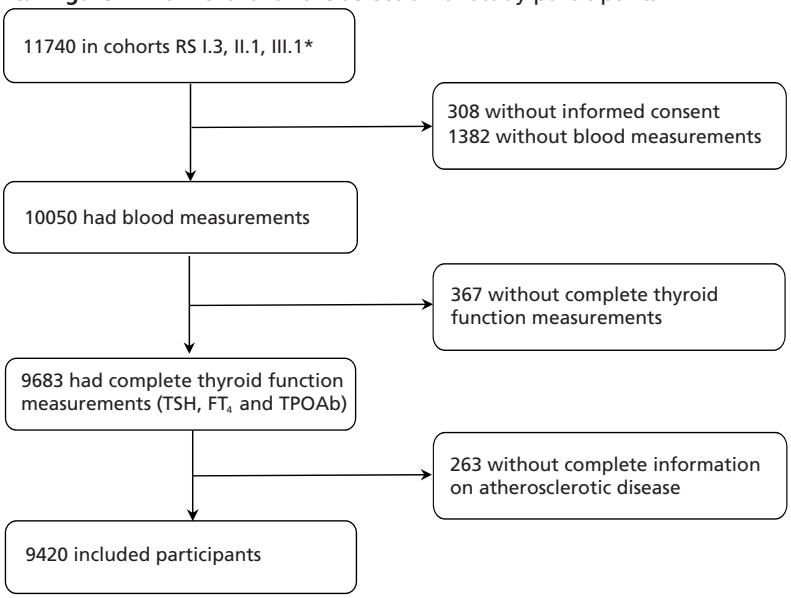
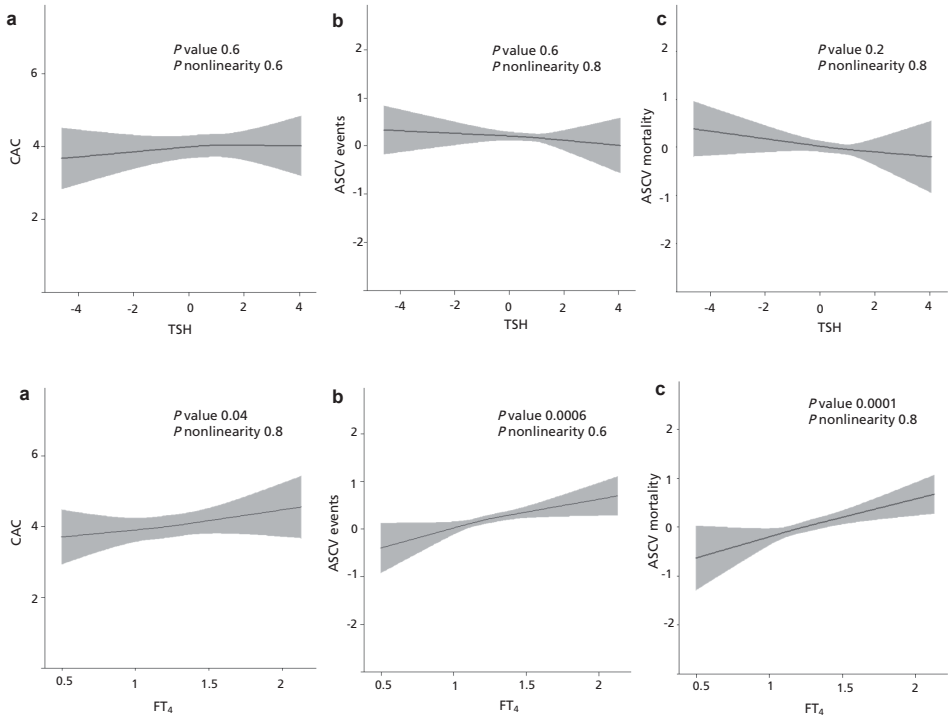
SUPPLEMENTAL MATERIAL

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.

CHAPTER 2.4

THYROID FUNCTION AND CARDIOVASCULAR DISEASE: IS THERE A MEDIATING ROLE OF COAGULATION?

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Submitted

ABSTRACT

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

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

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

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

INTRODUCTION

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

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

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

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

METHODS

Study population

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

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

Assessment of thyroid function

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

Assessment of coagulation factors

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

Assessment of cardiovascular disease

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

Additional measurements

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

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

Statistical analysis

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

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

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

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

RESULTS

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

Table 1. Baseline characteristics of 5918 participants

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

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

Thyroid function and coagulation factors

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

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

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

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

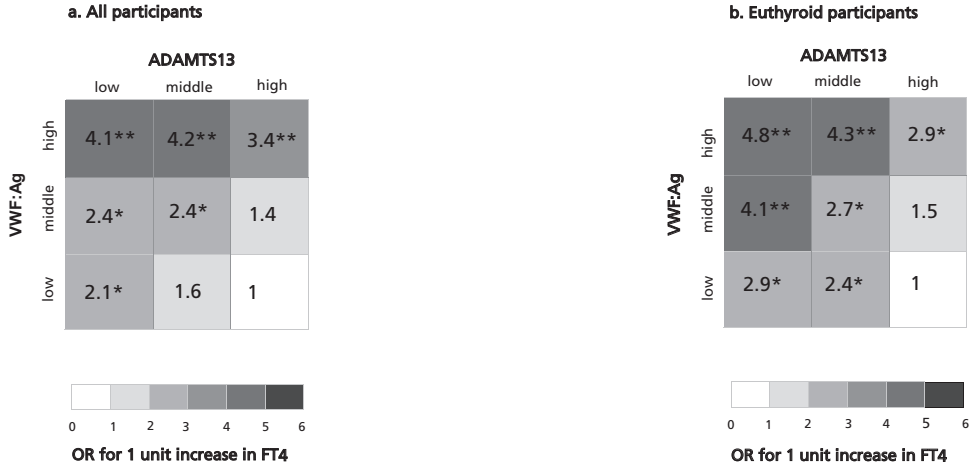
after excluding participants with cancer at baseline (Supplemental Table 2). No evidence of effect modification was observed. TPOAb positivity was not associated with the coagulation factors (Supplemental Table 3). Results remained similar or became stronger after restricting the analyses to euthyroid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

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

Thyroid function, coagulation factors, and cardiovascular disease

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

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



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

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

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

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

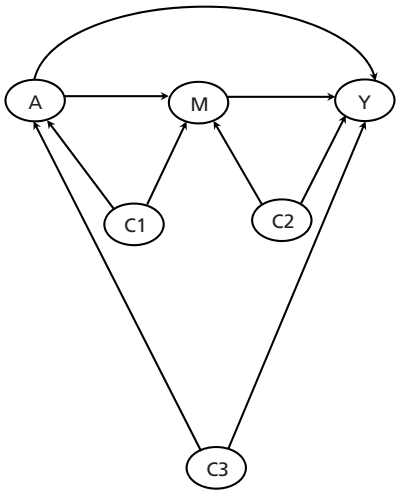
Adjusted for age, sex, cohort, smoking, alcohol intake, body mass index, diabetes, total cholesterol, triglycerides, systolic blood pressure, use of antihypertensive medications, use of lipid-lowering medications, use of anticoagulant medications, and blood group. HRs of TSH are denoted per 1 unit increase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

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

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

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

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



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

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

DISCUSSION

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

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

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

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

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

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

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

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

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

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

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

Conclusions

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

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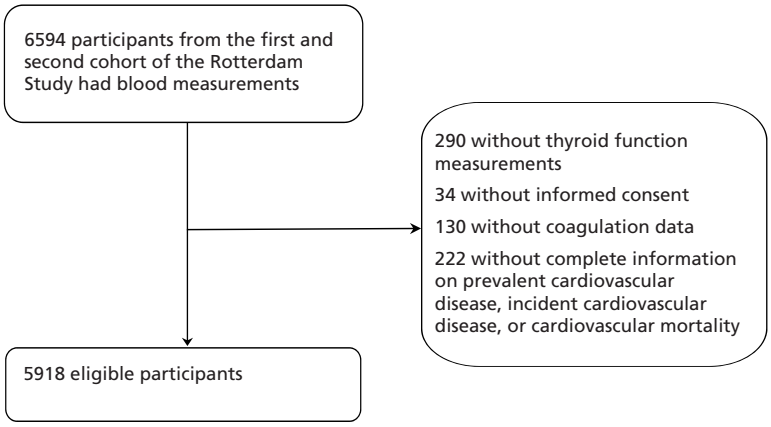
SUPPLEMENTAL MATERIAL

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



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

Supplemental Table 1. Association between thyroid function and coagulation factors

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

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

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

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

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

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

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

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

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

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

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

CHAPTER 2.5

THYROID FUNCTION AND ATRIAL FIBRILLATION: IS THERE A MEDIATING ROLE OF EPICARDIAL ADIPOSE TISSUE?

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ABSTRACT

Background The underlying mechanism of the association between thyroid function and atrial fibrillation (AF) is poorly understood, but epicardial adipose tissue (EAT) could be a promising mediator.

Methods In 1995 participants (mean age, 64.5 years) from the population-based Rotterdam Study, we measured thyroid function (thyroid-stimulating hormone [TSH], free thyroxine [FT_4]), and performed computed tomography to quantify EAT volumes. All participants were followed for the occurrence of AF. We assessed associations of TSH and FT_4 with EAT and AF, and performed causal mediation analysis to decompose the overall effect of thyroid function on AF with EAT as mediator.

Results Higher FT_4 levels were associated with larger EAT volumes in persons with large waist circumferences, defined by sex-specific cutoffs (0.08 mL more EAT per 1 standard deviation increase in FT_4; 95% confidence interval [95% CI], 0.02 to 0.14), but not in persons with a normal waist circumference. In persons with a large waist circumference, higher FT_4 levels were associated with a higher AF risk (hazard ratio, 1.50; 95% CI, 1.22 to 1.83). We found no evidence of a mediating role of epicardial adipose tissue in the association of thyroid function with AF (mediated interaction, 1.6%; pure indirect effect, 3.2%). The estimate of reference interaction of EAT with thyroid function on AF risk was more substantial (10.8%), but statistically nonsignificant.

Conclusions Higher FT_4 levels are associated with larger EAT volumes in persons with abdominal obesity. We report no mediating role of EAT in the association of thyroid function with AF, but found evidence for a suggested interaction of FT_4 with EAT volumes on AF risk.

INTRODUCTION

The association of high and high-normal thyroid function with atrial fibrillation (AF) has been established in several large studies and meta-analyses.[1-3] Possible pathophysiological mechanisms include direct effects of thyroid hormone on the sympathetic nervous system and indirect effects through accumulation of cardiovascular risk factors. However, the link between thyroid function and AF seems largely independent of traditional cardiovascular risk factors such as blood pressure, diabetes and cholesterol.[1,2] This suggests that yet unexplored cardiovascular risk factors or alternative pathways could mediate the association of thyroid dysfunction with AF. Further elucidation of these mediators is not only important for pathophysiological understanding but, possibly, also for future treatment decisions (ie, treatment targeted at thyroid dysfunction, modifiable mediators or both).

Against this background, epicardial adipose tissue (EAT) might be of great interest. EAT is a rapidly emerging risk factor for cardiovascular disease, and particularly for AF.[4,5] Thyroid hormones may be linked to EAT through several mechanisms. For example, thyroid hormones act on pathways leading to atherogenesis, including endothelial damage and increased procoagulation factors,[6] which are in turn closely related to EAT increase. Thyroid hormones may also exert direct effects on EAT by activating adipose tissue, mainly brown adipose tissue. Thyroid hormones are important for energy regulation and thermogenesis. Thyroid hormone excess leads to an increase in basal metabolic rate and thermogenesis, and patients with hyperthyroidism often present with weight loss. However, thyroid hormones have also shown to play a role in brown adipose tissue activity and white adipose tissue browning.[7] In mice, lack of thyroid hormone reduces activity of brown adipose tissue, while hyperthyroidism shows an increase in brown adipose tissue mass.[8] EAT is generally perceived as solely consisting of white adipose tissue.[4] This concept is under debate since brown adipose tissue-specific genes, *UCP1* gene in particular, have been identified in human EAT, suggesting that EAT additionally possesses brown adipose tissue-like characteristics.[4]

Hence, EAT may represent a potential explanation for the association of high thyroid function with AF. In the past years, several small studies (n<100 participants) have indicated a possible role of hypothyroidism or hyperthyroidism on EAT formation and progression.[9-13] This association has neither been explored in larger studies, nor has it been investigated in the full range of thyroid function in the general population. Hence, we aimed to assess the association of thyroid function with EAT

measured by computed tomography and to investigate whether EAT is a mediator of the association of thyroid function with AF in a large population-based cohort study.

METHODS

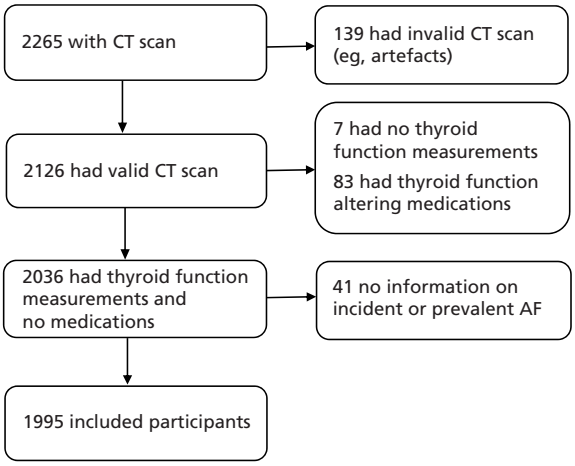
Study Population

The study was performed in the context of the Rotterdam Study (RS), a prospective population-based cohort study that investigates determinants and occurrence of cardiovascular, neurological, ophthalmologic, psychiatric, and endocrine diseases in the middle-aged and elderly population. The aims and design of the Rotterdam Study have been described in detail elsewhere.[14] We included participants from two independent cohorts within the Rotterdam Study. The first cohort (RS I) includes participants aged 55 years and older and baseline data were collected from 1990 to 1993. The second cohort (RS II) includes participants aged ≥55 years, and baseline data were collected from 2000 to 2001. Between 2003 and 2006, all participants who visited the research center were invited to undergo a multidetector computed tomography (MDCT) examination on which the amount of epicardial fat was assessed. This was part of a larger project on the assessment of vascular calcification. In total, 2524 participants were scanned.

For the current study, we included all participants from the Rotterdam Study, cohort I wave 3 and cohort II wave 1, with available thyroid function measurements, CT EAT measurements (assessed after laboratory measurement in all participants) and data on AF incidence. We excluded participants using thyroid function altering medication (levothyroxine, anti-thyroid drugs, amiodarone or corticosteroids) and with prevalent AF at baseline. Eligible participants were followed-up for incident AF events from CT EAT measurement onward. Detailed information on the selection of study participants is provided in Figure 1.

The study protocol was approved by the Medical Ethics Committee of the Erasmus University and by the Ministry of Health, Welfare and Sport of the Netherlands, implementing the "Wet Bevolkingsonderzoek: ERGO (Population Studies Act: Rotterdam Study)". All included participants provided a written informed consent in accordance with the Declaration of Helsinki to participate in the study and to obtain information from their family physicians.

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



Baseline measurements for the present study were performed during the cohort I wave 3 and cohort II wave 1 of the Rotterdam Study. Thyroid function was assessed at baseline. Epicardial adipose tissue volumes were measured by CT scans, which were performed in cohort I wave 4 and cohort II wave 2 of the Rotterdam Study. The median time between CT scans and laboratory measurements was 4.6 years. Eligible participants were followed-up for incident AF events from CT assessment onward. Abbreviations: CT, computed tomography; AF, atrial fibrillation.

Assessment of thyroid function

Thyroid function was measured through thyroid-stimulating hormone (TSH) and free thyroxine (FT_4) using the same methods and assay for all cohorts (The electrochemiluminescence immunoassay for thyroxine and thyrotropine, ECLIA, Roche) in serum samples stored at -80°C. We determined the reference values for normal range TSH as 0.4 to 4.0 mIU/L and FT_4 as 11 to 25 pmol/L (alternatively, 0.85 to 1.95 ng/dL) according to national guidelines as well as our previous studies.[1,15]

Assessment of epicardial fat volume

Non-contrast MDCT images were acquired using 16-slice (n=593) or 64-slice (n=1402) MDCT scanners (Somatom Sensation 16 or 64, Siemens, Forchheim, Germany). Detailed information on the imaging parameters are described elsewhere.[16] We used an ECG-gated cardiac scan to visualize the epicardium, and applied a previously described, fully automatic tool to quantify the amount of epicardial fat in milliliters.[17]

Assessment of atrial fibrillation

For the assessment of AF during follow-up, three methods are used in the Rotterdam Study and have been described in detail previously.[18,19] First, electrocar-

diograms (ECGs) were recorded at study entry and at each follow-up examination and analyzed with the Modular ECG Analysis System (MEANS).[20,21] All ECGs with a diagnosis of AF, atrial flutter, or any other rhythm disorder were reviewed by two independent research physicians who were blinded to the MEANS diagnosis. In case of disagreement, a senior specialist was consulted and the final decision was made. Second, information on AF was obtained through general practitioners' records, which includes their own results as well as hospitals discharge letters and outpatient clinic reports. Third, the national medical registry of all hospital discharge diagnoses was linked to the Rotterdam Study database. Any cases detected through linkage were verified by review of the medical records. Participants who developed AF as a consequence of severe systemic illness (eg, septic shock), resulting in death shortly after the detection of AF, were not considered to have AF. Furthermore, participants with transitory AF during myocardial infarction or after thoracic surgery were not considered as AF cases. All potential new diagnoses of AF were adjudicated by two independent research physicians, and in case of disagreement, consensus was sought. In case of persistent disagreement, a senior specialist made the final decision. Given that AF and atrial flutter are similar with respect to risk factors and consequences, these conditions were combined into a single composite outcome.[22] Follow-up for AF was complete until 1 January, 2014.

Additional measurements

We collected detailed information on cardiovascular risk factors and medication use in a standardized fashion by interview, physical examination, and blood sampling. Waist circumference was measured and expressed in centimeters. Waist circumference was stratified according to sex-specific clinical cutoffs. For women, normal waist circumference was defined as ≤88 cm, while large waist circumference was defined as >88 cm. For men, normal waist circumference was defined as ≤102 cm, while large waist circumference was defined as >102 cm. Systolic and diastolic blood pressure were measured twice at the right brachial artery using random-zero sphygmomanometer, and the mean of the two measurements was used for analyses. Fasting blood samples were obtained, and serum total cholesterol and high-density lipoprotein cholesterol were measured using an automatic enzymatic procedure (Hitachi analyzer, Roche Diagnostics). Information on lipid-lowering and antihypertensive medication use was derived from questionnaires and pharmacy information. Smoking information was derived from baseline questionnaires and

categorized into never, previous, and current smokers. Alcohol use information was derived from questionnaires and recorded as grams per day. History of diabetes mellitus was defined by a repeated (two measurements within one year) impaired fasting glucose ≥7 mmol/L or a non-fasting glucose of ≥7 mmol/L (when fasting samples were absent) or use of anti-glycemic medication at baseline. Prevalent coronary heart disease was ascertained as previously described and consisted of a prior myocardial infarction or revascularization.[19]

Statistical analysis

We analyzed the association of thyroid function (ie, FT_4 or TSH) with EAT with ordinary least-squared regression models. The association of thyroid function or EAT with AF was investigated by Cox-proportional hazards regression models. We used restricted cubic splines at three knots for all covariates in our analyses to assess and account for possible nonlinearity of the associations, but no evidence of nonlinearity was observed.

All primary analyses were performed for three models. Potential confounders were selected based on biological plausibility and previous literature (Supplemental Figures 1-4). The first model adjusted for age, sex, cohort, smoking, alcohol intake and time between laboratory measurement and CT scan. In the second model, we additionally adjusted for cardiovascular risk factors, including total cholesterol, high-density lipoprotein cholesterol, lipid-lowering medications, systolic blood pressure, diastolic blood pressure, antihypertensive medications, prevalent diabetes mellitus, and prevalent coronary heart disease. The third model additionally adjusted for waist circumference (within the waist circumference categories). Of note, the first model was regarded as the primary model for the analyses where thyroid function is the exposure, because all included covariates in the other models are more likely to be possible mediators rather than confounders. For the analyses where EAT is the exposure and AF is the outcome, we additionally adjusted for TSH and FT_4 and considered Model 2 as the primary model (Supplemental Material).

Due to the possible differential effect of thyroid function on adiposity in general, in contrast to EAT specifically, we tested interaction of TSH and FT_4 with waist circumference on the association with EAT and AF. There was a statistically significant interaction between FT_4 and waist circumference on EAT and on the risk of AF, and we therefore stratified the analyses according to sex-specific clinical cutoffs of waist circumference. We considered waist circumference rather than BMI as a marker of

obesity, because: (1) waist circumference is perceived as a better marker of visceral adiposity as compared to BMI and (2) waist circumference showed better statistical properties as compared to BMI (eg, statistical significance for the interaction terms while avoiding multicollinearity that occurred when introducing both waist circumference and BMI to the model). The association of thyroid function with waist circumference in our study is provided in Supplemental Table 1. There was no interaction of thyroid function with sex or age on any of the outcomes.

Given a possible synergic effect of thyroid function and EAT volumes on AF risk, our mediation analysis was based on the approach of a four-way decomposition, which combines methods assessing mediation and interaction. In the four-way decomposition approach, the overall effect of thyroid function on AF with EAT as mediator (with which the thyroid function may interact) is decomposed into 4 components: (1) the direct effect of thyroid function (ie, TSH or FT_4) on AF in the absence of EAT, (2) the interactive effect when the EAT is at the value it would be in the absence of the thyroid function, (3) a mediated interaction (due to mediation and interaction by EAT), and (4) a pure mediated effect.[23] These concepts will be referred to as controlled direct effect, reference interaction, mediated interaction, and pure indirect effect, respectively. The method assumes baseline covariates control for exposure-outcome, mediator-outcome, and exposure-mediator confounding and that there is no mediator-outcome relationship affected by exposure. In order not to violate the final condition, we used the predicted value of these factors on EAT: total cholesterol, high-density lipoprotein cholesterol, lipid-lowering medications, diastolic blood pressure, systolic blood pressure, use of antihypertensive medications, prevalent CHD, and prevalent diabetes mellitus as covariates. We performed a sensitivity analysis including these variables separately in the mediation analysis (Supplemental Table 2), but no meaningful differences were detected.

We used Z scores of FT_4, TSH and EAT for all analyses, after log-transformation, when appropriate. Statistical analyses were conducted using R statistical software (rms, Himsc, visreg packages, R-project, Institute for Statistics and Mathematics, R Core Team (2013), Vienna, Austria, version 3.0.2).

RESULTS

We included 1995 participants with valid CT-scans, thyroid function measurements, and information on AF follow-up that did not use thyroid-function altering medica-

tion (Figure 1). Baseline characteristics of study participants, total and stratified by waist circumference, are shown in Table 1. During an overall median follow-up of 12.9 (interquartile range, 12.07 to 13.70) years, 109 of 1189 participants with a normal waist circumference and 87 of 806 participants with a large waist circumference developed AF.

Table 1. Baseline characteristics of study participants*

	Total sample	Small waist circumference	Large waist circumference
Number	1995	1189	806
Age, years	64.5 (6.4)	64.4 (6.3)	64.7 (6.5)
Age, range	55-94	55-90	55-94
Women, n (%)	1018 (51.0)	516 (43.4)	502 (62.3)
TSH, mIU/L, median (IQR)	1.84 (1.27 – 2.72)	1.78 (1.22 – 2.61)	1.92 (1.33 – 2.90)
FT_4, pmol/L	15.6 (2.1)	15.8 (2.1)	15.4 (2.2)
Waist circumference, cm	93.4 (11.0)	87.7 (8.4)	101.7 (8.8)
Body mass index, kg/m^2	27.0 (3.8)	25.0 (2.5)	29.9 (3.5)
Diabetes mellitus, n (%)	194 (9.7)	64 (5.4)	130 (16.1)
Total cholesterol, mmol/L	5.82 (0.96)	5.79 (0.94)	5.86 (0.98)
Use of lipid-lowering medications	256 (12.8)	120 (10.1)	136 (16.9)
High-density lipoprotein cholesterol, mmol/L	1.39 (0.37)	1.44 (0.39)	1.31 (0.34)
Systolic BP, mmHg	141.5 (20.6)	139.5 (20.7)	144.5 (20.2)
Diastolic BP, mmHg	78.4 (10.8)	77.6 (10.8)	79.6 (10.8)
Use of antihypertensive medications, n (%)	511 (25.6)	229 (19.3)	282 (35.0)
Smoking, n (%)			
Current	348 (17.3)	217 (18.3)	131 (16.3)
Past	1039 (52.1)	605 (50.9)	434 (53.8)
Never	608 (30.6)	366 (30.8)	240 (29.9)
Alcohol intake, median, IQR	10.0 (1.4 – 20.0)	10.0 (1.9 – 20.0)	9.7 (1.0 – 20.0)
Prevalent coronary heart disease, n (%)	104 (5.2)	61 (5.1)	43 (5.3)
Epicardial fat volume, median, IQR	101.4 (80.0 – 130.4)	92.9 (72.9 – 118.4)	115.4 (93.7 – 148.2)
Time between laboratory measurement and scan (years) median, IQR	4.6 (4.4 – 4.8)	4.6 (4.4 – 4.8)	4.6 (4.3 – 4.6)

*Values are means (standard deviation), unless otherwise specified. Abbreviations: BP, blood pressure; TSH, thyroid-stimulating hormone; FT_4, free thyroxine; IQR, interquartile range.

Thyroid function and EAT

There was no association of TSH or FT_4 with EAT in participants with a normal waist circumference (Table 2). In participants with a large waist circumference, higher values of FT_4, but not TSH, were associated with a larger volume of EAT (β, 0.08 per standardized FT_4; 95% confidence interval [95% CI], 0.02 to 0.14; Table 2). This is in contrast to the association of FT_4 with waist circumference, where higher FT_4 levels were associated with a lower risk of having a large waist circumference (odds ratio, 0.84; 95% CI, 0.74 to 0.94 per one Z score increase of FT4, Supplemental Table 1).

Table 2. Association of TSH or FT_4 with EAT stratified for waist circumference*

	β (95% CI) Model 1	β (95% CI) Model 2	β (95% CI) Model 3
Small waist circumference (TN, 1189)			
TSH	0.03 (-0.01; 0.07)	0.01 (-0.03; 0.06)	0.03 (-0.01; 0.07)
FT_4	0.02 (-0.04; 0.08)	0.03 (-0.02; 0.09)	0.03 (-0.01; 0.03)
Large waist circumference (TN, 806)			
TSH	0.01 (-0.05; 0.06)	-0.00 (-0.05; 0.05)	-0.00 (-0.05; 0.05)
FT_4	**0.08 (0.02; 0.14)**	**0.09 (0.02; 0.15)**	**0.10 (0.04; 0.16)**

Model 1: age, sex, cohort, alcohol intake, smoking, and time between laboratory measurement and scan. Model 2: Model 1, total cholesterol, high-density lipoprotein cholesterol, use of lipid-lowering medications, systolic blood pressure, diastolic blood pressure, use of antihypertensive medications, prevalent diabetes mellitus, and prevalent coronary heart disease. Model 3: Model 2, and WC at baseline. *For both thyroid function parameters and epicardial adipose tissue, Z scores were used in the analysis. WC was stratified according to sex-specific clinical cutoffs. For women, small WC was defined as ≤88 cm, while large WC was defined as >88 cm. For men, small WC was defined as ≤ 102 cm, while large WC was defined as >102 cm. Abbreviations: TSH, thyroid-stimulating hormone; FT_4, free thyroxine; EAT, epicardial fat tissue; CI, confidence interval; WC, waist circumference; TN, total number.

Thyroid function, EAT, and AF

In participants with a normal waist circumference, TSH and FT_4 were not associated with incident AF while larger volumes of EAT were associated with an increased risk of AF with a hazard ratio (HR) of 1.53 per standardized EAT (95% CI, 1.19 to 1.97) (Table 3). In participants with large waist circumference, TSH was not associated with AF risk. However, FT_4 and EAT were both associated with AF risk with HRs of 1.45 (95% CI, 1.20 to 1.76) and 1.38 (95% CI, 1.00 to 1.89), respectively (Table 3).

None of the estimated excess risks of the 4 components was statistically significant (Table 4). The largest proportion attributable to the effect of FT_4 on AF in participants with a large waist circumference was the controlled direct effect (84.4%; excess risk, 0.424; 95% CI, -0.065 to 0.711; Table 4). The remainder of the

Table 3. Association of TSH, FT_4 and EAT with atrial fibrillation, stratified by waist circumference

	HR (95% CI) Model 1	HR (95% CI) Model 2	HR (95% CI) Model 3
Small waist circumference (Events/TN, 109/1189)			
TSH	1.10 (0.89; 1.35)	1.10 (0.90; 1.35)	1.12 (0.91; 1.38)
FT_4	1.09 (0.90; 1.33)	1.09 (0.90; 1.33)	1.10 (0.90; 1.34)
EAT	**1.50 (1.18; 1.91)**	**1.53 (1.19; 1.97)**	**1.48 (1.12; 1.96)**
Large waist circumference (Events/TN, 87/806)			
TSH	0.90 (0.74; 1.09)	0.90 (0.74; 1.10)	0.88 (0.72; 1.07)
FT_4	**1.45 (1.20; 1.76)**	**1.46 (1.19; 1.78)**	**1.50 (1.22; 1.83)**
EAT	**1.37 (1.01; 1.86)**	**1.38 (1.00; 1.89)**	1.22 (0.87; 1.70)

Model 1: age, sex, cohort, alcohol intake, smoking, and time between laboratory measurement and scan. Model 2: Model 1, total cholesterol, high-density lipoprotein cholesterol, use of lipid-lowering medications, systolic blood pressure, diastolic blood pressure, use of antihypertensive medications, prevalent diabetes mellitus, and prevalent coronary heart disease. Model 3: Model 2, and WC at baseline. For all EAT analyses, the models are additionally adjusted for TSH and FT_4. WC was stratified according to sex-specific clinical cutoffs. For women, small WC was defined as ≤88 cm, while large WC was defined as >88 cm. For men, small WC was defined as ≤102 cm, while large WC was defined as >102 cm. Abbreviations: TSH, thyroid-stimulating hormone; FT_4, free thyroxine; EAT, epicardial fat tissue; TN, total number; CI, confidence interval; WC, waist circumference.

Table 4. Proportions of the effect of FT_4 on AF due to mediation and/or interaction with EAT

	Excess risk (95% CI)	Proportion attributable
Small WC		
CDE	0.077 (-0.150; 0.285)	89.7%
INT_{ref}	-0.001 (-0.062; 0.018)	-1.0%
INT_{med}	0.002 (-0.009; 0.009)	2.5%
PIE	0.008 (-0.016; 0.025)	8.8%
Total	0.085 (-0.151; 0.276)	100%
Large WC		
CDE	0.424 (-0.065; 0.711)	84.4%
INT_{ref}	0.054 (-0.138; 0.242)	10.8%
INT_{med}	0.008 (-0.030; 0.038)	1.6%
PIE	0.016 (-0.024; 0.036)	3.2%
Total	0.502 (0.085; 0.733)	100%

WC was stratified according to sex-specific clinical cutoffs. For women, small WC was defined as ≤88 cm, while large WC was defined as >88 cm. For men, small WC was defined as ≤102 cm, while large WC was defined as >102 cm. Analyses for the association of FT_4 with EAT and AF were adjusted for age, sex, cohort, alcohol intake, and smoking. Analyses for the association of EAT with AF were additionally adjusted for total cholesterol, high-density lipoprotein cholesterol, lipid-lowering medications, systolic blood pressure, diastolic blood pressure, use of antihypertensive medications, prevalent diabetes mellitus, prevalent coronary heart disease, TSH and FT_4 levels. Abbreviations: FT_4 free thyroxine; AF, atrial fibrillation; EAT, epicardial fat tissue; CI, confidence interval; CDE, Controlled direct effect; INT_{ref}, reference interaction; INT_{med}, mediated interaction; PIE, pure indirect effect; TSH, thyroid stimulating hormone; WC, waist circumference.

overall effect was due to reference interaction (ie, the interactive effect between FT_4 and EAT when the EAT is at the value it would be in the absence of the thyroid function) with a proportion attributable of 10.8% (excess risk, 0.054, 95% CI, -0.138 to 0.242). The natural indirect effect (ie, sum of pure indirect effect and mediated interaction) was 0.024 (95% CI, -0.043 to 0.063; Table 4).

DISCUSSION

In this large sample of community-dwelling middle-aged and elderly subjects, higher FT_4 levels among participants with a large waist circumference were associated with larger EAT volumes. Among participants with a normal waist circumference, we observed no association between thyroid function and EAT volumes. The known relation between higher thyroid function and AF was not mediated by EAT.

Similar to previous studies, we found high and high-normal thyroid hormone levels to be associated with an increased risk of AF.[1,24] Prior prospective research has also reported a positive association of EAT volumes with AF risk.[25] Interestingly, a thyroid hormone-dependent gene, namely uncoupling protein-1 (UCP1) gene, is highly expressed in EAT,[26,27] thus suggesting a potential direct effect of thyroid hormones on mitochondrial uncoupling and in turn on EAT activation. In our study, we indeed describe an association of higher FT_4 levels with larger EAT volumes, mainly among participants with a large waist circumference. Therefore, we can speculate that higher FT_4 levels among participants with abdominal obesity may additionally increase the likelihood of having larger EAT volumes and the risk of developing AF. Due to the negative feedback mechanism of hypothalamic-pituitary-thyroid axis, one would expect TSH levels to be inversely associated with EAT. However, TSH levels were not associated with EAT volumes in our study. We hypothesize that this may reflect a dysregulation of the hypothalamic-pituitary-thyroid axis by overproduction of leptin in the setting of abdominal obesity.[28,29]

The relation of thyroid function with obesity is complex.[30] As described in previous literature and confirmed in our study, FT_4 levels are known to be negatively associated with abdominal obesity, which is in turn linked to increased EAT volumes.[30] However, in our study, there was a positive association of FT_4 levels with EAT volumes, which remained consistent after additionally adjusting for waist circumference. These results indicate that waist circumference does not explain the link between thyroid hormone and EAT. The opposing association of FT_4 levels with

EAT volumes as compared to FT_4 levels with waist circumference could suggest that the role of thyroid hormones on body fat distribution depends on the location and composition of visceral adipose tissue (eg, presence of brown adipose tissue-like characteristics or lack thereof). Along with other stimuli, increased thyroid hormones can enhance the transdifferentiation of white adipocytes to brown adipocytes in the EAT of obese patients. However, further research is needed to elucidate the exact mechanisms underlying the association of thyroid function with abdominal obesity and EAT.

Our study suggests that abdominal obesity may potentiate the effect of FT_4 on the development of AF. Among our participants with abdominal obesity, we found no evidence that the association between FT_4 and AF was mediated by EAT. Alternative mechanisms other than EAT could, therefore, explain the link between thyroid function and AF. For example, thyroid hormones may contribute to the initiation and maintenance of AF via the activation of automatic foci in the pulmonary veins,[31,32] the stimulation of sympathetic nervous system,[33,34] the elevation of left atrial pressure secondary to ventricular hypertrophy, and atrial ischemia secondary to an increased heart rate,[35] among others. On the other hand, we observed a notable proportion of reference interaction (10.8%), which indicates that the occurrence of AF can be partly explained by synergistic effects between thyroid hormones and EAT, though the 95% CI was quite wide. The results of our mediation analyses, however, should be interpreted with caution, as we may have lacked a sufficient sample size to detect statistically significant findings.

To our knowledge, this is the largest population-based cohort study investigating the relation of thyroid function with EAT volumes. Moreover, this is the first study that explores the potential mediating role of EAT in the association between thyroid function and AF. The mediation analysis was based on the approach of a four-way decomposition, which unifies within a single framework the methods assessing mediation and interaction.[23] The detailed information on potential confounders allowed us to perform multivariate-adjusted analyses. Another strength of our study is the comprehensive adjudication of end points. AF cases were extensively evaluated at baseline and during follow-up. EAT was assessed by using a standardized computed tomography-based procedure. Though most previous studies have utilized ultrasound measurements to evaluate EAT, computed tomography is considered superior to the ultrasound in the detection and, particularly, in the quantification of EAT.[13,36]

Several limitations of our study should also be considered. Thyroid function and EAT volumes were assessed only once and we had no information regarding their changes over time. However, due to the intra-individual variability of TSH and FT_4 levels, the lack of repeated measurements would tend to underestimate rather than overestimate the association of thyroid function with EAT and AF.[37] EAT volumes were assessed after the thyroid function measurements (median time between CT scans and laboratory measurements was 4.6 years). However, we adjusted all analyses for the time interval between the measurements. Another limitation is the lack of information on serum triiodothyronine levels. However, TSH and FT_4 represent the most relevant measurements of thyroid function in clinical practice. The majority of our participants were white middle-aged and older adults, limiting the generalizability of our findings to other populations. Lastly, given the observational study design, we cannot rule out the possibility of residual or unmeasured confounding.

Conclusions

Our findings suggest that in subjects with abdominal obesity, FT_4 measurement can help identify those with larger EAT volumes and higher risk of AF. Our results do not suggest EAT as a mediating factor between thyroid function and AF. However, the occurrence of AF may be influenced by potential synergic effects between thyroid hormones and EAT. Future research is warranted to replicate our results and provide further insight into the relation between thyroid function and AF.

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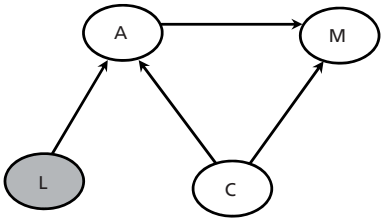
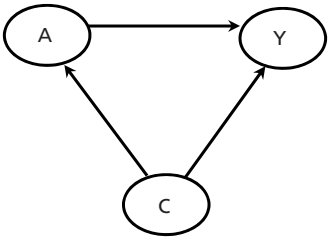
SUPPLEMENTAL MATERIAL

Supplemental Figure 1. Directed acyclic graph for the association of free thyroxine (FT_4) with epicardial adipose tissue.



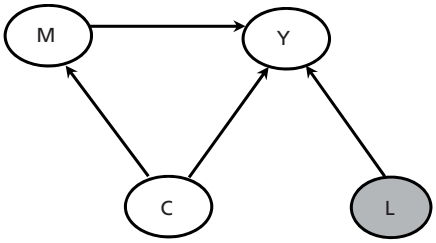
Corresponds to Table 2, Model 1. A, FT_4; M, epicardial adipose tissue; C, age, sex, cohort, smoking, alcohol intake, and time between measurements; L, waist circumference. Effect modification of FT_4 by waist circumference (interaction FT_4*waist circumference, *P* value <0.05) on M, not included in the primary model.

Supplemental Figure 2. Directed acyclic graph for the association of FT_4 with atrial fibrillation.



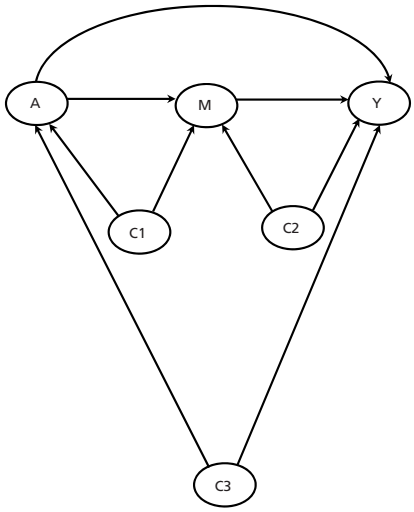
Corresponds to Table 3, Model 1. A, FT_4; Y, atrial fibrillation; C, age, sex, cohort, smoking, alcohol intake, and (large) waist circumference. Effect modification of FT_4 by waist circumference (interaction FT_4 * waist circumference, *P* value <0.05) on Y.

Supplemental Figure 3. Directed acyclic graph for the association of epicardial adipose tissue with atrial fibrillation.



Corresponds to Table 3, Model 2. M, epicardial adipose tissue; Y, atrial fibrillation; C, (1) age, sex, cohort, smoking, alcohol intake, free thyroxine, thyroid-stimulating hormone, and time between measurements, (2) total cholesterol, high-density lipoprotein cholesterol, use of lipid-lowering medications, diastolic blood pressure, systolic blood pressure, use of antihypertensive medications, prevalent coronary heart disease, and prevalent diabetes mellitus; L, waist circumference (omitted in this model due to collinearity with M). No interactions observed.

Supplemental Figure 4. Final directed acyclic graph, stratified by waist circumference dichotomized according to sex-specific clinical cutoffs.



A, FT_4; M, epicardial adipose tissue; Y, atrial fibrillation; C1, age, sex, cohort, smoking, alcohol intake, and time between measurements; C2, age, sex, cohort, smoking, alcohol intake, and predicted values using the following variables: total cholesterol, high-density lipoprotein cholesterol, use of lipid-lowering medications, diastolic blood pressure, systolic blood pressure, use of antihypertensive medications, prevalent coronary heart disease and prevalent diabetes mellitus (sensitivity analysis with original covariates); C3, age, sex, cohort, smoking, and alcohol intake.
Building final directed acyclic graph for mediation analysis
Conditions:
(1) The effect the exposure A has on the outcome *Y* is unconfounded conditional on *C*;
(2) The effect the mediator M has on the outcome *Y* is unconfounded conditional on (*C*, *A*);
(3) The effect the exposure A has on the mediator *M* is unconfounded conditional on *C*;
(4) None of the mediator-outcome confounders are themselves affected by the exposure.
Condition 4 is violated due to the relationship of A with several individual variables C; however, A is not associated with the composite of these variables. Due to the interaction of free thyroxine with waist circumference, all analyses are stratified by large and small waist circumference (sex-specific clinical cutoffs).

Supplemental Table 1. Association of Z scores of TSH or FT_4 with waist circumference*

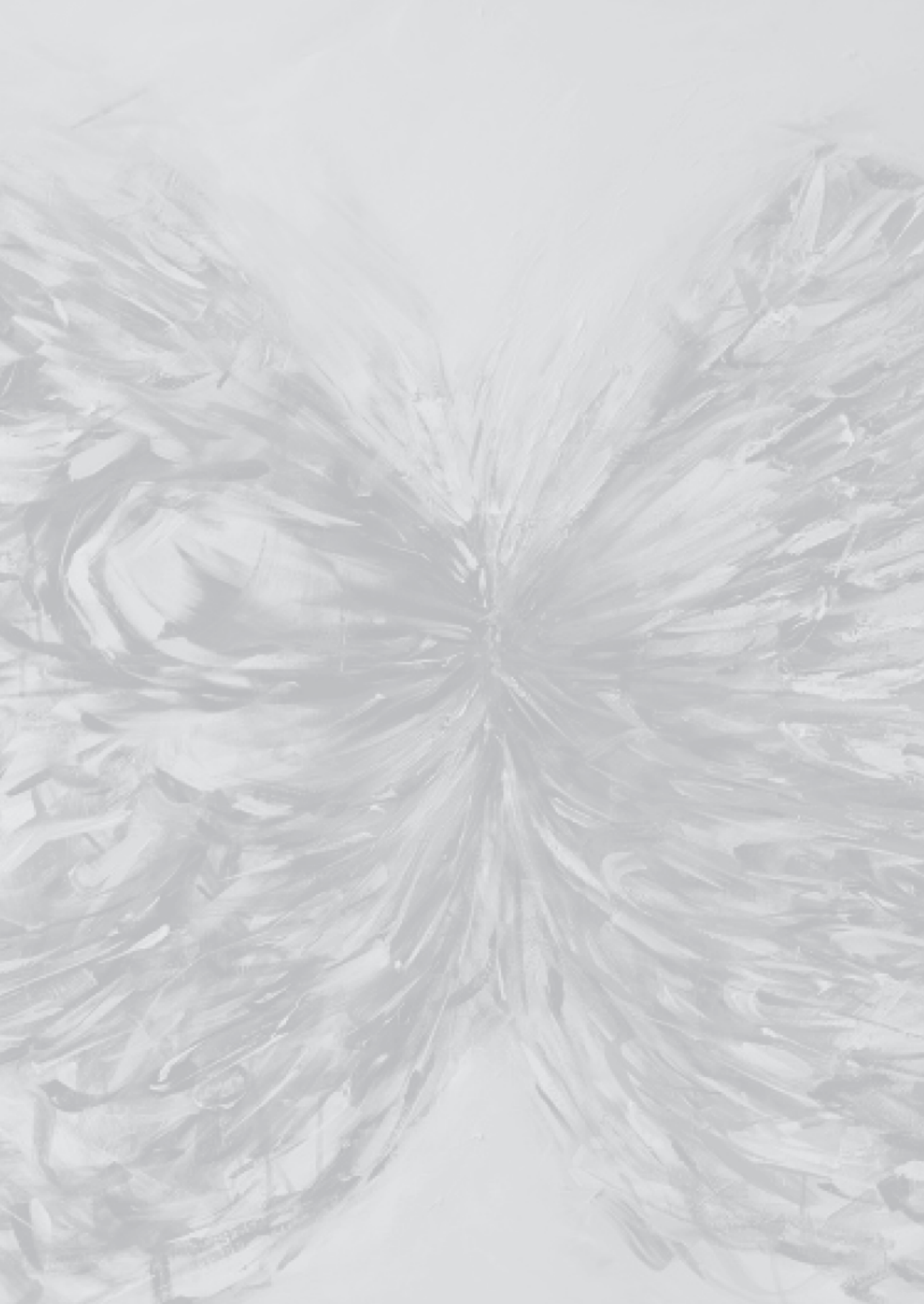
	OR (95% CI) Model 1	OR (95% CI) Model 2
TSH	1.17 (1.06; 1.28)	1.13 (1.02; 1.25)
FT_4	0.82 (0.73; 0.92)	0.84 (0.74; 0.94)

Model 1: age, sex, cohort, alcohol intake, smoking, and time between laboratory measurement and scan. Model 2: Model 1, total cholesterol, high-density lipoprotein cholesterol, use of lipid-lowering medications, systolic blood pressure, diastolic blood pressure, use of antihypertensive medications, prevalent diabetes mellitus, and prevalent coronary heart disease. *WC was stratified according to sex-specific clinical cutoffs. For women, small WC was defined as ≤88 cm, while large WC was defined as >88 cm. For men, small WC was defined as ≤102 cm, while large WC was defined as >102 cm. Abbreviations: TSH, thyroid-stimulating hormone; FT_4, free thyroxine; OR, odds ratio; CI, confidence interval; WC, waist circumference.

Supplemental Table 2. Proportions of the effect of Z scores FT_4 on AF due to mediation and/or interaction with EAT, using all confounders of M→Y relation separately

	Excess risk (95% CI)	Proportion attributable
Small waist circumference		
CDE	0.076 (-0.154; 0.305)	84.3%
INT_{ref}	-0.001 (-0.061; 0.024)	-1.2%
INT_{med}	0.004 (-0.011; 0.014)	4.0%
PIE	0.012 (-0.014; 0.031)	12.9%
Total	0.090 (-0.140; 0.306)	100%
Large waist circumference		
CDE	0.394 (-0.043; 0.675)	77.6%
INT_{ref}	0.080 (-0.149; 0.282)	15.6%
INT_{med}	0.013 (-0.038; 0.045)	2.5%
PIE	0.022 (-0.019; 0.046)	4.3%
Total	0.508 (0.063; 0.765)	100%

WC was stratified according to sex-specific clinical cutoffs. For women, small WC was defined as ≤88 cm, while large WC was defined as >88 cm. For men, small WC was defined as ≤102 cm, while large WC was defined as >102 cm. Abbreviations: CDE, controlled direct effect; CI, confidence interval; EAT, epicardial fat tissue; FT_4, free thyroxine; INT_{ref}, reference interaction; INT_{med}, mediated interaction; PIE, pure indirect effect; TSH, thyroid-stimulating hormone; WC, waist circumference.



CHAPTER 3

THYROID FUNCTION AND GENERAL HEALTH

CHAPTER 3.1

THYROID FUNCTION ASSOCIATED WITH FRAILTY INDEX, A MEASURE OF FRAILTY AND GENERAL HEALTH

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ABSTRACT

Background Thyroid hormones affect metabolism in various tissues, organs and systems. However, the overall impact of thyroid function on an individual's vulnerability to adverse outcomes remains unclear. We therefore aimed to investigate the cross-sectional and prospective association of thyroid function with frailty index, a well-established measure of overall health, in a population-based, prospective cohort study.

Methods Participants of the Rotterdam Study with baseline measurements of thyroid function and frailty index were eligible. The frailty index was measured at baseline and after a median follow-up time of 10.1 (interquartile range, 5.7 to 10.8) years. A higher frailty index indicated a worse health state. We assessed the association of thyroid function with frailty at baseline, frailty at follow-up, and frailty changes over time, adjusting for age, sex, cohort, smoking, alcohol intake, and education.

Results We included 9640 participants (mean age, 64.9 years). There was a U-shaped association of TSH (*P* value, 0.0003) and FT_4 (*P* value, <0.0001) with frailty at baseline. There was no association of TSH, but a positive association of FT_4 with frailty at follow-up and frailty changes over time (β, 1.22; 95% confidence interval, 0.73 to 1.72 per 1 unit FT_4).

Conclusions In this population-based study, participants with low and high thyroid function were more likely to be frail than participants with normal thyroid function. However, only those with higher FT_4 levels had an increased risk of becoming more frail over time. The identification of FT_4 as a potential marker of health deterioration could have future implications regarding the prediction and prevention of frailty.

INTRODUCTION

Frailty is a condition of reduced physiological reserves, decreased resistance to stressors and enhanced vulnerability to poor health outcomes, such as diseases, disability, falls, institutionalization and death.[1] With the aging of the population, the prevalence of frailty is expected to rise.[2] Therefore, various tools are being utilized to evaluate and identify vulnerable subjects.[3] One of the most common measurements is the frailty index, which has been validated as a robust predictor of adverse outcomes in many patient and community settings.[3-6] The frailty index, also known as the "multidomain phenotype", was developed to reflect the multidimensional and dynamic nature of frailty. It is composed of >30 items covering a broad range of health domains, and it is considered a useful tool to quantify overall health and its changes over time.[7,8]

Thyroid hormones, which are key regulators of metabolism, are likely to be implicated in the development of frailty.[9] So far, variations in thyroid hormone levels have been linked to alterations in cardiometabolic, cognitive and musculoskeletal functioning, which in turn contribute to a reduction in physiological capacity and resistance to stressors.[9] Most previous research, however, has focused on the system-specific effects of thyroid function, suggesting that lower thyroid hormone levels are associated with a higher risk of metabolic outcomes (ie, diabetes, dyslipidemia, and nonalcoholic fatty liver disease),[10-12] whereas higher thyroid hormone levels are associated with a higher risk of cognitive decline, atrial fibrillation, and osteoporosis.[13-17] Meanwhile, the overall impact of thyroid function on general health remains to be clarified. This could be important to further improve the prediction and prevention of health deterioration over time.

To date, only very few studies have investigated the association of thyroid function with frailty assessed either by the "physical phenotype" [18] or the Frail scale,[19] with inconsistent results. In a cross-sectional study assessing frailty by the Frail scale, higher free thyroxine (FT_4) levels were associated with an increased frailty risk, but there was no association for thyroid-stimulating hormone (TSH).[19] Another study assessing frailty by the "physical phenotype" showed that men with a low thyroid function (ie, highest TSH quintile) and women with a high thyroid function (ie, lowest TSH quintile) had an increased frailty risk.[18] Notably, both the Frail scale and the physical phenotype are derived from only 5 items mainly reflecting the physical aspect of frailty.[20,21] What previous research is lacking, however, is the utilization

of a multidimensional tool that would be able to capture the pleiotropic effects of thyroid hormones on general health.

Therefore, in a large population-based prospective study of middle-aged and elderly subjects, we aimed to investigate the cross-sectional and prospective association of thyroid function with the frailty index, a well-established measure of overall health.

METHODS

Study population

The Rotterdam Study is a prospective population-based cohort study that aims to investigate 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.[22] The Rotterdam Study was initiated in 1989, including 7983 participants ≥55 years of age (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 ≥45 years of age was added (RS III). Study participants undergo extensive follow-up medical examinations every 3 to 5 years.

For the current study, baseline measurements were performed during the third visit of the first cohort (n=4797) and the first visits of the second (n=3011) and third (n=3932) cohorts of the Rotterdam Study (Supplemental Figure 1). A total of 9640 participants with data available on thyroid function and frailty index at baseline were considered eligible. Of these, 6416 participants had repeated measurements on frailty index (Table 1).

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. In accordance with the Declaration of Helsinki, all participants provided written informed consent.

Assessment of thyroid function

Thyroid function was assessed at baseline in three study cohorts using the same method and assay. Concentrations of TSH, FT_4, and thyroid peroxidase antibodies (TPOAbs) were measured on baseline serum samples stored at -80°C using the electrochemiluminescence immunoassay ECLIA Roche. The reference ranges of serum

Table 1. Baseline characteristics of 9640 participants*

	Total	Follow-up available	Died before follow-up	No follow-up available
Number	9640	6416	2364	860
Age, years	64.9 (9.7)	61.8 (7.8)	74.6 (8.4)	62.2 (8.6)
Women, n (%)	5467 (56.7)	3709 (57.8)	1233 (52.2)	525 (61)
Smoking, n (%)				
current	2042 (21.2)	1321 (20.6)	506 (21.4)	215 (25.0)
former	4549 (47.2)	3059 (47.7)	1124 (47.5)	366 (42.6)
never	3010 (31.2)	2018 (31.5)	714 (30.2)	278 (32.3)
Education, n (%)				
Elementary	1189 (12.3)	595 (9.3)	469 (19.8)	125 (14.5)
Lower secondary	3874 (40.2)	2555 (39.8)	957 (40.5)	362 (42.1)
Higher secondary	2787 (28.9)	1897 (29.6)	670 (28.3)	220 (25.6)
Tertiary	1720 (17.8)	1331 (20.7)	244 (10.8)	145 (16.9)
TSH, mIU/L, median (IQR)	1.9 (1.2-2.8)	1.9 (1.3-2.8)	1.8 (1.1-2.6)	1.9 (1.2-2.8)
FT_4, ng/dL	1.2 (0.1)	1.2 (0.1)	1.2 (0.2)	1.2 (0.1)
TPOAb positive, n (%)	1272 (13.2)	870 (13.6)	282 (11.9)	120 (14.0)
TPOAb, kU/ml, median (IQR)	7.6 (5.0-13.6)	7.7 (5.0-13.8)	6.5 (5.0-12.6)	8.7 (5.2-14.7)
Use of thyroid medication, n (%)	308 (3.2)	210 (3.3)	71 (3.0)	27 (3.1)
Thyroid surgery, n (%)	167 (1.7)	100 (1.6)	49 (2.1)	18 (2.1)
Frailty index†	17.1 (8.7)	15.0 (7.1)	22.7 (10.4)	16.7 (8.0)

*Data are mean (standard deviation), unless otherwise specified. †To increase the interpretability of the risk estimates, the frailty index score was multiplied by 100. Abbreviations: TSH, thyroid-stimulating hormone; IQR, interquartile range; FT_4, free thyroxine; TPOAb, thyroid peroxidase antibodies (cutoff 35 kU/ml).

TSH (0.40 to 4.0 mIU/L) and serum FT_4 (11 to 25 pmol/L; alternatively, 0.86 to 1.94 ng/dL) were determined based on national guidelines and our previous studies.[10,11,23] The time of blood sampling was recorded. 99% of the blood samplings were performed between 8.00 ^{AM} and 11.00 ^{AM}.

Assessment of frailty index

Frailty was assessed by the frailty index, which is defined as the accumulation of health deficits including symptoms, signs, diseases and functional impairments.[7] A 45-item frailty index has been recently validated in the Rotterdam Study and has been described extensively elsewhere.[5] In short, health deficits were selected using a stepwise procedure, on the basis of the following predefined criteria: (1) the deficit is associated with health; (2) the prevalence or severity of the deficit generally increases with age; (3) the deficit is not too exceptional (ie, prevalence <5 %) or too

common (ie, prevalence >80%).[4,5] In case of a high correlation between variables of the same domain (r >0.7), only the one with the highest correlation with age was eventually included in the score.[4,5] To be able to evaluate changes of frailty over time, we used a slightly adapted version of the Rotterdam Study frailty index score that consisted of 38 health-related variables covering various health domains, including functional status (n=13), health conditions (n=6), diseases (n=6), cognition (n=6), mood (n=4), and nutritional status (n=3).[24] The remaining 7 items (namely vitamin D, sex hormone binding globulin, mobility, uric acid, pro-B-type natriuretic peptide, C-reactive protein, and homocysteine) were not assessed at follow-up and were therefore removed from the original Rotterdam Study frailty index score.[24] To obtain a stable frailty index score, it is recommended to have data available on at least 20 items.[4] Therefore, participants of the Rotterdam Study with <20 observed items were excluded. For individuals with data available on ≥20 items, missing values were imputed using multiple imputation.[5] Deficits were dichotomized or categorized into a score ranging from 0 (deficit absent) to 1 (deficit present) (Supplemental Table 1). Per person, the frailty index score was calculated as the sum of present deficits divided by the total number of potential deficits. For instance, if 10 out of 38 deficits were present, the frailty index would be 10/38. A higher score of the frailty index indicated a worse health state. In order to increase the interpretability of our risk estimates, frailty index score was multiplied by 100.

Additional measurements

The baseline home interview provided extensive information on medical history, tobacco smoking, alcohol consumption, education level, and medication. Smoking habits were categorized as current, past, and never smoking. Education level was classified as low, intermediate, and high.

Statistical analysis

We performed ordinary least-squares linear regression, using restricted cubic splines with three knots to allow for potential nonlinearity. First, we cross-sectionally investigated the association of thyroid function (ie, TSH and FT_4 levels) with the frailty index at baseline. Second, we investigated the association of thyroid function with the frailty index at follow-up. Third, we prospectively investigated the association of thyroid function with changes in the frailty index over time (calculated by subtracting the frailty index at baseline from the frailty index at follow-up). Potential

confounders were selected on the basis of biological confounding plausibility. The first analysis was adjusted for age, sex, cohort, smoking status, alcohol intake, and education level. The second and third analyses were additionally adjusted for the frailty index at baseline and time interval between the measurements of the frailty index. To assess the potential role of thyroid autoimmunity on frailty, we also investigated the cross-sectional and prospective association of TPOAb with the frailty index, additionally adjusting for TSH or FT_4 levels. TSH and TPOAb values were logarithmically transformed, because of their skewed distribution. All models were tested for effect modification by separately adding product interaction terms of TSH, FT_4 or TPOAbs with each covariate of the multivariable model, but none of the interaction terms were significant.

Multiple imputations were performed for covariates with missing data (<5% for all covariates). Statistical analyses were performed using R statistical software (rms package, R project, Institute for Statistics and Mathematics, R Core Team, version 3.2.2) and SPSS version 21 (IBM SPSS).

Sensitivity analyses

We performed several analyses to test the robustness of our findings. (1) We reran the cross-sectional analysis using the original 45-item frailty index instead of the adapted frailty index. (2) We restricted the cross-sectional analysis to participants with both baseline and prospective data on the frailty index. (3) We restricted the cross-sectional and prospective analyses to: (i) participants without past thyroid surgery and not using thyroid medications; (ii) participants with thyroid function within the reference range, without past thyroid surgery and not using thyroid medications. (4) To address the issue of attrition, we used the inverse probability weighting method. We fitted two logistic regression models predicting the possibility of having follow-up data. The first model used as covariates the baseline frailty index, age, sex, cohort, smoking status, alcohol intake, education level, and TSH. The second model used as covariate only TSH. The stabilized weights for each participant were calculated as the predicted probability of the second model divided by that of the first model. Subsequently, we used the stabilized weights to examine the association of TSH with frailty changes over time. The analyses were also repeated for FT_4. (5) In our prospective analysis, we added product interaction terms of thyroid parameters with the frailty index at baseline to test for effect modification by the baseline health status of participants. (6) To explore a potential

influence of the time of blood sampling or thyroid autoimmunity on our results, our cross-sectional and prospective analyses were additionally adjusted for the time of blood withdrawal (recorded in hours and minutes) or TPOAb levels.

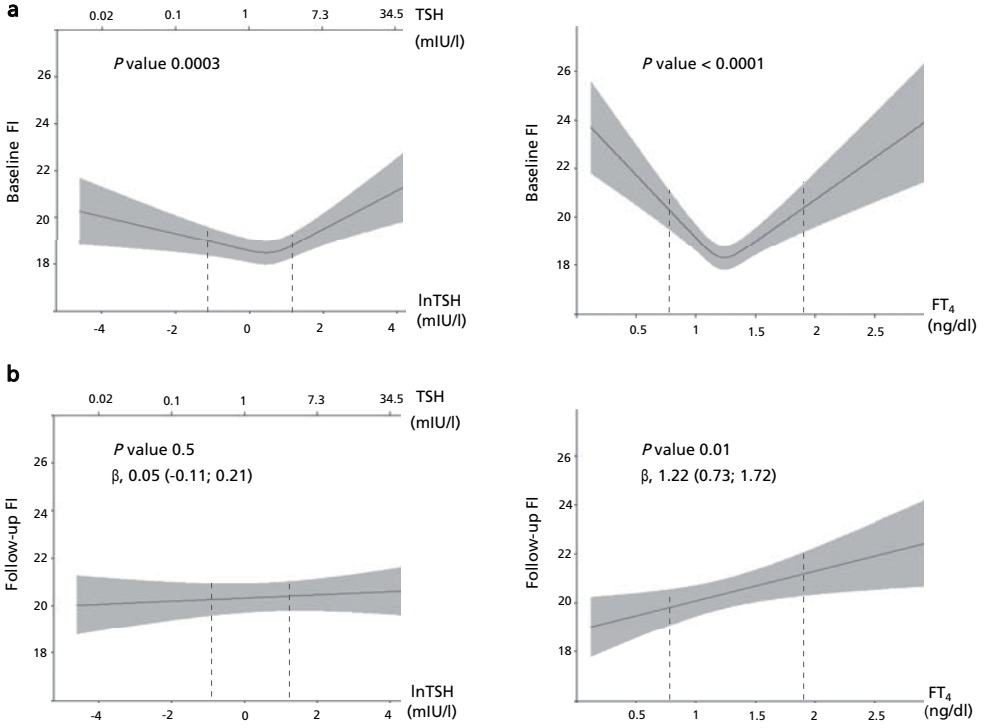
RESULTS

Baseline characteristics of 9640 eligible participants are shown in Table 1. The mean age was 64.9 years and 56.7% were women. The median TSH was 1.9 mIU/L, with an interquartile range of 1.2 to 2.8 mIU/L. Of participants, 3.0% had TSH below, 86.8% within, and 10.2% above the reference range. The mean (standard deviation) FT_4 was 1.2 (0.1) ng/dL. Of participants, 1.2% had FT_4 below, 98.4% within, and 0.4% above the reference range. The mean (standard deviation) frailty index was 17.1 (8.7), with a range of 0 to 66.4 (Table 1). After a median follow-up time of 10.1 (interquartile range, 5.7 to 10.8) years, the frailty index was remeasured in 6416 participants. Of participants, 2364 died before having a follow-up frailty measurement. The remaining 860 participants did not have complete follow-up data available on frailty (Table 1). The median TSH and mean FT_4 concentrations at baseline were very similar among participants who died, those with repeated measurements of frailty, and those without follow-up data available on frailty (Table 1). Participants with prospective data had a lower frailty index than those without prospective data (Table 1).

Cross-sectional analysis: thyroid function and frailty index at baseline

There was a U-shaped association of both TSH (*P* value, 0.0003) and FT_4 levels (*P* value, <0.0001) with the baseline frailty index (Figure 1a). Results remained similar after using the original 45-item frailty index (Supplemental Figure 2a), after excluding participants without prospective data on frailty (Supplemental Figure 2b), after excluding participants with known thyroid disease (Supplemental Figure 2c), and after additionally adjusting for the time of blood withdrawal or TPOAb levels. Among euthyroid participants, there was a U-shaped association of FT_4 with frailty index (*P* value, <0.0001), but no association of TSH with frailty index (*P* value, 0.3) (Supplemental Figure 3a).

Figure 1. Association of thyroid function with the frailty index.



a. Cross-sectional association of thyroid function with frailty index at baseline (TN, 9640); b. Association of thyroid function with the frailty index at the end of the follow-up (TN, 6416). We used linear regression models with restricted cubic splines. Predicted means of frailty index (black lines) with 95% confidence intervals (gray areas) are plotted against TSH and FT_4 concentrations. Dashed lines indicate the limits of TSH or FT_4 reference ranges. A higher value of frailty index represents a worse health state. *P* values are for the plotted association. Abbreviations: TSH, thyroid-stimulating hormone; FT_4, free thyroxine; FI, frailty index, TN, total number.

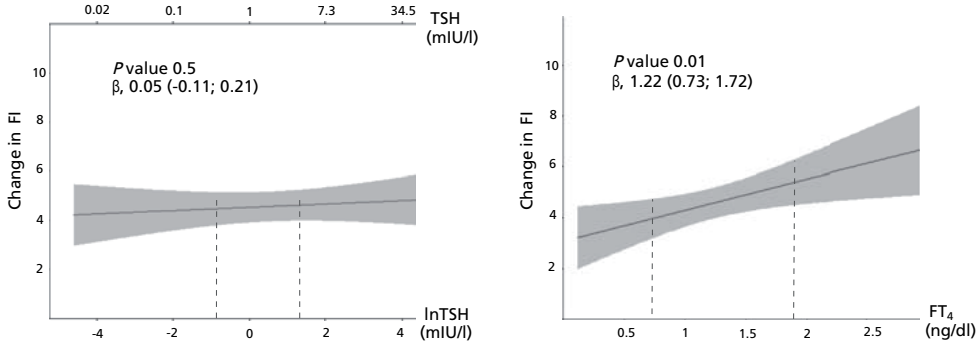
Thyroid function and frailty index at follow-up

TSH was not associated with frailty index at follow-up (β, 0.05; 95% confidence interval [95% CI], -0.11 to 0.21 per 1 unit logTSH) (Figure 1b). Increasing FT_4 levels were associated with a higher frailty index at follow-up (β, 1.22; 95% CI, 0.73 to 1.72 per 1 unit FT_4) (Figure 1b).

Prospective analysis: thyroid function and changes in frailty index

There was no association of TSH (β, 0.05; 95% CI, -0.11 to 0.21 per 1 unit logTSH) and a positive association of FT_4 with frailty changes over time (β, 1.22; 95% CI, 0.73 to 1.72 per 1 unit FT_4; Figure 2). Results remained similar after excluding participants with known thyroid disease (Supplemental Figure 2d), after additionally adjusting

Figure 2. Prospective association of thyroid function with changes in the frailty index.



Total number, 6416. Changes in the frailty index were calculated by subtracting the frailty index at baseline from the frailty index at follow-up. We used linear regression models with restricted cubic splines. Predicted means of frailty index (black lines) with 95% confidence intervals (gray areas) are plotted against TSH and FT_4 concentrations. Dashed lines indicate the limits of TSH or FT_4 reference ranges. A higher value of frailty index represents a worse health state. *P* values are for the plotted association. Abbreviations: TSH, thyroid-stimulating hormone; FT_4, free thyroxine; FI, frailty index.

for the time of blood withdrawal or TPOAb levels. The association became stronger after the inverse probability weighting (β, 0.19; 95% CI, -0.03 to 0.42 per 1 unit logTSH; β, 1.99; 95% CI, 0.97 to 3.0 per 1 unit FT_4). Among euthyroid participants, the association was not statistically significant (β, 0.18; 95% CI, -0.11 to 0.48 per 1 unit logTSH; β, 1.0; 95% CI, -0.17 to 2.18 per 1 unit FT_4) (Supplemental Figure 3b). Also, the interaction terms of TSH and FT_4 with the frailty index at baseline were not statistically significant.

TPOAb and frailty index

In the cross-sectional analysis, TPOAb were not associated with frailty index (β, -0.01; 95% CI,-0.15 to 0.13 per 1 unit logTPOAb; Supplemental Figure 4). In the prospective analysis, there was an inverse U-shaped association of TPOAb with frailty changes over time (*P* value, 0.0002; Supplemental Figure 4). Results remained similar after additionally adjusting for TSH or FT_4.

DISCUSSION

In this large population-based cohort study, participants with low and high thyroid function were more likely to be frail than participants with normal thyroid function.

However, only those with higher FT_4 levels had an increased risk of becoming more frail over time.

Thyroid hormones exert pleiotropic effects on nearly all organs and systems,[9,10,12-14] the resultant of which can be reflected in overall health. However, whereas most previous research has focused on the system-specific effects of thyroid function,[13,14,16,17,23,25-27] our study provides novel insights into the impact of thyroid function on general health. Most importantly, our findings suggest that high circulating FT_4 levels can contribute to health deterioration over time. This can be attributed to the combination of many deleterious system-specific effects of excess thyroid hormones, as arrhythmias, hemodynamic changes, hypercoagulability, neurodegeneration, and reduction in bone mineral density. In line, large prospective population-based studies have reported that subjects with high FT_4 levels have an increased risk of developing a broad range of adverse outcomes, including atrial fibrillation, chronic kidney disease, age-related macular degeneration, dementia, osteoporosis, and fractures.[13,14,16,17,25,26] A more general pathway linking high thyroid function to frailty could be related to the perturbation of the prooxidant-antioxidant balance.[28,29] Excess circulating thyroid hormones stimulate the production of reactive oxygen species via accelerating basal metabolism and increasing oxygen consumption.[30] In turn, reactive oxygen species predispose to altered gene expression, mitochondrial dysfunction, and cumulative cellular damage,[31] which increase the susceptibility to physical, cognitive, and functional decline. Conversely, low thyroid function can reduce the frailty risk via decreasing basal metabolic rate and promoting energy conservation.[32] As shown in experimental research, age-related chronic disorders occur less often in the mutant hypothyroid dwarf mice than in the wild-type mice.[33]

Thyroid autoimmunity could additionally be involved in the development of frailty. To date, the association of thyroid autoimmunity with frailty risk has been investigated in only one population-based study, reporting a low frailty risk in TPOAb-positive women.[34] However, this study was cross-sectional, assessed frailty by the physical phenotype and included only women aged ≥65 years of age (n=641). We addressed some limitations of this study, by exploring the prospective association of TPOAb levels with the risk of frailty assessed by the multidomain phenotype, in a much larger population of >6000 middle-aged and elderly men and women (n=6416). Our results point toward the possibility of protective autoimmunity [35] and

confirm that the association of TPOAbs with frailty risk is independent of thyroid function.

During follow-up, the frailty risk increased with higher FT_4 levels. Based on the negative feedback mechanism of the hypothalamus-pituitary-thyroid axis, one would expect an increased frailty risk with lower TSH levels. However, TSH was not associated with frailty risk in our study. Similarly, many other population-based cohort studies have suggested that in middle-aged and elderly subjects, FT_4 rather than TSH levels can predict various adverse outcomes, including atrial fibrillation, dementia and mortality.[15,27,36] These observations may reflect an alteration in the TSH-FT_4 set point of the negative feedback mechanism, due to the ageing process.[37] After restricting the study population to euthyroid participants, the association of thyroid function with frailty risk attenuated and/or lost statistical significance. This suggests that elevated levels of FT_4 have a larger effect on frailty risk over time as compared to FT_4 levels within the reference range.

Our cross-sectional and prospective analyses examined the relationship of thyroid function with the likelihood of being frail and the risk of becoming more frail over time, respectively. Cross-sectional designs, however, do not provide evidence on the temporal relationship between the exposure and outcome. Therefore, the results of our cross-sectional analysis may be partly influenced by reverse causation. In other words, health-related problems underlying a high frailty index can potentially alter thyroid function parameters. Notably, our participants with low thyroid function had an increased likelihood of being frail, but did not have an increased risk of becoming more frail over time. This can be explained by the condition of non-thyroidal illness syndrome, which is typically characterized by low thyroid hormones and normal TSH levels, secondary to a poor health status.[38]

Alternatively, the discrepancy between our cross-sectional and prospective findings could have been explained by the selective dropout of participants with low thyroid function. This is unlikely, given that the median TSH levels and the mean FT_4 levels among participants with prospective data were similar to those without prospective data. Another important issue is whether the participants of our prospective analysis were representative of the baseline sample population. Indeed, participants with prospective data had a lower baseline frailty index than did those without prospective data, which indicates that the more frail participants at baseline may have died during follow-up. However, we do not expect our conclusions to be compromised by the selective dropout of frail participants for several reasons.

First, we obtained consistent results after restricting our cross-sectional analyses to participants with complete follow-up data on the frailty index. Second, the product interaction term of thyroid function with the frailty index at baseline was not statistically significant, suggesting that our prospective findings were independent of the baseline health status of participants. Third, we addressed the issue of attrition by using the inverse probability weighting method. Originally, the effect of FT_4 on frailty seemed to wane over time, as it was smaller in the prospective than in the cross-sectional analysis. However, the effect of FT_4 on frailty became stronger after the inverse probability weighting, indicating that the selective dropout of participants may have led to an underestimation rather than an overestimation of our prospective results.

To the best of our knowledge, this is the first population-based cohort study that explores the relationship of thyroid function with the frailty index. The latter represents a well-validated frailty measure that is considered useful to evaluate overall health and trajectories of health over time.[3] Our frailty index data were available at two time points with a long follow-up time interval, allowing us to explore the relation between thyroid function variations and health changes over time. The frailty index characteristics of our population were similar to most other populations of similar age.[4,6,39] Moreover, our study is the largest investigation on thyroid function and frailty. The large sample size enabled us to perform multiple sensitivity analyses. Additionally, to our knowledge, our study is the first to examine the prospective association of TPOAb levels with frailty risk. Other strengths include the well-characterized population-based study sample, the laboratory assessment of thyroid parameters and the available data on potential confounding factors.

Several limitations should also be mentioned. Considering the observational character of our study, one can argue that reverse causation may have affected even our prospective findings. This is very unlikely, given that non-thyroidal illness syndrome is typically characterized by low thyroid hormones;[38] whereas we found an increased frailty risk among participants with high rather than low FT_4 levels. Moreover, we did not have repeated measurements of thyroid function. This, however, would tend to underestimate the association between thyroid function and frailty risk, based on the low intra-individual variability of TSH and FT_4 levels.[40] Also, we did not measure serum triiodothyronine levels. Nevertheless, TSH and FT_4 are considered the most relevant measurements of thyroid function in clinical practice. In certain circumstances (eg, pregnancy or critical illnesses), substances interfering

with the FT_4 immunoassay can alter the affinity of thyroid hormones to plasma proteins. In our study, there were no data available on thyroid hormone-binding proteins. However, the concentrations of these proteins were most likely unaltered, given that our population consists of community-dwelling middle-aged and elderly individuals. Moreover, the possibility of residual confounding cannot be ruled out, even though we adjusted for various potential confounders. Lastly, our findings require confirmation in other ethnicities, given that the Rotterdam Study includes predominantly white participants.

Conclusions

In this large population-based cohort study, participants with low and high thyroid function are more likely to be frail than are participants with normal thyroid function. However, only those with higher FT_4 levels have an increased risk of becoming more frail over time. Our study provides novel insights into the possible impact of thyroid function on overall health, suggesting that elevated circulating FT_4 levels can constitute a useful marker of health deterioration. Therefore, our findings may have future implications regarding the prediction and prevention of frailty. Further studies are warranted to replicate our results in other population settings.

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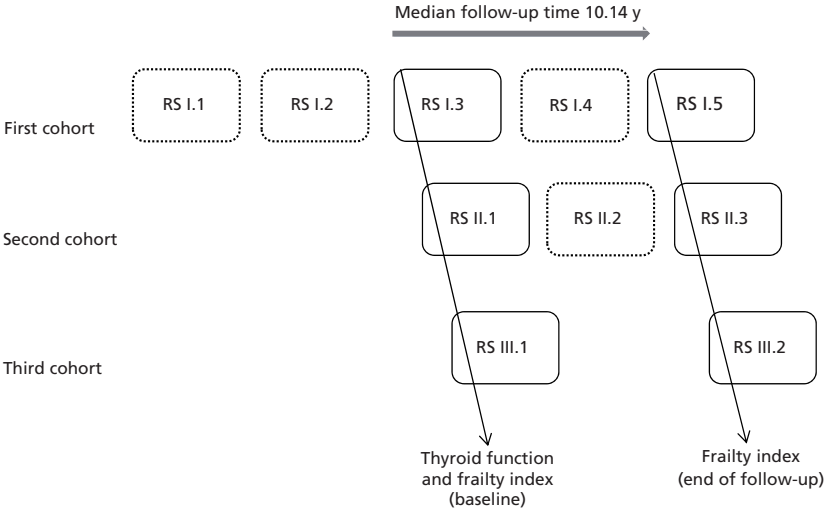
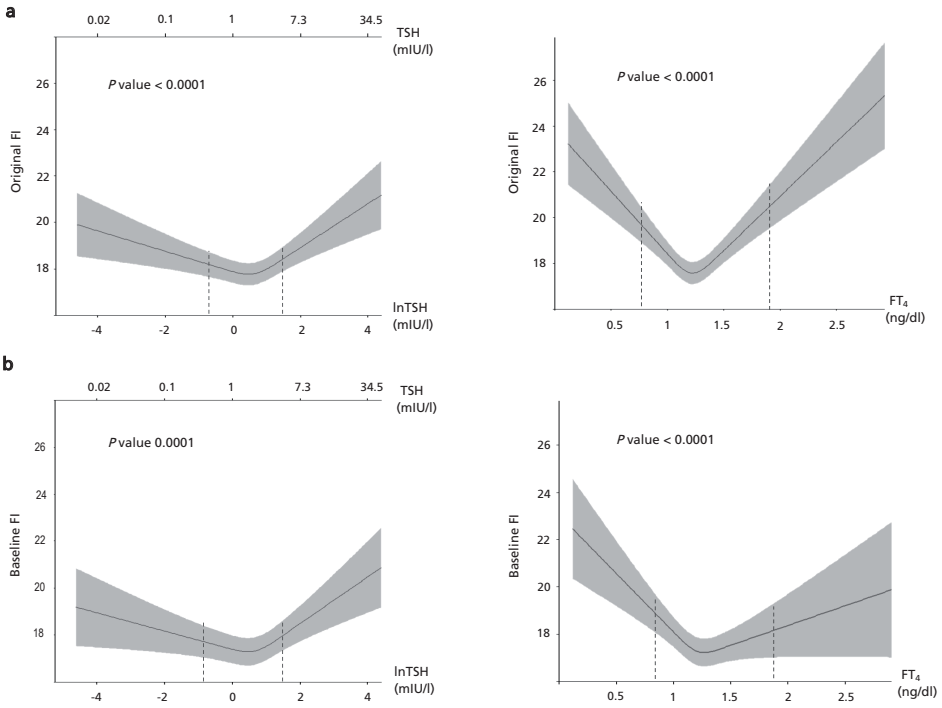
SUPPLEMENTAL MATERIAL

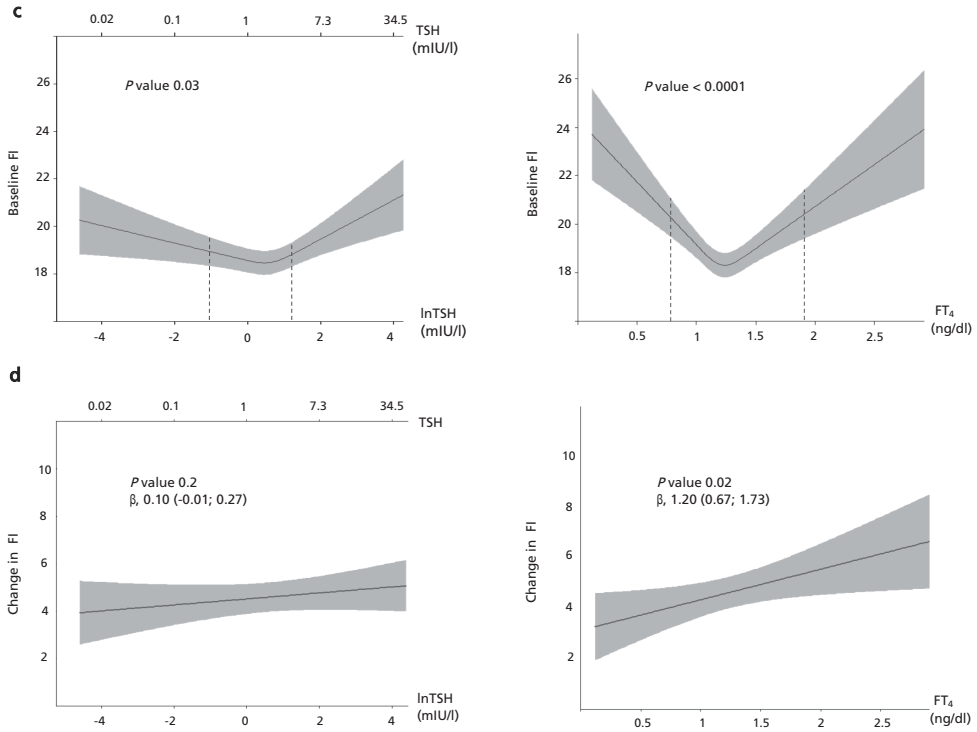
Supplemental Figure 1. Measurements of thyroid function and frailty index.



Thyroid function and frailty index were measured at baseline, during the first visit of the third cohort (RS I.3), the first visits of the second and third cohorts (RS II.1, RS III.1). Frailty index was remeasured at the end of the follow-up, during the fifth visit of the first cohort (RS I.5), the third visit of the second cohort (RS II.3) and the second visit of the third cohort (RS III.2). Abbreviation: RS, Rotterdam Study.

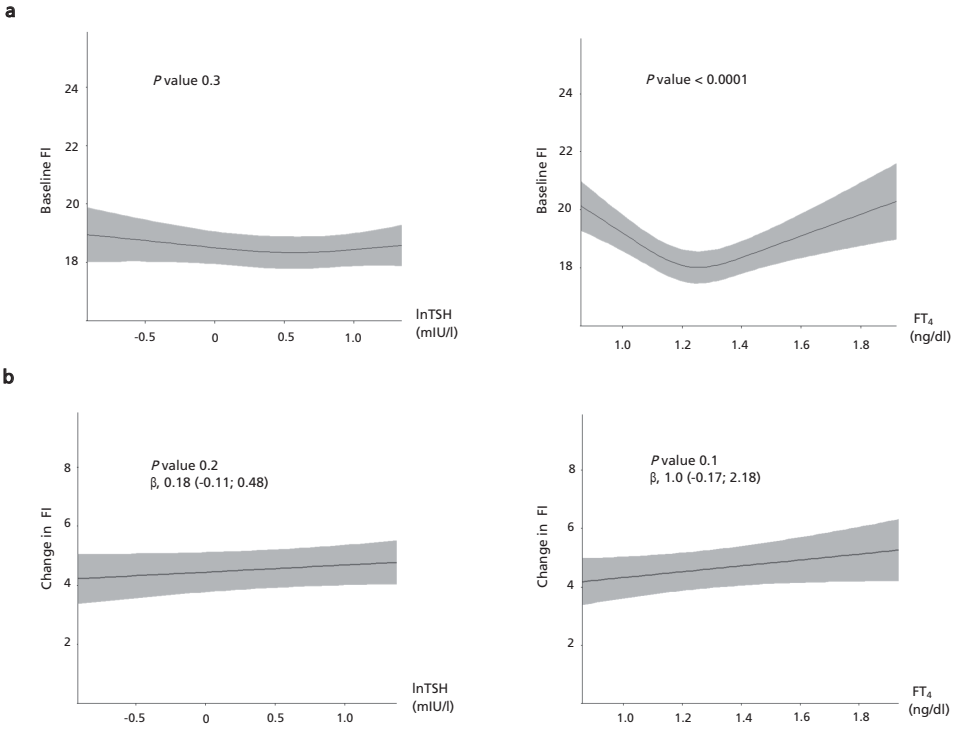
Supplemental Figure 2. Sensitivity analyses.





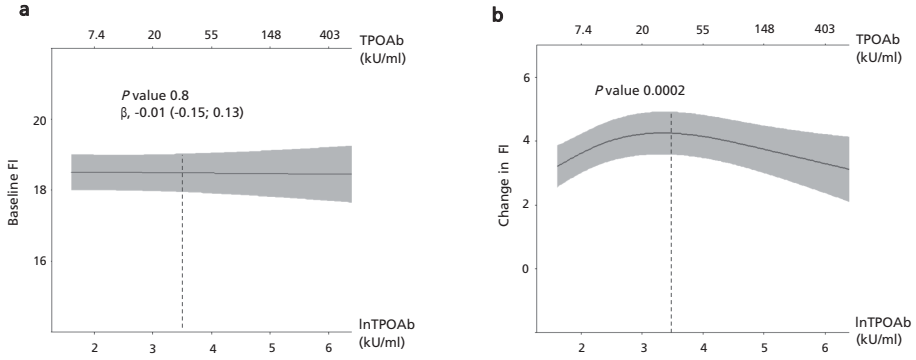
a. Cross-sectional association of thyroid function with original frailty index score (TN, 9640); b. Cross-sectional association of thyroid function with frailty index, restricted to participants with follow-up data on frailty index (TN, 6416); c. Cross-sectional association of thyroid function with frailty index at baseline, after excluding participants with past thyroid surgery and users of thyroid medication (TN, 9199); d. Prospective association of thyroid function with changes in frailty index, after excluding participants with past thyroid surgery and users of thyroid medication (TN, 6126). The changes in frailty index were calculated by subtracting the frailty index at baseline from frailty index at follow-up. We used linear regression models with restricted cubic splines. Predicted means of frailty index (black lines) with 95% confidence intervals (gray areas) are plotted against TSH and FT_4 concentrations. Dashed lines indicate the limits of TSH or FT_4 reference ranges. A higher value of frailty index represents a worse health state. *P* values are for the plotted association. Abbreviations: TSH, thyroid-stimulating hormone; FT_4, free thyroxine; FI, frailty index; TN, total number.

Supplemental Figure 3. Association of thyroid function with frailty index among euthyroid participants.



a. Cross-sectional association of thyroid function with frailty index at baseline (TN, 8038); b. Prospective association of thyroid function with changes in frailty index (TN, 5403). The changes in frailty index were calculated by subtracting the frailty index at baseline from frailty index at follow-up. We used linear regression models with restricted cubic splines. Predicted means of frailty index (black lines) with 95% confidence intervals (gray areas) are plotted against TSH and FT_4 concentrations. A higher value of frailty index represents a worse health state. *P* values are for the plotted association. Abbreviations: TSH, thyroid-stimulating hormone; FT_4, free thyroxine; FI, frailty index; TN, total number.

Supplemental Figure 4. Association of TPOAb with frailty index.



a. Cross-sectional association of TPOAb with frailty index at baseline (TN, 9640); b. Prospective association of TPOAb with changes in frailty index (TN, 6416). The changes in frailty index were calculated by subtracting the frailty index at baseline from frailty index at follow-up. We used linear regression models with restricted cubic splines. Predicted means of frailty index (black lines) with 95% confidence intervals (gray areas) are plotted against TPOAb concentrations. A higher value of frailty index represents a worse health state. Dashed lines indicate the cutoff of TPOAb positivity (35 kU/ml). *P* values are for the plotted association. Abbreviations: TPOAb, thyroid peroxidase antibodies; FI, frailty index; TN, total number.

Supplemental Table 1. Items of the frailty index score

	Items	Additional information on the items	Cutoff values
1	Dressing and grooming [a]	Able to get the clothes from closets or drawers; able to dress; able to shampoo the hair; able to comb the hair or do the make up	without any difficulty = 0; with some difficulty = 0.33; with much difficulty = 0.66; unable to do = 1
2	Arising [a]	Able to stand up from a straight chair without using the arms for support; able to get in and out of bed	without any difficulty = 0; with some difficulty = 0.33; with much difficulty = 0.66; unable to do = 1
3	Eating [a]	Able to cut meat; able to lift a full cup or glass to the mouth; able to open a new carton of milk	without any difficulty = 0; with some difficulty = 0.33; with much difficulty = 0.66; unable to do = 1
4	Walking [a]	Able to walk outdoors on flat ground; able to climb up five steps	without any difficulty = 0; with some difficulty = 0.33; with much difficulty = 0.66; unable to do = 1
5	Hygiene [a]	Able to wash and dry the entire body; able to take a shower or bath	without any difficulty = 0; with some difficulty = 0.33; with much difficulty = 0.66; unable to do = 1
6	Reach [a]	Able to reach and get down a 1 kg object from just above the head; able to bend down to pick up clothing from the floor	without any difficulty = 0; with some difficulty = 0.33; with much difficulty = 0.66; unable to do = 1
7	Grip [a]	Able to open a car door; able to open jars which have been previously opened	without any difficulty = 0; with some difficulty = 0.33; with much difficulty = 0.66; unable to do = 1
8	Riding a bike [b]	Able to ride a bike	without any difficulty = 0; with some difficulty = 0.33; with much difficulty = 0.66; unable to do = 1
9	Telephone [b]	Able to use the telephone	without any difficulty = 0; with some difficulty or using a customized phone = 0.33; with much difficulty = 0.66; unable to do = 1
10	Meal [b]	Able to prepare meals	without any difficulty = 0; with some difficulty = 0.33; with much difficulty = 0.66; unable to do = 1
11	Gardening [b]	Able to maintain a garden	without any difficulty = 0; with some difficulty = 0.33; with much difficulty = 0.66; unable to do = 1
12	Landry [b]	Able to do the laundry	without any difficulty = 0; with some difficulty = 0.33; with much difficulty = 0.66; unable to do = 1
13	Financial [b]	Able to do finances	without any difficulty = 0; with some difficulty = 0.33; with much difficulty = 0.66; unable to do = 1
14	Depressed affect [c]	I felt that I could not shake off the blues even with help from family or friends; I felt depressed; I thought my life had been a failure; I felt lonely; I had crying spells; I felt sad	rarely or none of the time = 0; some or a little of the time = 0.33; occasionally or a moderate amount of time = 0.66; most or all of the time = 1

Supplemental Table 1. Items of the frailty index score (continued)

	Items	Additional information on the items	Cutoff values
15	Positive affect [c]	I felt that I was just as good as other people; I felt hopeful about the future; I was happy; I enjoyed life	rarely or none of the time = 1; some or a little of the time = 0.66; occasionally or a moderate amount of time = 0.33; most or all of the time = 0
16	Somatic and retarded activity [c]	I did not feel like eating; my appetite was poor; I had trouble keeping my mind on what I was doing; I felt that everything I did was an effort; I felt fearful; my sleep was restless; I talked less than usual; I could not get going	rarely or none of the time = 0; some or a little of the time = 0.33; occasionally or a moderate amount of time = 0.66; most or all of the time = 1
17	Interpersonal [c]	I was bothered by things that usually don't bother me; people were unfriendly; I felt that people dislike me	rarely or none of the time = 0; some or a little of the time = 0.33; occasionally or a moderate amount of time = 0.66; most or all of the time = 1
18	Falling	How often did you fall over the past 12 months?	no falling = 0; less than once a month = 0.5; more than once a month = 1
19	Joint complains	Did you have joint pain or other complaints from the knees, hips, back or hands?	no = 0; yes = 1
20	Forgetfulness	Do you sometimes forget what you were about to do?	no = 0; yes = 1
21	Aphasia	Do you have difficulties finding the right words?	no = 0; yes = 1
22	Liver enzymes [d]	Aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transferase	all values within the range = 0; abnormal values = 1
23	Creatinine [d]		Males: 65-115 µmol/L = 0; other values = 1 Females: 55-90 µmol/L = 0; other values = 1
24	Hyperlipidemia [d]		statin use and/or cholesterol levels >6.5 mmol/L; no statin use and cholesterol levels 2.9-6.5 mmol/L
25	High density lipoprotein [d]		values ≥1.55 = 0; values <1.55 = 1
26	Systolic blood pressure [d]	Measured three times, average was taken	values 90-140 = 0; values 140-160 = 0.5; values <90 = 0.5; values >160 = 1
27	Mini Mental State Examination		unimpaired >25 = 0; impaired ≤25 =1
28	Letter-Digit Substitution Test	The number of correct digits	above the mean or less than 1 sd below the mean = 0; 1 sd below the mean = 0.5; 2 sd below the mean = 1
29	Stroop test		above the mean or less than 1 sd above the mean = 0; 1 sd above the mean = 0.5; 2 sd above the mean = 1
30	Word Fluency test		above the mean or less than 1 sd below the mean = 0; 1 sd below the mean = 0.5; 2 sd below the mean = 1
31	Cancer		no = 0; yes = 1
32	Lung condition (COPD/Asthma)		no = 0; yes = 1

Supplemental Table 1. Items of the frailty index score (continued)

	Items	Additional information on the items	Cutoff values
33	Coronary heart disease	Prevalent coronary heart disease	no = 0; yes = 1
34	Stroke	Prevalent stroke	no = 0; yes = 1
35	Diabetes mellitus		no = 0; high glucose levels= 0.5; yes = 1
36	BMI	BMI < 18.5 = underweight BMI ≤25 and ≥18.5 = normal weight BMI <25 and ≤30 = overweight BMI <30 = obese	normal weight = 0; overweight = 0.5; obese or underweight = 1
37	Hospital admission	Last 12 months	no = 0; yes = 1
38	Age-related macular degeneration	Fundus photography after pharmacologic mydriasis. The eyes of each participant were graded and classified separately. The eye with the more severe grade was used to classify the person.	0 = 5-year risk of developing advanced age-related macular degeneration in at least one eye is 0.5%; 0.25 = 5-year risk is 3%; 0.50 = 5-year risk is 12% ; 0.75 = 5-year risk is 25%; 1= 5-year risk is 50%

The original frailty index score, which was designed and validated among 11539 participants of the RS, consisted of 45 variables. However, 7 items from the original RS frailty index (namely vitamin D, sex hormone binding globulin, mobility, uric acid, proBNP, C-reactive protein and homocysteine) were not assessed at follow-up. In order to assess frailty changes over time, these items were removed, resulting in an adapted RS frailty index that consisted of the remaining 38 health-related variables. Sources: [a] Stanford Health Assessment Questionnaire; [b] Lawton Instrumental Activities of Daily Living scale; [c] The CESD scale: a self-report depression scale; [d] Serum blood measurement; cutoff values derived from the Laboratory guide Erasmus Medical Center Rotterdam. Abbreviations: sd, standard deviation; RS, Rotterdam Study; BMI, body mass index.

CHAPTER 3.2

IDENTIFICATION OF GAIT ASPECTS RELATED TO THYROID FUNCTION

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ABSTRACT

Background Gait is an important health indicator and poor gait is strongly associated with disability and risk of falls. Thyroid dysfunction is suggested as a potential determinant of gait deterioration, but this has not been explored in a population-based study.

Methods We therefore investigated the association of thyroid function with gait patterns in 2645 participants from the Rotterdam Study with data available on TSH (thyroid-stimulating hormone), FT_4 (free thyroxine), and gait, without known thyroid disease or dementia. The primary outcome was global gait (standardized Z score), while secondary outcomes included gait domains (rhythm, variability, phases, pace, base of support, tandem, turning), and velocity. Gait was assessed by electronic walkway.

Results Multivariable regression models revealed an inverted U-shaped association of TSH (*P* value, <0.001), but no association of FT_4 concentrations with global gait (*P* value, 0.2). TSH levels were positively associated with base of support (*P* value, 0.01) and followed an inverted U-shaped curve with tandem (*P* value, 0.002) and velocity (*P* value, 0.02). Clinical and subclinical hypothyroidism were associated with worse global gait than euthyroidism (β, -0.61; 95% confidence interval, -1.03 to -0.18; and β, -0.13; 95% confidence interval, -0.26 to -0.00; respectively). In euthyroid participants, higher thyroid function was associated with worse gait patterns.

Conclusions Both low and high thyroid function are associated with alterations in global gait, tandem, base of support, and velocity.

INTRODUCTION

Gait is an important marker of general health. Disturbances in gait gradually increase with advancing age and affect approximately one third of community-dwelling individuals older than 60 years.[1] Gait impairment has a substantial impact on quality of life and is strongly associated with increased risk of falls, which can in turn cause soft-tissue injuries, fractures, and death.[2,3] Quantitative gait assessment comprises many parameters that can be summarized into seven independent domains, namely rhythm, variability, phases, pace, base of support, tandem, and turning (Figure 1).[4,5] These gait domains reflect distinct functional abilities and their investigation is crucial to identify novel modifiable contributors to gait deterioration.[5]

Thyroid hormones regulate metabolism in most tissues, including neurological and musculoskeletal systems, whose integrated functioning is reflected in gait.[6-8] As gait disturbances, thyroid dysfunction increases in prevalence with advancing age. However, the clinical symptoms of thyroid dysfunction become less pronounced among older adults[9] and this may result in a diagnostic delay and increased risk of systemic complications. Research to date has suggested a possible role of thyroid

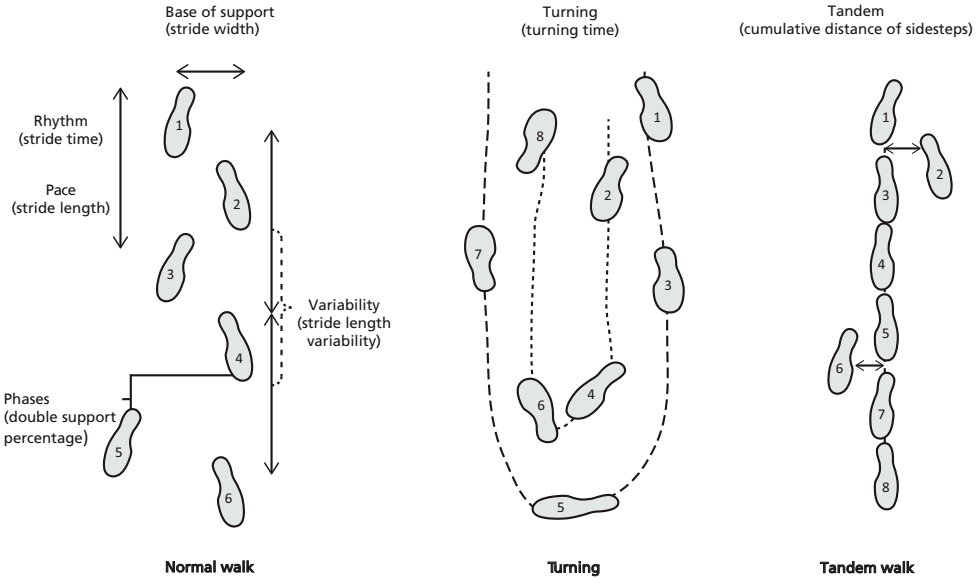


Figure 1. Walking conditions.
The three walking conditions, including five gait domains for normal walk (rhythm, variability, phases, pace, base of support), one for turn (turning) and one for tandem walk (tandem).

dysfunction in gait impairment. Adult mice lacking the thyroid-hormone activating enzyme type 2 deiodinase have shown progressive gait impairment in the late stages of life.[10] In humans, several case series[11,12] and case reports[13-15] have shown a restoration of gait disturbances after treatment of thyroid disease.

Thyroid function in the general population has been linked to gait velocity, which constitutes only one of the parameters in the pace domain.[16,17] However, the link of thyroid function with gait and its spatiotemporal aspects remains unexplored. Therefore, we aimed to investigate the association of thyroid function with global gait and its separate domains, in a large population-based cohort of middle-aged and elderly subjects.

METHODS

Study population

The Rotterdam Study is an ongoing prospective population-based cohort study that investigates chronic diseases in the middle-aged and elderly. The objectives and study design of the Rotterdam Study have been described in detail elsewhere.[18] Rotterdam Study was initiated in 1990, including 7983 participants aged 55 years or older (RS I). In 2000, the cohort was expanded with 3011 participants aged 55 or older (RS II). In 2006, a third cohort of 3932 participants aged 45 years and over was added (RS III). As of now, the Rotterdam Study comprises a total of 14926 participants, who undergo extensive follow-up medical examinations every 3 to 5 years. From 2009 onwards, quantitative gait assessment was included in the study protocol. Between March 2009 and March 2012, 3651 participants of the Rotterdam Study were invited for gait assessment. An overview on the selection of study participants can be found in the flowchart (Supplemental Figure 1).

A total of 2857 subjects had complete information on thyroid function and gait. Of these, we excluded 212 subjects with at least one out of several conditions: (1) dementia diagnosis (n=14); (2) thyroid medication use (n=79); (3) history of thyroid disease (n=192) and (4) previous thyroid surgery (n=33) (Supplemental Figure 1). The remaining 2645 eligible participants were enrolled in the study.

The Medical Ethics Committee of the Erasmus University and the Ministry of Health, Welfare and Sport of the Netherlands have approved the study protocols, implementing the "Wet Bevolkingsonderzoek: ERGO (Population Studies Act: Rotterdam Study)". The methods were performed in accordance with the approved

guidelines. All included participants provided written informed consent in accordance with the Declaration of Helsinki.

Assessment of thyroid function

Thyroid function tests were performed during the third visit of the first cohort (RS I.3), the first visit of the second cohort (RS II.1) and the first visit of the third cohort (RS III.1) using the same method and assay. Concentrations of thyroid-stimulating hormone (TSH), free thyroxine (FT_4) and thyroid peroxidase antibodies (TPOAb) were measured on baseline serum samples stored at -80°C using the electrochemiluminescence immunoassay, ECLIA, Roche. We determined the reference range of serum TSH as 0.40–4.0 mIU/L and serum FT_4 as 11–25 pmol/L (alternatively 0.86–1.94 ng/dL), according to national guidelines and our previous studies.[19,20] Euthyroidism was defined as serum TSH within the reference range. Subclinical hypothyroidism was defined as serum TSH >4.0 mIU/L and FT_4 levels within the reference range. Overt hypothyroidism was defined as serum TSH >4.0 mIU/L and FT_4 levels <11 pmol/L. Subclinical hyperthyroidism was defined as serum TSH <0.40 mIU/L and FT_4 levels within the reference range. Overt hyperthyroidism was defined as serum TSH <0.40 mIU/L and FT_4 levels >25 pmol/L. TPOAb positivity was defined as TPOAb levels above the cutoff of 35 kU/ml, in accordance with the recommendations of the assay manufacturer.[19,20]

Assessment of gait

Quantitative gait assessment was performed during the fifth visit of the first cohort (RS I.5), the third visit of the second cohort (RS II.3) and the first visit of the third cohort (RS III.1). Gait was evaluated using a 5.79 m long walkway (GAITRite Platinum; CIR systems, Sparta, NJ: 4.88-m active area; 120 Hz sampling rate). The reliability and validity of this device have been previously established.[4,21-23] The standardized gait protocol comprises three walking conditions: normal walk, turning, and tandem walk (Figure 1). In the normal walk, participants walked at their usual pace across the walkway. This walk was repeated eight times, of which the first recording was considered a practice walk and excluded from the analyses. In turning, participants walked at their usual pace, turned halfway, and returned to the starting position. In the tandem walk, participants walked heel-to-toe on a line across the walkway. Based on the recorded footfalls, the walkway software calculated thirty gait parameters, including twenty five from the normal walk, two from turning,

and three from the tandem walk. Subsequently, principal component analysis (PCA) was performed to avoid multiple testing and collinearity across the variables. While capturing the largest amount of variance, PCA summarizes gait parameters into seven independent gait domains: rhythm, variability, phases, pace, base of support, tandem, and turning.[5] Rhythm reflects cadence and stride time; variability reflects variations in length and time among strides; phases reflects double support time and double support as a percentage of the gait cycle; pace reflects stride length and gait velocity; base of support reflects stride width and stride width variability; tandem reflects errors in tandem walking; turning reflects turning time and the number of turn steps.[5] When necessary, gait domains were inverted so that lower values represent "worse" gait. Global gait was calculated by averaging gait domains into a standardized Z score.[5] Gait velocity was additionally included in our analysis in order to compare our findings with previous studies investigating the association between thyroid function and gait velocity.[16,17]

Additional measurements

The baseline home interview provided information on medical history, tobacco smoking, alcohol consumption, education level, medication, knee and hip pain or stiffness. Participants were categorized based on their smoking status (current, past and never smokers) and education level (low, intermediate and high). Height and weight were measured during the examinations at the research center. Stroke cases were reviewed and verified by an experienced vascular neurologist using hospital letters, information from practitioners and nursing home physicians. Depressive disorders were evaluated based on the Centre for Epidemiological Studies Depression Scale (CESD) questionnaire. A score above 16 was considered indicative of a depressive disorder.[24] Cerebellar cortical volume and intracranial volume were examined by standardized magnetic resonance imaging (MRI) scanning of the brain.[18]

Statistical analysis

We investigated the association of thyroid parameters (TSH, FT_4, and TPOAb positivity) with global gait and spatiotemporal gait components, by performing ordinary least-squares linear regression. The primary outcome was global gait, while secondary outcomes included gait domains (ie, rhythm, variability, phases, pace, base of support, tandem, and turning), and gait velocity. We fitted restricted cubic splines to allow for potential nonlinearity. Moreover, we evaluated global gait and gait ve-

locity throughout thyroid function categories, with euthyroid subjects as reference group. Next, we examined the association of thyroid function with gait in euthyroid participants. In addition, we performed a sensitivity analysis excluding participants with prevalent stroke (n=66) and Parkinson's disease (n=3).

All analyses were adjusted for potential confounding by age, sex, cohort, smoking status, alcohol intake (Model 1). As thyroid function measurement preceded the gait assessment, we also adjusted for the time interval between measurements. In Model 2, we additionally adjusted for covariates that could be either confounders or mediators, including education level, height, weight, knee pain or stiffness, hip pain or stiffness, prevalent stroke, CESD depression score, cerebellar cortical volume, intracranial volume, TPOAb concentrations. Step count and mean step size can affect the score of tandem walk. Therefore, all models including tandem walk were further adjusted for step count and mean step size.

TSH values were logarithmically transformed, because of its skewed distribution. The assumption of normally distributed residuals was checked and met. All models were tested for effect modification by separately adding product interaction terms of the exposure (TSH or FT_4 or TPOAb) with covariates of the multivariable model, but none of the interaction terms were significant. Multiple imputations were performed for covariates with missing data (less than 4.6% for all covariates). A *P* value (two-tailed) <0.05 was considered statistically significant. Statistical analyses were conducted using R statistical software (rms package, R project, Institute for Statistics and Mathematics, R Core Team, version 3.2.2) and IBM SPSS version 21 (IBM Corp).

RESULTS

We included a total of 2645 eligible participants with data available on thyroid function and gait, without known thyroid disease or dementia (Supplemental Figure 1). The baseline characteristics of the study population are shown in Table 1. The mean age was 59.6 years and 52.6% were females (Table 1).

Thyroid function and global gait

Our results did not change after primary and additional adjustments for potential confounders; therefore, we further report only the most adjusted model (Model 2). TSH concentrations within the full range followed an inverted U-shaped curve with global gait (*P* value, <0.001; Figure 2a). However, there was no association of

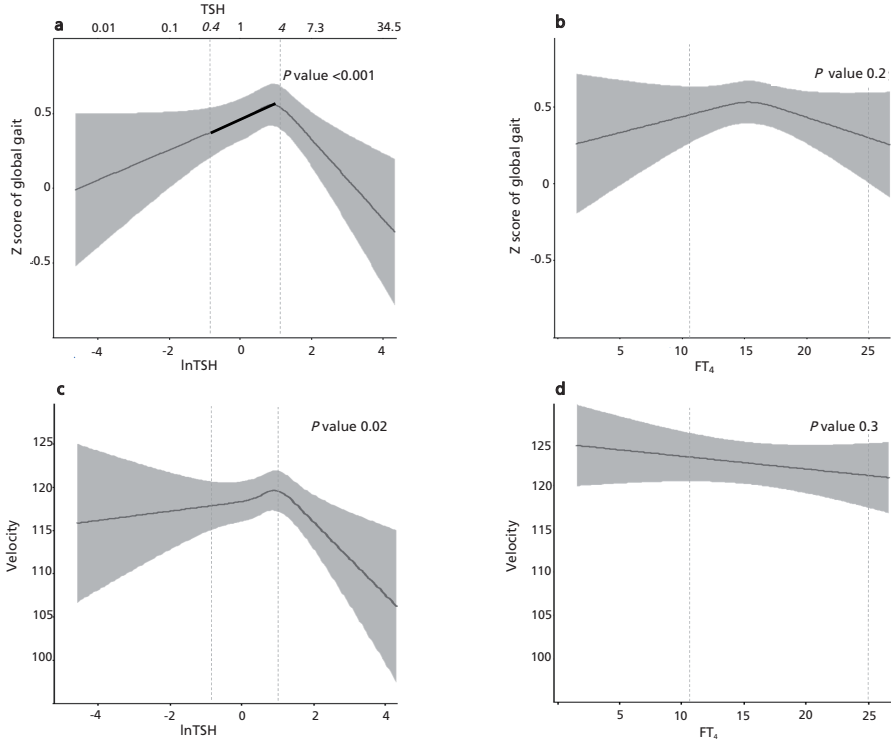
Table 1. Baseline characteristics of 2645 participants*

Age, years	59.6 (6.6)
Women, n (%)	1392 (52.6)
Smoking, n (%)	
current	561 (21.2)
past	1242 (47.0)
never	842 (31.8)
Alcohol intake >14 drinks/week, n (%)	565 (21.4)
Education level, n (%)	
low	195 (7.4)
intermediate	1821 (68.8)
high	629 (23.7)
Height, cm	170.0 (9.2)
Weight, kg	78.4 (14.1)
Knee pain or stiffness, n (%)	693 (26.2)
Hip pain or stiffness, n (%)	401 (15.2)
Past stroke, n (%)	66 (2.5)
CESD depressive symptoms, n (%)	298 (11.3)
Cerebellar cortical volume, ml	99.3 (10.6)
Intracranial volume, ml	1479.6 (159.6)
TSH, mIU/L, median (IQR)	1.9 (1.3-2.8)
FT_4, pmol/L	15.5 (2.1)
TPOAb positive, n (%)	312 (11.8)

*Data are presented as mean (standard deviation), unless otherwise specified. Abbreviations: CESD, Centre for Epidemiological Studies Depression Scale; TSH, thyroid-stimulating hormone; IQR, interquartile range; FT_4, free thyroxine; TPOAb, thyroid peroxidase antibodies (cutoff 35 kU/ml).

FT_4 concentrations with global gait (*P* value, 0.2; Figure 2b). When we restricted the analysis to euthyroid participants, higher TSH concentrations were associated with a better global gait (β, 0.08; 95% confidence interval [95% CI], 0.02 to 0.13 per 1 unit logTSH). Moreover, there was a borderline statistically significant association between FT_4 levels within the normal range and global gait (β, -0.05; 95% CI, -0.10 to 0.00 per 1 pmol/L FT_4; Figure 2 and Supplemental Table 1). Clinical and subclinical hypothyroidism were associated with a worse global gait than euthyroidism (β, -0.61; 95% CI, -1.03 to -0.18 and β, -0.13; CI, -0.26 to -0.00, respectively; Figure 3a). No association was observed between TPOAb and global gait in the main analysis or after restricting to euthyroid participants (Supplemental Table 2). Results remained similar after excluding participants with prevalent stroke and Parkinson's disease (Supplemental Figure 2).

Figure 2. Association of thyroid function with global gait and velocity.

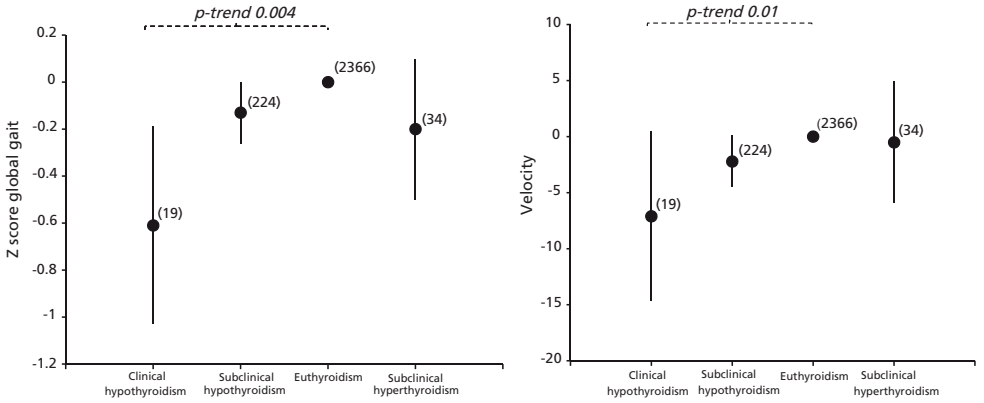


Adjusted for age, sex, cohort, smoking, alcohol intake, education level, height, weight, time interval between thyroid function measurement and gait assessment, knee pain or stiffness, hip pain or stiffness, prevalent stroke, CESD depression score, cerebellar cortical volume, intracranial volume, and thyroid peroxidase antibodies. We utilized linear regression models with restricted cubic splines. Predicted means of Z score global gait and velocity (black lines) with 95% CI (gray areas) are plotted against TSH/FT_4 concentrations. Dashed lines indicate the limits of TSH or FT_4 reference ranges. A higher value of global gait represents better gait.

Thyroid function, gait domains and gait velocity

TSH levels were positively linearly associated with base of support (*P* value, 0.01; Figure 4e) and followed an inverted U-shaped curve with respect to tandem (*P* value, 0.002; Figure 4f) and gait velocity (*P* value, 0.02; Figure 2c). In euthyroid participants, higher TSH levels were associated with higher base of support (β, 0.07; 95% CI, 0.01 to 0.14) and tandem (β, 0.06; 95% CI, 0.01 to 0.12), whereas higher FT_4 levels were associated with lower gait velocity (β, -0.96; 95% CI, -1.85 to -0.07; Supplemental Table 1). Clinical and subclinical hypothyroidism were associated with lower gait velocity than euthyroidism, with borderline statistical significance (β, -7.11; 95% CI, -14.69 to 0.49 and β, -2.22; 95% CI,- 4.50 to 0.05, respectively). Gait velocity decreased gradually from euthyroidism to clinical hypothyroidism (*P* for trend, 0.01) (Figure 3b).

Figure 3. Association of thyroid status categories with global gait and velocity.



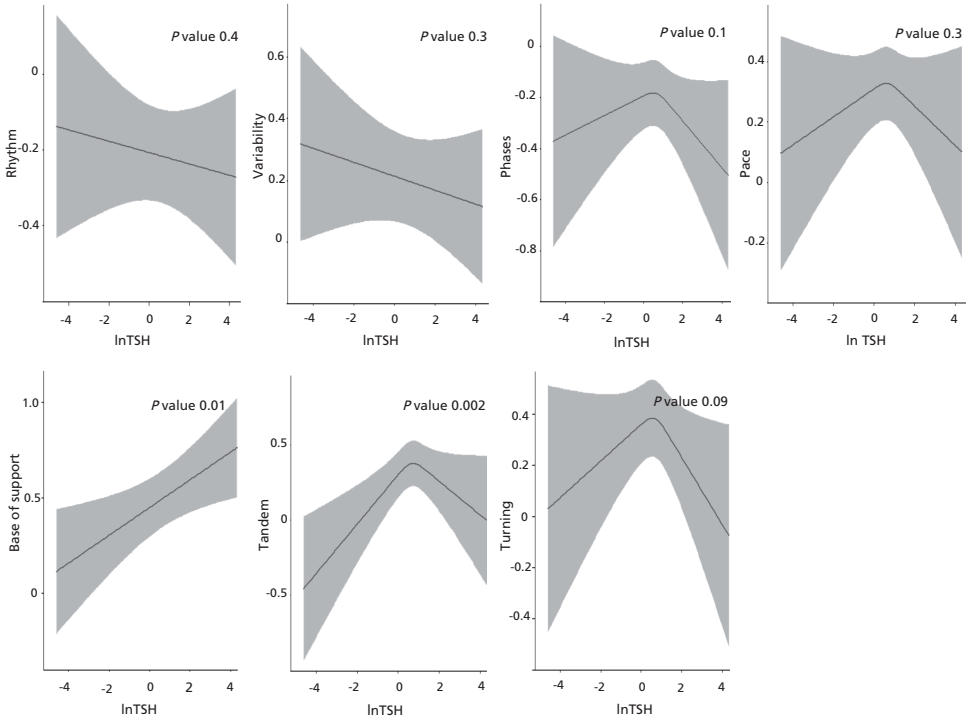
Adjusted for age, sex, cohort, smoking, alcohol intake, education level, height, weight, time interval between thyroid function measurement and gait assessment, knee pain or stiffness, hip pain or stiffness, prevalent stroke, CESD depression score, cerebellar cortical volume, intracranial volume, and thyroid peroxidase antibodies. Differences in Z score of global gait and velocity are plotted against thyroid status categories, with euthyroid subjects as reference. Euthyroidism was defined as TSH (thyroid-stimulating hormone) within reference range (0.4 to 4.0 mIU/l); clinical hypothyroidism as TSH >4.0 mU/L and FT_4 (free thyroxine) <11 pmol/L; subclinical hypothyroidism as TSH >4.0 mU/L and FT_4 11 to 25 pmol/L; clinical hyperthyroidism as TSH <0.4 mU/L and FT_4 >25 pmol/L; subclinical hyperthyroidism as TSH < 0.4 mU/L and FT_4 11 to 25 pmol/L. None of the participants had clinical hyperthyroidism. Error bars represent the 95% confidence intervals around the standardized β (black dots). Within brackets: Total number. A higher value of global gait represents better gait.

DISCUSSION

In a large cohort of middle-aged and elderly subjects, we reported an inverted U-shaped association between TSH concentrations and global gait, indicating that both low and high thyroid function are associated with worse gait. TSH levels were positively associated with base of support and followed an inverted U-shaped curve with tandem and gait velocity. In euthyroid subjects, higher thyroid function was associated with worse gait patterns.

The association between thyroid function and gait could be explained by different pathophysiological mechanisms, particularly involving the neurological and musculoskeletal systems. Low and high thyroid function may increase the risk of stroke via unfavorable cardiovascular risk profile and atrial fibrillation, respectively.[25,26] Low thyroid function can additionally induce immune-mediated cerebellar degeneration.[14] Furthermore, low and high thyroid function can lead to a dysregulation of the neurotransmission systems and subsequent depressive symptoms.[27] Low and high thyroid function may also contribute to myopathy and fractures, by affecting muscle mass and bone mineral density.[28,29] In turn, stroke, cerebellar degeneration,

Figure 4. Association of TSH with the seven gait domains.



Adjusted for age, sex, cohort, smoking, alcohol intake, education level, height, weight, time interval between thyroid function measurement and gait assessment, knee pain or stiffness, hip pain or stiffness, prevalent stroke, CESD depression score, cerebellar cortical volume, intracranial volume, and thyroid peroxidase antibodies. The model including tandem walk was additionally adjusted for step count and mean step size. Point estimates are reported as predicted means (black lines) of gait domains with 95% CI (gray areas). A higher value of gait domains represents better gait.

depression, myopathy and fractures are all implicated in gait deterioration.[14,25-29] In our study, adjustments for stroke, cerebellar cortical volume, TPOAb, CESD depression score, hip and knee pain or stiffness (proxy for musculoskeletal dysfunction) did not change the results, suggesting that the association between thyroid function and gait patterns is independent of these factors. Alternative underlying pathways can explain the association. The most plausible may be peripheral neuropathy, given that thyroid dysfunction has been commonly associated with axonal degeneration and nerve conduction abnormalities.[28,30,31] Both hypothyroid and hyperthyroid patients usually experience symmetric distal sensory disturbances that can resolve after treatment of thyroid dysfunction.[28,32] Also, genetic disorders affecting thyroid hormone transport and metabolism may play a role in gait impairment.[33] However, the exact mechanisms through which thyroid function could affect the gait patterns

remain unexplored and further studies should be directed towards unravelling the underlying pathophysiology.

Although gait is a multidimensional concept, gait assessment in prior comparable studies has been limited to the measurement of gait velocity.[16,17] A relatively small study (n=602) reported an association of high-normal FT_4 levels with slower walk.[17] A second study reported a faster walk in individuals with mildly elevated TSH levels (4.5-7.0 mIU/L) compared with euthyroid individuals.[16] Our conclusions are in line with the results of the first study, but do not support those of the second study. Most likely, the discrepancy between our results and those of the second study may be attributable to differences in TSH reference ranges and thyroid status definitions. In the second study, participants with TSH levels between 4.5 and 7.0 mIU/L were considered to have mild subclinical hypothyroidism, though they lacked FT_4 measurements. Instead, we used both TSH and FT_4 measurements to define the thyroid status of our participants. Therefore, our conclusions may add valuable information to the ongoing debate on the effects of untreated or undetected subclinical hypothyroidism. Most importantly, our large population-based cohort study extends the previous literature by addressing for the first time the association of thyroid function with global gait and gait domains. Our results indicate the importance of comprehensive gait evaluation, as we observe a stronger association of thyroid status with global gait than with gait velocity.

We were able to identify tandem, base of support and gait velocity as spatiotemporal gait aspects related to thyroid function. Likewise, past case reports have described hypothyroid patients with a "wide-based gait" and tandem walking errors on neurological examination.[12-15] In addition, adult mice lacking type 2 deiodinase walked slower and with wider base of support than the wild-type mice.[10] Our results confirm these findings in the setting of a general population cohort study. Of note, the identification of thyroid-related gait domains may provide valuable hints on the pathways linking thyroid function to gait. Tandem, base of support, and gait velocity have been associated with distinct brain structures (ie, prefrontal regions, parietal cortex, pallidum, putamen, and cerebellum), executive functioning and balance, that might be specific targets of thyroid hormone action.[4,15,34-38]

A limitation of our study is its cross-sectional design, which does not enable us to draw conclusions on causality. Though it is more likely that thyroid function affects gait than vice-versa, one could also hypothesize that health problems underlying gait abnormalities may alter thyroid parameters in the setting of non-thyroidal

illness syndrome (NTIS). This condition is characterized by low thyroid hormones and normal TSH levels.[39] Instead, we reported a nonlinear association between TSH levels and global gait. Also, NTIS is typical in critically ill patients, whereas the Rotterdam Study consists of community-dwelling adults.[39] Therefore, NTIS is unlikely to be the explanation of our findings. Furthermore, turning and tandem walk lacked repeated measurements, which would have reduced the intra-individual variability. However, we did perform up to eight consecutive recordings of the normal walk, and used a well validated instrument for an objective gait evaluation in three walking conditions. Also, the Rotterdam Study does not have data available on serum triiodothyronine levels, which is a limitation for most population-based studies. However, TSH and FT_4 concentrations are considered as the most relevant measurements of thyroid function in clinical practice. Moreover, Rotterdam Study includes predominantly Caucasians over 45 years old, which limits the generalizability of our findings to other populations. Lastly, the possibility of residual confounding cannot be excluded, even though we controlled for multiple potential confounders.

Conclusions

Both low and high thyroid function are associated with worse gait patterns. There is an inverted U-shaped association of TSH levels with global gait, tandem, and gait velocity, as well as a positive association of TSH levels with base of support. Subjects with clinical and subclinical hypothyroidism have worse gait patterns than euthyroid individuals. These conclusions might have future implications regarding the prevention and treatment of thyroid and gait disorders. Further studies are needed to confirm our findings, determine the underlying mechanisms linking thyroid function to gait patterns and subsequently investigate the possible motor benefits of thyroid treatment.

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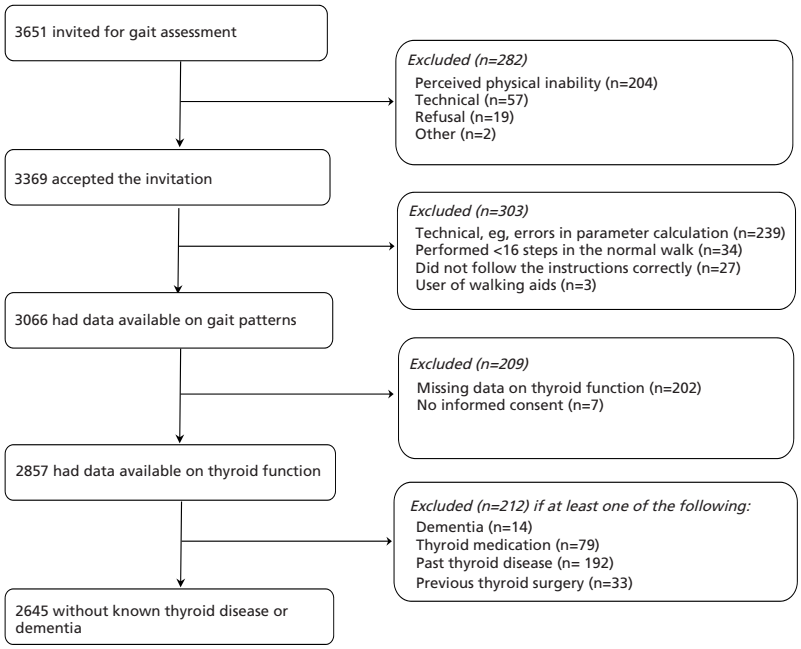
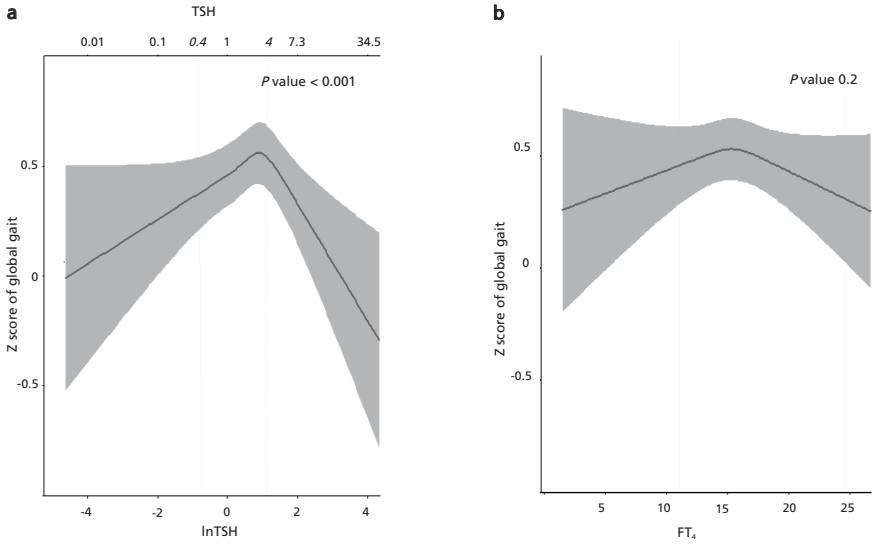
SUPPLEMENTAL MATERIAL

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



Supplemental Figure 2. Association of thyroid function with global gait, after excluding participants with prevalent stroke and Parkinson's disease.



Supplemental Table 1. Association of thyroid function with global gait, gait domains and gait velocity in euthyroid participants*

	TSH β (95% CI)	FT_4 β (95% CI)
Global gait	**0.08 (0.02; 0.13)**	-0.05 (-0.10; 0.00)
Rhythm	0.00 (-0.06; 0.06)	-0.02 (-0.07; 0.03)
Variability	0.02 (-0.04; 0.08)	-0.01 (-0.06; 0.04)
Phases	0.02 (-0.03; 0.08)	-0.05 (-0.10; 0.00)
Pace	0.05 (0.00; 0.10)	-0.02 (-0.07; 0.02)
Base of support	**0.07 (0.01; 0.14)**	0.01 (-0.04; 0.06)
Tandem†	**0.06 (0.01; 0.12)**	-0.03 (-0.08; 0.02)
Turning	-0.02 (-0.08; 0.04)	-0.01 (-0.06; 0.05)
Velocity	0.87 (-0.12; 1.87)	**-0.96 (-1.85;-0.07)**

A higher value of gait represents better gait. Analyses are adjusted for age, sex, cohort, smoking, alcohol intake, education level, height, weight, time interval between thyroid function measurement and gait assessment, knee pain or stiffness, hip pain or stiffness, prevalent stroke, CESD depression score, cerebellar cortical volume, intracranial volume, and thyroid peroxidase antibodies. *Euthyroidism was defined as TSH within the reference range (0.4 to 4.0 mIU/L). †Additionally adjusted for step count and step size within tandem walk. Abbreviations: TSH, thyroid-stimulating hormone, is per one unit increase of log transformed TSH (mIU/L); FT_4, free thyroxine, is per one unit increase of FT_4 (pmol/L); β, regression coefficient; CI, confidence interval.

Supplemental Table 2. Association of TPOAb positivity* with global gait

	β (95% CI)
All participants	-0.05 (-0.16; 0.06)
Euthyroid participants†	-0.06 (-0.19; 0.06)

A higher value of global gait represents better gait. Analyses are adjusted for age, sex, cohort, smoking, alcohol intake, education level, height, weight, time interval between thyroid function measurement and gait assessment, knee pain or stiffness, hip pain or stiffness, prevalent stroke, CESD depression score, cerebellar cortical volume, intracranial volume, and lnTSH. *TPOAb >35 kU/ml were regarded as positive. †Euthyroidism was defined as TSH within the reference range (0.4 to 4.0 mIU/L). Abbreviations: TPOAb, thyroid peroxidase antibodies; TSH, thyroid-stimulating hormone.

CHAPTER 3.3

DIFFERENCES IN TOTAL LIFE EXPECTANCY AND LIFE EXPECTANCY WITH AND WITHOUT CARDIOVASCULAR DISEASE WITHIN THE REFERENCE RANGE OF THYROID FUNCTION

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ABSTRACT

Background Variations in thyroid function within reference ranges are associated with an increased risk of cardiovascular disease (CVD) and mortality. However, the impact of thyroid function on life expectancy and the number of years lived with and without CVD remains unknown. In a large population-based prospective cohort study, we therefore aimed to investigate the association of thyroid function with total life expectancy and life expectancy with and without CVD among euthyroid individuals.

Methods We included participants of the Rotterdam Study without known thyroid disease and with thyroid-stimulating hormone (TSH) and free thyroxine (FT_4) levels within the reference ranges. Multistate life tables were used to calculate total life expectancy and life expectancy with and without CVD among TSH and FT_4 tertiles. Life expectancy estimates in men and women aged 50 years and older were obtained using prevalence, incidence rates and hazard ratios for 3 transitions (healthy to CVD, healthy to death, and CVD to death), adjusting for sociodemographic and cardiovascular risk factors.

Results The mean (standard deviation) age of 7785 participants was 64.7 (9.8) years and 52.5% were women. Over a median follow-up of 8.1 (interquartile range 2.7 to 9.9) years, we observed 789 incident CVD events and 1357 deaths. Compared with those in the lowest tertile, men and women in the highest TSH tertile lived 2.0 (95% confidence interval [95% CI], 1.0 to 2.8) and 1.4 (95% CI, 0.2 to 2.4) years longer, respectively; of which 1.5 (95% CI, 0.2 to 2.6) and 0.9 (95% CI, -0.2 to 2.0) years longer without CVD. Compared with those in the lowest tertile, the difference in life expectancy for men and women in the highest FT_4 tertile was -3.2 (95% CI,-5.0 to -1.4) and -3.5 (95% CI, -5.6; -1.5), respectively; of which -3.1 (95% CI, -4.9 to -1.4) and -2.5 (95% CI, -4.4 to -0.7) years without CVD.

Conclusions At the age of 50 years, participants with low-normal thyroid function live up to 3.5 years longer overall and up to 3.1 years longer without CVD than participants with high-normal thyroid function. These findings provide supporting evidence for a reevaluation of the current reference ranges of thyroid function and can help inform preventive and clinical care.

INTRODUCTION

Thyroid dysfunction is one of the most common endocrine disorders.[1] Clinical thyroid dysfunction is characterized by thyroid-stimulating hormone (TSH) and free thyroxine (FT_4) levels outside the reference ranges, whereas subclinical thyroid dysfunction is characterized by TSH levels outside the reference range combined with FT_4 levels within the reference range. At present, the reference ranges for TSH and FT_4 levels are statistically determined on the basis of the 2.5th and 97.5th percentiles of an apparently healthy population. This arbitrary approach, however, has been recently challenged by studies suggesting that the current reference ranges of thyroid function may need to be reevaluated by additionally taking into account the risk of clinical outcomes.[2-5] In view of the ongoing debate on redefining the reference ranges of TSH and FT_4 levels, there is a need for novel insights about the qualitative and quantitative impact of thyroid function on an individual's life and health.

The cardiovascular system represents a major target of thyroid hormone action.[6] Both clinical and subclinical thyroid dysfunction have been associated with an increased risk of coronary heart disease,[7,8] heart failure[9] and mortality.[7,8,10,11] These deleterious effects of thyroid dysfunction might also be extended to the euthyroid range. Many studies conducted in middle-aged and elderly euthyroid individuals have reported an increased risk of cardiovascular disease (CVD) and mortality with lower TSH and/or higher FT_4 levels.[2-5,12-16] Other studies do not find an association,[17-19] probably due to the relatively small proportion of events,[17,18] insufficient sample sizes[19] or short term follow-up.[17] However, it remains unclear whether there are meaningful differences in the remaining years of life lived with and without CVD within the reference range of thyroid function. Therefore, in a large population of euthyroid subjects, we aimed to investigate the association of thyroid function with total life expectancy (LE) and LE with and without CVD.

METHODS

Study population

This study was embedded within the Rotterdam Study, a large prospective population-based cohort study. The objectives and design have been described in detail previously.[20] The Rotterdam Study was initiated in 1989, including 7983 participants aged 55 years or older. In 2000, the study was extended with a second cohort of 3011 subjects. In 2006, a third cohort of 3932 subjects aged 45 years or older was added.

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 (1997-1999, n=4797) and the first visit of the second (2000-2001, n=3011) and third (2006-2008, n=3932) cohorts of the Rotterdam Study. The original cohort during these three visits included a total of 11740 participants, of which 10050 had available blood measurements. Thyroid function measurements were performed in a random sample of 9702 participants. Of these, we excluded 16 participants without complete follow-up data, 1346 with TSH or FT_4 outside the normal reference ranges and 555 with past thyroid disease or taking thyroid medications. The remaining 7785 participants were eligible for the analysis (Supplemental Figure 1). Follow-up started at the date of thyroid function assessment.

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

Assessment of thyroid function

Thyroid function was assessed during the third visit of the first cohort (RS I.3) and the first visit of the second (RS II.1) and third (RS III.1) cohorts using the same method and assay. Measurements of TSH and FT_4 were performed in baseline serum samples stored at -80°C using the electrochemiluminescence immunoassay ECLIA Roche. The reference ranges of TSH (0.40–4.0 mIU/L) and FT_4 (0.86–1.94 ng/dL, alternatively 11–25 pmol/L) were determined based on national guidelines and our previous studies.[21,22]

Assessment of CVD and mortality

Outcome measures were incident nonfatal CVD, fatal CVD and overall mortality. CVD was defined as presence of coronary heart disease, stroke or heart failure. Coronary heart disease was defined as coronary revascularization (as a proxy for significant coronary artery disease), fatal or nonfatal myocardial infarction or fatal coronary heart disease.[23] Based on the World Health Organization criteria, stroke was defined as a syndrome of rapidly developing symptoms, with an apparent vascular cause of focal or global cerebral dysfunction lasting 24 hours or longer or leading to death.[20,24] Based on the European Society of Cardiology criteria, heart

failure 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.[25] Prevalent CVD was assessed at baseline through interview and medical records. After enrollment, participants were continuously monitored for incident CVD through linkage of the study database with files from general practitioners and hospital records.

Information on mortality was obtained from municipality records, general practitioners, and reports of medical specialists. The underlying cause of death was ascertained independently by 2 research physicians and subsequently validated by a medical specialist.[23]

Additional measurements

The baseline home interview provided information on medical history, medication use, tobacco smoking, alcohol consumption, education level and marital status.[20] Smoking habits were categorized as current, former and never smoking. Education level was divided into four categories: elementary, lower secondary, higher secondary and tertiary education, in accordance with the standard international classification of education.[26] Marital status was categorized as single, married, widowed and divorced/separated. Serum glucose and lipid levels were measured by an automated enzymatic procedure (Mannheim System). Anthropometric measurements were performed in the research center by trained medical staff. Body mass index was calculated as weight in kilograms divided by height in meters squared. Blood pressure was measured in the sitting position on the right arm and calculated as the mean of two measurements using a random-zero sphygmomanometer. Diabetes mellitus was defined as fasting serum glucose level of 7 mmol/L or more, non-fasting plasma glucose level of 11.1 mmol/L or more (when fasting samples were absent) or the use of antidiabetic medications.[20]

Statistical analysis

Total LE and the number of years lived with and without CVD were calculated among tertiles of TSH and FT_4, by using multistate lifetables. Differences in LE were evaluated using the lowest tertile as reference. Multistate life tables combined information from participants in 3 possible health states, namely "free of CVD", "CVD", and "death". Possible transitions of participants were: (1) from free of CVD to CVD (incident CVD); (2) from free of CVD to death (mortality among those with-

out CVD); (3) from CVD to death (mortality among those with CVD). Backflows were not allowed and only the first event into a state was considered.[27] To calculate LE, we followed a similar approach to previous studies.[28,29]

Due to the known gender differences in LE, analyses were performed separately among men and women. We first calculated the prevalence of TSH tertiles among participants with and without CVD, categorized in 10-year age groups. In each transition, we calculated age-specific incidence rates. Next, we applied Poisson regression with Gompertz distribution to compute hazard ratios (HRs) of the association between TSH tertiles and incident CVD or mortality. The confidence intervals of LE estimates were calculated using Monte Carlo method with 10000 bootstrap simulations.[30] Moreover, we repeated the analyses for the FT_4 tertiles.

Analyses were adjusted for potential confounders, which were selected on the basis of biological plausibility and previous literature. Model 1 was adjusted for age and cohort. Model 2 was adjusted for age, cohort, smoking, alcohol intake, education level, marital status, diabetes mellitus, body mass index, systolic blood pressure, total cholesterol, triglycerides, and use of antihypertensive and lipid-lowering medications.

Multiple imputations were performed in case of missing covariates (<5% for all covariates). Statistical analyses were conducted using IBM SPSS version 21 (IBM Corp), STATA version 13 for Windows (StataCorp, College Station, Texas) and @RISK software (Palisade).

Sensitivity analysis

Several sensitivity analyses were performed: (1) To account for potential reverse causation, we excluded CVD events (n=179) or deaths (n=293) that occurred during the first 2 years of follow-up; (2) We excluded participants using thyroid function-altering medications (ie, amiodarone and corticosteroids) (n=137); (3) To exclude any potential bias caused by presence of cancer at baseline, we additionally adjusted our analyses for prevalent cancer at baseline; (4) To detect a potential influence of follow-up duration on our results, we performed the analyses restricting the length of follow-up to 8 years (median follow-up time).

To additionally explore the association between thyroid status categories (ie, hypothyroidism, euthyroidism, hyperthyroidism) and LE with and without CVD, we extended the study population, including participants of the Rotterdam Study with data available on thyroid function and CVD, without past thyroid disease and not

using thyroid function-altering medications (ie, thyroid medications, amiodarone or corticosteroids). Participants were categorized on the basis of their thyroid status. Euthyroidism was defined as serum TSH levels within the reference range. Hypothyroidism (clinical and subclinical combined) was defined as high TSH combined with low or normal FT_4. Hyperthyroidism (clinical and subclinical combined) was defined as low TSH combined with high or normal FT_4. Total LE and LE with and without CVD were calculated in men and women, among thyroid status categories, by using multistate lifetables. Differences in LE were evaluated using the euthyroid category as reference.

RESULTS

Baseline characteristics of 7785 eligible participants are presented in Table 1. The mean (standard deviation) age of participants was 64.7 (9.8) years and 52.5% were women. Over a median follow-up time of 8.1 (interquartile range, 2.7 to 9.9) years, 789 incident CVD events and 1357 deaths occurred. Both models yielded similar estimates, therefore we further report the results of the most adjusted model (Model 2).

Association of thyroid function within the reference range with the risk of CVD and death

The association of TSH tertiles with the risk of incident CVD was not statistically significant (highest versus lowest TSH tertile: HR, 0.93; 95% CI, 0.79 to 1.11) (Table 2). Compared with the lowest tertile, the highest TSH tertile was associated with a lower risk of mortality among participants without CVD (HR, 0.76; 95% CI, 0.64 to 0.91) and with CVD (HR, 0.82; 95% CI, 0.67 to 1.01) (Table 2).

The highest FT_4 tertile was associated with a 1.32 times higher risk of incident CVD than the lowest tertile (95% CI, 1.10 to 1.58) (Table 2). Compared with the lowest tertile, the highest FT_4 tertile was also associated with a 1.64 times higher risk of mortality among participants with CVD (95% CI, 1.32 to 2.02) and a 1.45 times higher risk of mortality among participants without CVD (95% CI, 1.21 to 1.73) (Table 2).

Results for TSH and FT_4 analyses did not change substantially after the events that occurred during the first 2 years of follow-up were excluded (Supplemental Table 1). Also, results remained similar after excluding users of thyroid function-altering medications and additionally adjusting for the presence of prevalent cancer at baseline (Supplemental Table 2).

Table 1. Baseline characteristics of 7785 participants*

	Men	Women
Number	3699	4086
Age, years	64.3 (9.3)	65.0 (10.2)
Smoking, n (%)		
current	906 (24.5)	828 (20.3)
former	2180 (58.9)	1553 (38.0)
never	613 (16.6)	1705 (41.7)
Education, n (%)		
Elementary	342 (9.2)	605 (14.8)
Lower secondary	1051 (28.4)	2060 (50.4)
Higher secondary	1354 (36.6)	924 (22.6)
Tertiary	952 (25.7)	497 (12.2)
Marital status, n (%)		
Single	115 (3.1)	252 (6.2)
Married	3155 (85.3)	2515 (61.6)
Widowed	227 (6.1)	897 (22.0)
Divorced/Separated	202 (5.5)	422 (10.3)
Diabetes mellitus, n (%)	497 (13.4)	464 (8.9)
BMI, kg/m^2	27.0 (3.5)	27.3 (4.6)
Systolic blood pressure, mm Hg	141.0 (20.2)	137.9 (21.5)
Use of antihypertensive medications, n (%)	816 (22.1)	942 (23.1)
Total cholesterol, mmol/l	5.4 (0.9)	5.9 (1.0)
Triglycerides, mmol/l	1.6 (0.9)	1.5 (0.7)
Use of lipid-lowering medications, n (%)	666 (18)	583 (14.3)
Prevalent cancer, n (%)	201 (5.4)	250 (6.1)
TSH, mIU/L, median (IQR)	1.8 (1.2-2.4)	1.9 (1.3-2.6)
FT_4, pmol/L	15.9 (2.0)	15.6 (1.9)

*Data are presented as mean (standard deviation), unless otherwise specified. Abbreviations: BMI, body-mass index; TSH, thyroid-stimulating hormone; IQR, interquartile range; FT_4, free thyroxine.

Association of thyroid function within the reference range with total LE and LE with and without CVD

Total LE increased significantly from the lowest to the middle TSH tertile and did not change substantially from the middle to the highest TSH tertile (Figure 1). Compared with those in the lowest tertile, men in the highest TSH tertile lived 2.0 (95% CI, 1.0 to 2.8) years longer overall, of which, 1.5 (95% CI, 0.2 to 2.6) years longer without CVD and 0.5 (95% CI, -0.5 to 1.4) years longer with CVD (Table 3). Compared with those in the lowest tertile, women in the highest TSH tertile lived 1.4 (95% CI, 0.2 to 2.4) years longer overall, of which, 0.9 (95% CI, -0.2 to 2.0) years longer without CVD and 0.5 (95% CI, -0.5 to 1.2) years longer with CVD (Table 3).

Table 2. HRs for incident CVD and death among TSH and FT_4 tertiles

			TSH		FT_4	
Transition	**Cases/PY**	**TSH/FT_4 tertiles**	**HR (95% CI) Model 1**	**HR (95% CI) Model 2**	**HR (95% CI) Model 1**	**HR (95% CI) Model 2**
Incident CVD	789/38417	Tertile 1	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)
		Tertile 2	0.85 (0.72; 1.01)	0.86 (0.72; 1.01)	1.16 (0.97; 1.38)	1.15 (0.97; 1.38)
		Tertile 3	0.93 (0.79; 1.10)	0.93 (0.79; 1.11)	**1.33 (1.11; 1.58)**	**1.32 (1.10; 1.58)**
Mortality among those without CVD	801/41130	Tertile 1	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)
		Tertile 2	**0.74 (0.62; 0.87)**	**0.75 (0.64; 0.89)**	**1.20 (1.00; 1.44)**	1.19 (0.99; 1.42)
		Tertile 3	**0.72 (0.61; 0.86)**	**0.76 (0.64; 0.91)**	**1.50 (1.26; 1.79)**	**1.45 (1.21; 1.73)**
Mortality among those with CVD	556/8718	Tertile 1	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)
		Tertile 2	0.95 (0.77; 1.15)	0.92 (0.75; 1.13)	1.24 (0.99; 1.55)	**1.27 (1.01; 1.60)**
		Tertile 3	**0.81 (0.62; 0.91)**	0.82 (0.67; 1.01)	**1.59 (1.29; 1.95)**	**1.64 (1.32; 2.02)**

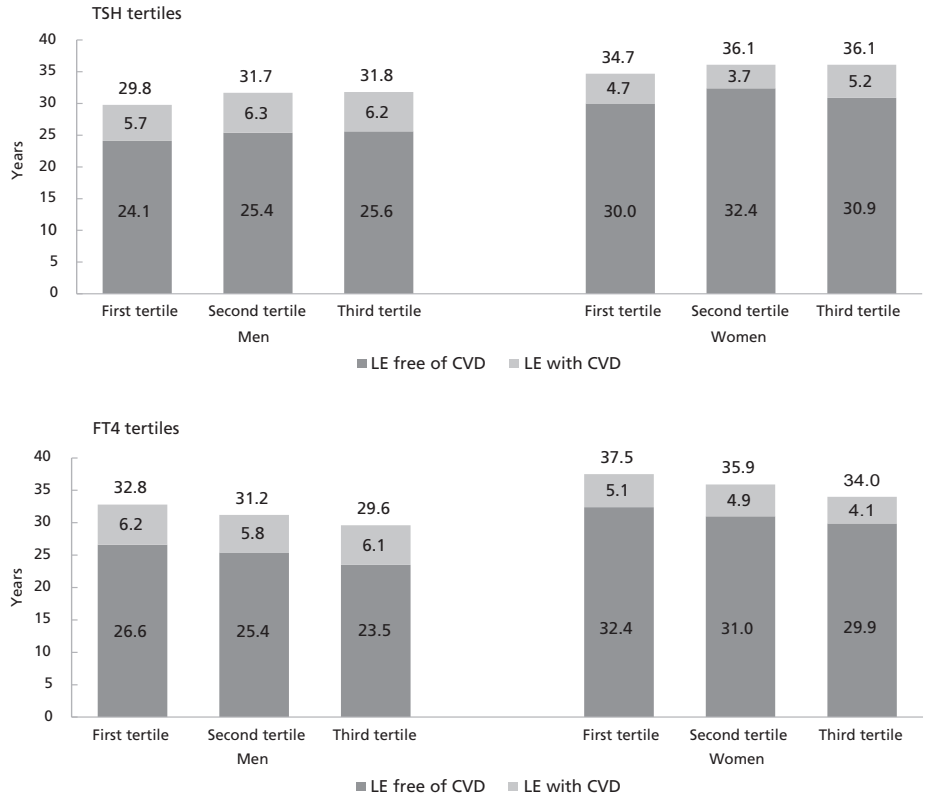
Model 1: age, sex, and cohort. Model 2: Model 1, smoking, alcohol intake, education level, marital status, diabetes mellitus, body mass index, systolic blood pressure, total cholesterol, triglycerides, use of antihypertensive medications, and use of lipid-lowering medications. Abbreviations: HR, hazard ratio; TSH, thyroid-stimulating hormone; FT_4, free thyroxine; PY, person-years; CI, confidence interval; CVD, cardiovascular disease.

Total LE decreased progressively with increasing FT_4 tertiles (Figure 1). Compared with those in the lowest tertile, the differences in LE for men in the highest FT_4 tertile was -3.2 (95% CI, -5.0; -1.4) years overall; of which -3.1 (95% CI, -4.9 to -1.4) years without CVD and -0.1 (95% CI, -1.7 to 1.6) years with CVD (Table 3). Compared with those in the lowest tertile, the difference in LE for women in the highest FT_4 tertile was -3.5 (95% CI, -5.6 to -1.5) years fewer overall, of which -2.5 (95% CI, -4.4 to -0.7) years without CVD and -1.0 (95% CI, -2.4 to 0.4) years with CVD (Table 3). Results were consistent over the length of follow-up of 8 years (Supplemental Table 3).

Association of thyroid status with total LE and LE with and without CVD

Compared with their euthyroid counterparts, hypothyroid men and women lived 0.3 (95% CI, -1.7 to 1.9) and 1.1 (95% CI, -0.4 to 2.3) years longer, respectively (Supplemental Table 4). The difference in LE for hyperthyroid men was -1.4 (95% CI, -4.4 to 2.0) years compared with euthyroid men. However, these results were not statistically significant. The difference in LE for hyperthyroid women was 2.3 (95% CI, 0.2 to 4.4) years without CVD and -1.9 (95% CI, -3.1 to -0.4) years with CVD, compared with euthyroid women (Supplemental Table 4).

Figure 1. Life expectancy with and without CVD at age 50 years among TSH and FT_4 tertiles, in men and women.



Abbreviations: LE, life expectancy; CVD, cardiovascular disease; TSH, thyroid-stimulating hormone; FT_4, free thyroxine.

DISCUSSION

In a large prospective population-based cohort study among middle-aged and elderly participants, we investigated differences in LE with and without CVD within the reference range of thyroid function. Participants with low-normal thyroid function lived up to 3.5 years longer overall and up to 3.1 years longer without CVD than participants with high-normal thyroid function. Total LE in euthyroid participants increased from the lowest to the middle TSH tertile but did not change substantially from the middle to the highest TSH tertile. Total LE in euthyroid participants decreased progressively with increasing FT_4 tertiles. Overall, there were no meaningful sex differences throughout TSH and FT_4 tertiles.

LE without CVD is the resultant of 2 components: risk of incident CVD (transition 1) and risk of mortality among participants without CVD (transition 2). Compared

Table 3. LE at age 50 years among TSH and FT_4 tertiles, in men and women*

TSH/FT_4 tertiles	Total LE	Differences in total LE†	LE free of CVD	Differences in LE free of CVD†	LE with CVD	Differences in LE with CVD†
TSH tertiles						
Men						
Tertile 1	**29.8 (29.2; 30.2)**	Reference	**24.1 (23.5; 24.6)**	Reference	**5.7 (5.2; 6.2)**	Reference
Tertile 2	**31.7 (30.9; 32.6)**	**1.9 (1.1; 3.1)**	**25.4 (24.5; 26.2)**	1.3 (-0.0; 2.3)	**6.3 (5.6; 7.0)**	0.6 (-0.3; 1.4)
Tertile 3	**31.8 (30.9; 32.5)**	**2.0 (1.0; 2.8)**	**25.6 (24.6; 26.5)**	**1.5 (0.2; 2.6)**	**6.2 (5.5; 7.2)**	0.5 (-0.5; 1.4)
Women						
Tertile 1	**34.7 (34.2; 35.3)**	Reference	**30.0 (29.5; 30.5)**	Reference	**4.7 (4.2; 5.3)**	Reference
Tertile 2	**36.1 (35.4; 36.9)**	**1.4 (0.5; 2.4)**	**32.4 (31.5; 33.2)**	**2.4 (1.4; 3.3)**	**3.7 (3.0; 4.3)**	**-1.0 (-2.0; -0.1)**
Tertile 3	**36.1 (35.2; 36.8)**	**1.4 (0.2; 2.4)**	**30.9 (30.0; 32.0)**	0.9 (-0.2; 2.0)	**5.2 (4.4; 5.9)**	0.5 (-0.5; 1.2)
FT_4 tertiles						
Men						
Tertile 1	**32.8 (31.8; 34.0)**	Reference	**26.6 (25.6; 27.5)**	Reference	**6.2 (5.3; 7.1)**	Reference
Tertile 2	**31.2 (30.1; 32.6)**	-1.6 (-3.5; 0.2)	**25.4 (23.9; 26.8)**	-1.2 (-3.2; 0.5)	**5.8 (4.7; 7.0)**	-0.4 (-1.6; 1.0)
Tertile 3	**29.6 (28.5; 30.8)**	**-3.2 (-5.0; -1.4)**	**23.5 (22.3; 24.6)**	**-3.1 (-4.9;-1.4)**	**6.1 (5.0; 7.3)**	-0.1 (-1.7; 1.6)
Women						
Tertile 1	**37.5 (36.5; 38.7)**	Reference	**32.4 (31.5; 33.4)**	Reference	**5.1 (4.3; 6.1)**	Reference
Tertile 2	**35.9 (34.5; 37.6)**	-1.6 (-3.15; 0.0)	**31.0 (29.7; 32.4)**	-1.4 (-3.0; 0.2)	**4.9 (3.8; 6.2)**	-0.2 (-1.4; 1.1)
Tertile 3	**34.0 (32.8; 35.4)**	**-3.5 (-5.6; -1.5)**	**29.9 (28.7; 31.2)**	**-2.5 (-4.4;-0.7)**	**4.1 (3.3; 5.1)**	-1.0 (-2.4; 0.4)

*Data are given as years (95% confidence intervals). All life expectancies have been calculated with hazard ratios adjusted for age, cohort, smoking, alcohol intake, education level, marital status, diabetes mellitus, body mass index, systolic blood pressure, total cholesterol, triglycerides, use of antihypertensive medications, and use of lipid-lowering medications. †Differences in LE are calculated using the first tertile as reference. Abbreviations: LE, life expectancy; TSH, thyroid-stimulating hormone; FT_4, free thyroxine; CVD, cardiovascular disease.

with the lowest tertile, the highest FT_4 tertile was associated with a higher risk of incident CVD, meaning an earlier clinical manifestation of CVD and fewer years lived without CVD. The highest FT_4 tertile was also associated with an increased mortality risk among participants without CVD, resulting in a further decrease in total LE and LE without CVD. *LE with CVD* is the resultant of 2 components: risk of incident CVD (transition 1) and risk of mortality among subjects with CVD (transition 3). Compared with the lowest tertile, the highest FT_4 tertile was associated with a 1.32 times higher risk of incident CVD, meaning an earlier clinical manifestation of CVD and more years lived with CVD. However, participants with CVD in the highest FT_4 tertile had an even higher risk of mortality (ie, 1.64 times higher), which explains the decrease in the number of years lived with CVD.

Our study confirms prior research, suggesting that high-normal thyroid function is associated with an increased risk of CVD and mortality, independent of traditional cardiovascular risk factors.[2-5] Most importantly, it extends the previous literature by revealing considerable differences in LE within the reference range of thyroid function. These findings provide supporting evidence for a reevaluation of the current reference ranges of TSH and FT_4 measurements, implying the possibility of an upward shift of TSH and a downward shift of FT_4 reference ranges in middle-aged and elderly people. Further investigations are needed to determine the clinically relevant normal range of thyroid function.

Overactivity of thyroid gland is known to have a negative effect on overall health. Higher thyroid hormone concentrations have been associated with an increased heart rate (chronotropic effect), myocardial contractility (inotropic effect), and hypercoagulability, which may further predispose to CVD and mortality.[6,31] Moreover, elevated thyroid hormone levels can enhance oxygen consumption and production of reactive oxygen species, which may subsequently induce DNA damage and cell apoptosis.[32,33] Also, elevated thyroid hormone levels can affect cognition, nerve conduction, and bone mineral density, thus contributing to an increased risk of dementia, polyneuropathy, osteoporosis, and death.[34-36] However, the deleterious effects of high thyroid function could be also extended to the high-normal range of thyroid function.[2,37] Therefore, the aforementioned mechanisms could be additionally involved in the pathways linking high-normal thyroid function to a reduced life span.

Other mechanisms can explain the association of low-normal thyroid function with a prolonged life span. Low-normal thyroid function may promote energy conservation, which is necessary to adequately cope with acute and chronic stressors.[38] Low-normal thyroid function may also represent a heritable phenotype of exceptional longevity. In line with this hypothesis, Rozing et al[39] observed lower circulating thyroid hormone levels in middle-aged offspring of nonagenarian siblings compared with age-matched controls. In addition, decreased thyroid hormone levels in nonagenarian siblings have been associated with a prolonged life span in their parents.[40]

Based on the negative feedback mechanism of the hypothalamus-pituitary-thyroid axis, each individual is expected to have a unique set point of thyroid function, with an inverse relation between TSH and FT_4 concentrations. Our results were consistent with the feedback regulation because high-normal TSH levels and

low-normal FT_4 levels were both associated with an increased LE. However, LE in our participants was more strongly associated with FT_4 than with TSH levels. Likewise, previous cohort studies have reported a stronger association of adverse outcomes (including mortality) with FT_4 than with TSH levels, particularly within the euthyroid range.[2,4,13,14,22,35] Likely, genetic determinants and ageing can modify the TSH-FT_4 set point of the feedback mechanism among euthyroid individuals.[41,42] Various genetic polymorphisms that affect serum TSH but not FT_4 levels have been identified.[42]

In addition, we investigated the differences in LE with and without CVD among thyroid status categories (ie, hypothyroidism, euthyroidism, hyperthyroidism). In line with the results of our main analysis, we found that hypothyroid participants lived longer than euthyroid subjects. Among hyperthyroid participants, we observed sex differences in the number of years lived with and without CVD. However, these results should be interpreted with caution, owing to the relatively small number of participants with thyroid dysfunction and their increased susceptibility to receiving treatment and changing health behaviors over time. Future studies can explore more extensively the effect of thyroid disease on LE with and without CVD.

To the best of our knowledge, this is the first population-based cohort study that investigates differences in LE with and without CVD within the reference range of thyroid function. Strengths include the prospective study design, the long follow-up period, and the large number of participants with extensive and detailed information on covariates and outcomes. The large sample size allowed us to conduct multiple sensitivity analyses, which provided consistent findings. Events were adjudicated using standardized criteria.

Several limitations should also be considered. The Rotterdam Study includes predominantly whites older than 45 years; therefore, our findings require confirmation in other populations. Moreover, we lacked repeated measurements of thyroid function. Nevertheless, this is unlikely to have affected our results, given that the normal range of thyroid function is considered to be stable over time with a low intra-individual variability.[43] Furthermore, we did not have data available on serum triiodothyronine levels. However, TSH and FT_4 represent the most relevant measurements of thyroid function in clinical practice. Due to the observational character of our study, the possibility of residual confounding cannot be entirely ruled out.

Conclusions

At the age of 50 years, participants with low-normal thyroid function lived up to 3.5 years longer overall and up to 3.1 years longer without CVD than those with high-normal thyroid function. Our findings support a reevaluation of the current reference ranges of TSH and FT_4 measurements, implying the possibility of an upward shift of TSH and a downward shift of FT_4 current limits in middle-aged and elderly people. Future research is needed to replicate our findings and elucidate the exact mechanisms underlying the LE differences within the reference range of thyroid function.

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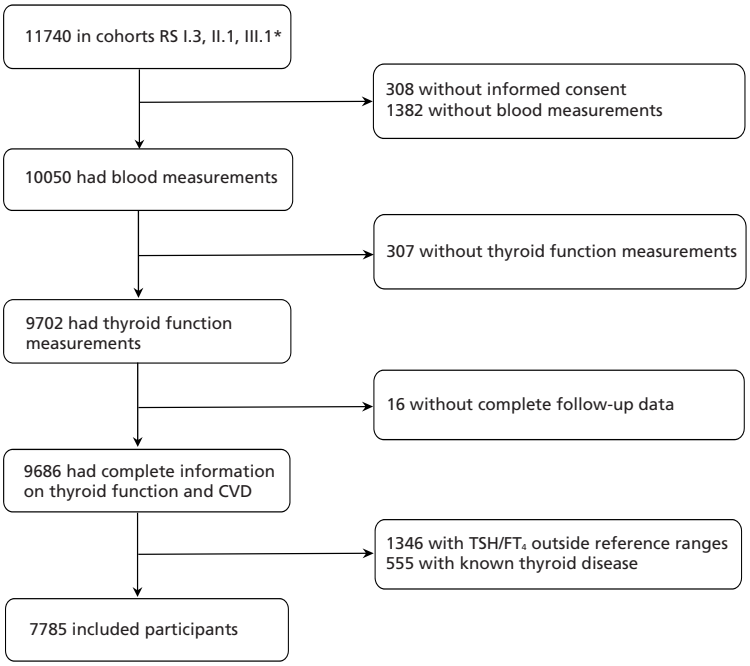
SUPPLEMENTAL MATERIAL

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. Abbreviations: RS, Rotterdam Study; CVD, cardiovascular disease; TSH, thyroid-stimulating hormone; FT_4, free thyroxine.

Supplemental Table 1. HRs of incident CVD and death among TSH and FT_4 tertiles, excluding the first 2 years of follow-up for CVD and death

			TSH		FT_4	
Transition	**Cases/PY**	**TSH/FT_4 tertiles**	**HR (95% CI) Model 1**	**HR (95% CI) Model 2**	**HR (95% CI) Model 1**	**HR (95% CI) Model 2**
Incident CVD	610/38139	Tertile 1	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)
		Tertile 2	0.83 (0.69; 1.01)	0.84 (0.69; 1.02)	1.14 (0.94; 1.40)	1.14 (0.93; 1.40)
		Tertile 3	0.95 (0.79; 1.16)	0.96 (0.79; 1.17)	**1.25 (1.02; 1.53)**	**1.24 (1.02; 1.52)**
Mortality among those without CVD	639/39950	Tertile 1	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)
		Tertile 2	**0.78 (0.65; 0.94)**	**0.80 (0.67; 0.96)**	1.21 (0.98; 1.47)	1.19 (0.97; 1.46)
		Tertile 3	**0.72 (0.60; 0.88)**	**0.77 (0.63; 0.94)**	**1.43 (1.18; 1.74)**	**1.35 (1.11; 1.65)**
Mortality among those with CVD	425/7753	Tertile 1	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)
		Tertile 2	0.98 (0.78; 1.23)	0.92 (0.73; 1.16)	1.29 (1.00; 1.67)	**1.31 (1.01; 1.68)**
		Tertile 3	**0.77 (0.61; 0.98)**	**0.76 (0.60; 0.97)**	**1.49 (1.17; 1.89)**	**1.49 (1.17; 1.90)**

Model 1: age, sex, and cohort. Model 2: Model 1, smoking, alcohol intake, education level, marital status, diabetes mellitus, body mass index, systolic blood pressure, total cholesterol, triglycerides, use of antihypertensive medications, and use of lipid-lowering medications. Abbreviations: HR, hazard ratio; CVD, cardiovascular disease; TSH, thyroid-stimulating hormone; FT_4, free thyroxine; PY, person-years; CI, confidence interval.

Supplemental Table 2. HRs for incident CVD and death among TSH and FT_4 tertiles, excluding users of thyroid function-altering medications and additionally adjusting for prevalent cancer

			TSH	FT_4
Transition	**Cases/PY**	**TSH/FT_4 tertiles**	**HR (95% CI)**	**HR (95% CI)**
Incident CVD	763/37814	Tertile 1	1 (Reference)	1 (Reference)
		Tertile 2	0.87 (0.73; 1.03)	1.14 (0.95; 1.37)
		Tertile 3	0.94 (0.78; 1.12)	**1.32 (1.10; 1.57)**
Mortality among those without CVD	771/40460	Tertile 1	1 (Reference)	1 (Reference)
		Tertile 2	**0.78 (0.65; 0.91)**	1.16 (0.96; 1.39)
		Tertile 3	**0.76 (0.64; 0.91)**	**1.43 (1.20; 1.72)**
Mortality among those with CVD	524/8445	Tertile 1	1 (Reference)	1 (Reference)
		Tertile 2	0.93 (0.76; 1.15)	1.24 (0.98; 1.56)
		Tertile 3	**0.80 (0.65; 1.00)**	**1.57 (1.26; 1.94)**

Hazard ratios are adjusted for age, sex, cohort, smoking, alcohol intake, education level, marital status, diabetes mellitus, body mass index, systolic blood pressure, total cholesterol, triglycerides, use of antihypertensive medications, use of lipid-lowering medications, and prevalent cancer. Abbreviations: HR, hazard ratio; CVD, cardiovascular disease; TSH, thyroid-stimulating hormone; FT_4, free thyroxine; PY, person-years; CI, confidence interval.

Supplemental Table 3. LE at age 50 years, among TSH and FT_4 tertiles, in men and women over 8 years of follow-up*

	Total LE	Differences in total LE	Differences in LE free of CVD	Differences in LE with CVD
TSH tertiles				
Men				
Tertile 1	29.6	Reference	Reference	Reference
Tertile 2	32.3	2.7	1.4	1.3
Tertile 3	31.5	2.0	1.3	0.6
Women				
Tertile 1	34.6	Reference	Reference	Reference
Tertile 2	36.3	1.7	3.1	-1.3
Tertile 3	36.2	1.6	1.4	0.2
FT_4 tertiles				
Men				
Tertile 1	31.8	Reference	Reference	Reference
Tertile 2	30.5	-1.2	-0.9	-0.3
Tertile 3	28.8	-3.0	-2.8	-0.2
Women				
Tertile 1	37.9	Reference	Reference	Reference
Tertile 2	35.8	-2.1	-1.5	-0.6
Tertile 3	33.9	-4.0	-2.7	-1.3

*Data are given as years. Differences are calculated using the first tertile as reference. All LEs have been calculated with hazard ratios adjusted for age, cohort, smoking, alcohol intake, education level, marital status, diabetes mellitus, body mass index, systolic blood pressure, total cholesterol, triglycerides, use of antihypertensive medications, and use of lipid-lowering medications. Abbreviations: LE, life expectancy; TSH, thyroid-stimulating hormone; FT_4, free thyroxine; CVD, cardiovascular disease.

Supplemental Table 4. LE at age 50 years, among thyroid status categories, in men and women*

	TN	Total LE	Differences in total LE	Differences in LE free of CVD	Differences in LE with CVD
Men					
Euthyroidism	3667	**31.0 (30.8; 31.3)**	Reference	Reference	Reference
Hypothyroidism†	245	**31.3 (29.5; 32.9)**	0.3 (-1.7; 1.9)	0.1 (-2.4; 2.5)	0.2 (-1.5; 2.1)
Hyperthyroidism†	80	**29.6 (26.6; 32.9)**	-1.4 (-4.4; 2.0)	-3.7 (-7.6; 0.1)	2.3 (-1.3; 6.4)
Women					
Euthyroidism	4028	**35.6 (35.3; 35.9)**	Reference	Reference	Reference
Hypothyroidism†	551	**36.7 (35.5; 37.8)**	1.1 (-0.4; 2.3)	0.8 (-0.7; 2.2)	0.3 (-0.8; 1.3)
Hyperthyrodism†	110	**36.0 (33.7; 38.4)**	0.4 (-2.1; 2.9)	**2.3 (0.2; 4.4)**	**-1.9 (-3.1;-0.4)**

*For this analysis, we included participants without known thyroid disease and not using thyroid function-altering medications (ie, thyroid medications, amiodarone, corticosteroids). Differences are calculated using the euthyroid category as reference. Data are given as years (95% confidence intervals). All LEs have been calculated with hazard ratios adjusted for age, cohort, smoking, alcohol intake, education level, marital status, diabetes mellitus, body mass index, systolic blood pressure, total cholesterol, triglycerides, use of antihypertensive medications, and use of lipid-lowering medications. †Includes subclinical and clinical range. Abbreviations: LE, life expectancy; TN, total number; CVD, cardiovascular disease.

CHAPTER 3.4

DIFFERENCES IN TOTAL LIFE EXPECTANCY AND LIFE EXPECTANCY WITH AND WITHOUT NON-COMMUNICABLE DISEASES WITHIN THE REFERENCE RANGE OF THYROID FUNCTION

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ABSTRACT

Background Variations in thyroid function within the reference range are associated with an increased risk of diseases and death. However, the impact of thyroid function on life expectancy (LE) and the number of years lived with and without non-communicable diseases (NCD) remains unknown. Therefore, we aimed to investigate the association of thyroid function with total LE and LE with and without NCD among euthyroid subjects.

Methods Participants of the Rotterdam Study without known thyroid disease and with thyroid-stimulating hormone (TSH) and free thyroxine (FT_4) levels within the reference ranges were eligible. NCD was defined as presence of cardiovascular disease, diabetes mellitus type 2, or cancer. We used multistate life tables to calculate the total LE and LE with and without NCD among TSH and FT4 tertiles, in men and women. LE estimates were obtained using prevalence, incidence rates and hazard ratios for three transitions (healthy to NCD, healthy to death and NCD to death). Analyses were adjusted for sociodemographic and cardiovascular risk factors.

Results The mean (standard deviation) age of 7644 participants was 64.5 (9.7) years and 52.2% were women. Over a median follow-up of 8 years, we observed 1396 incident NCD events and 1422 deaths. Compared with those in the lowest tertile, men and women in the highest TSH tertile lived 1.5 (95% confidence interval [95% CI], 0.8 to 2.3) and 1.5 (95% CI, 0.8 to 2.2) years longer, respectively; of which 1.4 (95% CI, 0.5 to 2.3) and 1.3 (95% CI, 0.3 to 2.1) years with NCD. Compared with those in the lowest tertile, the difference in LE for men and women in the highest FT_4 tertile was -3.7 (95% CI,-5.1 to -2.2) and -3.3 (95% CI, -4.7 to -1.9), respectively; of which -1.8 (95% CI, -3.1 to -0.7) and -2.0 (95% CI, -3.4 to -0.7) years without NCD.

Conclusions There are meaningful differences in total LE, LE with and without NCD within the reference ranges of thyroid function. These findings support a reevaluation of the current reference ranges of thyroid function.

INTRODUCTION

Non-communicable diseases (NCD) pose a global health threat, inflicting high disability rates and a huge economic burden.[1,2] According to the World Health Organization, NCD are a leading cause of deaths, accounting for approximately 65% of total mortality worldwide.[2,3] Cardiovascular disease (CVD), diabetes, cancer, and chronic kidney disease (CKD) account for a large proportions of deaths.[4] Among other factors, clinical and subclinical thyroid dysfunction are associated with the development of NCD and the risk of NCD mortality.[5-9]

In view of the ongoing debate on redefining the reference ranges of thyroid-stimulating hormone (TSH) and free thyroxine (FT_4) levels,[10] many prospective studies have suggested that variations in thyroid function within the reference range can contribute to the occurrence of chronic conditions and deaths.[11-13] Recently, it was reported that middle-aged and older adults with low-normal thyroid function have a longer total life expectancy (LE) and a longer LE without CVD than those with high-normal thyroid function.[14] Still, it remains unclear whether these differences in LE within the reference range of thyroid function reflect healthy years or years lived with NCD. Prospective studies in euthyroid subjects have suggested that high-normal thyroid function increases the risk of CVD, cancer or CKD,[12,13,15-17] while low-normal thyroid function has been associated with an increased risk of chronic metabolic diseases as diabetes.[11,18] Based on this evidence, it is challenging to determine the balance of overall benefits and risks for specific cutoffs of TSH and FT_4 levels within the reference range. In this respect, the utilization of multidimensional measures, as LE with and without NCD, can provide useful information on the qualitative and quantitative impact of thyroid function on general health.

In a large prospective population-based cohort study, we investigated the association of thyroid function within the reference range with the risk of incident NCD. Also, we investigated whether there are differences in the amount of years lived with and without NCD, within the reference range of thyroid function. We focused on chronic conditions that have been prospectively associated with thyroid function,[11,13,14] and have been highlighted as a global threat by the United Nations.[19] NCD was defined as the presence of CVD, diabetes, or cancer. Due to the incomplete data on the CKD diagnosis, we performed a secondary analysis which additionally included CKD in the definition of NCD.

METHODS

Study population

The Rotterdam Study is a large prospective population-based cohort study. The objectives and design have been described in detail previously.[20] The Rotterdam Study was initiated in 1989, including 7983 participants aged 55 years or older. In 2000, the study was extended with a second cohort of 3011 subjects. In 2006, a third cohort of 3932 subjects aged 45 years or older was added. 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 (1997-1999, n=4797) and the first visit of the second (2000-2001, n=3011) and third (2006-2008, n=3932) cohorts of the Rotterdam Study. The original cohort during these three visits included a total of 11740 participants, of which 10050 had available blood measurements. Thyroid function measurements were performed in a random sample of 9702 participants. Of these, we excluded 836 participants with past thyroid disease or taking thyroid medications, 182 participants without complete information on prevalent or incident NCD, and 1040 participants with TSH or FT_4 outside the normal reference ranges. The remaining 7644 participants were eligible for the analyses.

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

Assessment of thyroid function

Thyroid function was assessed at baseline during the third visit of the first cohort (RS I.3) and the first visit of the second (RS II.1) and third (RS III.1) cohorts using the same method and assay. Measurements of TSH and FT_4 were performed in baseline serum samples stored at -80°C using the electrochemiluminescence immunoassay ECLIA Roche. The reference ranges of TSH (0.40–4.0 mIU/L) and FT_4 (0.86–1.94 ng/dL, alternatively 11–25 pmol/L) were determined based on national guidelines and our previous studies.[17,18] Participants and family physicians were not informed about the thyroid function measurements results. The study investigators assessing the outcomes of interest were blinded to the thyroid status of participants.

Assessment of NCD and mortality

Outcome measures were incident nonfatal NCD, mortality among those with NCD and overall mortality. NCD was defined as presence of CVD, diabetes mellitus type 2, or cancer.

CVD was defined as presence of coronary heart disease, stroke or heart failure. Coronary heart disease was defined as coronary revascularization (as a proxy for significant coronary artery disease), fatal or nonfatal myocardial infarction or fatal coronary heart disease.[21] Based on the World Health Organization criteria, stroke was defined as a syndrome of rapidly developing symptoms, with an apparent vascular cause of focal or global cerebral dysfunction lasting 24 hours or longer or leading to death.[22] Based on the European Society of Cardiology criteria, heart failure was defined as the presence of typical symptoms and signs, as 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.[23] Prevalent CVD was assessed at baseline through interview and medical records. After enrollment, participants were continuously monitored for incident CVD through linkage of the study database with files from general practitioners and hospital records.

Diabetes mellitus was defined as fasting serum glucose level of 7 mmol/L or more, non-fasting plasma glucose level of 11.1 mmol/L or more (when fasting samples were absent) or the use of blood glucose lowering medications. Cases of type 2 diabetes were ascertained at baseline and during follow-up through general practitioners, hospital discharge letters, and serum glucose measurements from the Rotterdam Study visits. Information regarding the use of blood glucose lowering medications was derived from both structured home interviews and linkage to pharmacy records. Potential events of type 2 diabetes were independently adjudicated by two research physicians. In case of discrepancy, consensus was sought or an endocrinologist decided.[24]

Cancer events were classified according to the International Classification of Diseases (ICD) 10th edition. Cases of cancer were determined through general practitioners, hospital discharge letters and by linkage with a nationwide registry of histopathology and cytopathology in the Netherlands, Pathologisch-Anatomisch Landelijk Geautomatiseerd Archief (PALGA).[25] Two research physicians independently assessed the diagnosis of cancer. In case of discrepancy, consensus was sought or a cancer epidemiologist decided.

Chronic kidney disease

Creatinine measurements were calibrated by aligning the mean values of creatinine with creatinine values of participants of the National Health and Nutrition Examination Survey III in different sex and age groups (<60, 60–69, and ≥70 years old).[26] Estimated glomerular filtration rate (eGFR) was calculated according to the Chronic Kidney Disease Epidemiology Collaboration Equation.[27] To calculate the annual eGFR decline, we first subtracted the eGFR estimates of the follow-up examination from the eGFR estimates at baseline and then divided by the time between the two visits. CKD was defined as eGFR<60 ml/min per 1.73 m^2. Patients with incident CKD were defined as individuals free of CKD at baseline (eGFR>60 ml/min per 1.73 m^2) who had a decline in eGFR to <60 ml/min per 1.73 m^2 between the two periodic examinations.[28] To estimate the censoring date of the cases, we assumed a linear decrease in eGFR. Given this assumption, the date that each case had passed the eGFR threshold of 60 ml/min per 1.73 m^2 was taken as the censoring date and it was used to calculate the follow-up time for incident cases. For controls, the time spent between the two examinations was used as the follow-up time.

Additional measurements

The baseline home interview provided information on medical history, medication use, tobacco smoking, alcohol consumption, education level and marital status.[20] Smoking habits were categorized as current, former and never smoking. Education level was divided into four categories: elementary, lower secondary, higher secondary and tertiary education, in accordance with the standard international classification of education. Marital status was categorized as single, married, widowed and divorced/separated. Lipid levels were measured by an automated enzymatic procedure (Mannheim System). Anthropometric measurements were performed in the research center by trained medical staff. Body mass index was calculated as weight in kilograms divided by height in meters squared. Blood pressure was measured in the sitting position on the right arm and calculated as the mean of two measurements using a random-zero sphygmomanometer.

Statistical analysis

Total LE and the number of years lived with and without NCD were calculated among tertiles of TSH and FT_4, by using multistate lifetables.[29] Differences in LE were evaluated using the lowest tertile as reference. Multistate life tables combined

information from participants in 3 possible health states, namely "free of NCD", "NCD", and "death". Possible transitions of participants were: (1) from free of NCD to NCD (incident NCD); (2) from free of NCD to death (mortality among those without NCD); (3) from NCD to death (mortality among those with NCD). Backflows were not allowed and only the first event into a state was considered.[30] To calculate LE, we followed a similar approach to previous studies.[29,31] Due to the known gender differences in LE, analyses were performed separately among men and women. We first calculated the prevalence of TSH tertiles among participants with and without NCD, categorized in 10-year age groups. In each transition, we calculated age-specific incidence rates. Next, we applied Poisson regression with Gompertz distribution to compute hazard ratios (HRs) of the association between TSH tertiles and incident NCD or mortality, adjusting for potential confounders. The confidence intervals of LE estimates were calculated using Monte Carlo method with 10000 bootstrap simulations.[32] Furthermore, we repeated the analyses for the FT_4 tertiles.

Confounders were selected on the basis of biological plausibility and previous literature. Model 1 was adjusted for age, sex, and cohort. Model 2 was additionally adjusted for smoking, alcohol intake, education level, marital status, body mass index, systolic blood pressure, total cholesterol, triglycerides, and use of antihypertensive and lipid-lowering medications.

Multiple imputations were performed in case of missing covariates (<5% for all covariates). Statistical analyses were conducted using IBM SPSS version 21 (IBM Corp), STATA version 13 for Windows (StataCorp, College Station, Texas) and @RISK software (Palisade).

Several sensitivity analyses were performed: (1) To account for potential reverse causation, we excluded NCD events or deaths that occurred during the first 2 years of follow-up. (2) We excluded participants using thyroid function-altering medications, as amiodarone or corticosteroids. (3) To detect a potential influence of follow-up duration on our results, we performed the analyses restricting the length of follow-up to 8 years (median follow-up time). (4) We additionally included CKD in the definition of NCD. (5) Chronic obstructive pulmonary disease (COPD) was not included in the primary outcome of NCD, due to the lack of solid evidence on the association of thyroid function with incident COPD. However, we performed a sensitivity analysis in which we included COPD in the definition of NCD. COPD was diagnosed based on an obstructive pre-bronchodilator spirometry ($FEV_1/FVC < 0.70$) according to the GOLD guidelines.[33] The incident date of COPD was defined as the

date of the first obstructive lung function examination, the date of COPD diagnosis in the medical records or the date of the first prescription of COPD medications, whichever came first. (6) Lastly, we assessed the differences in LE with and without CVD, diabetes, and cancer, separately, among the TSH and FT_4 tertiles.

RESULTS

Baseline characteristics of 7644 eligible participants are presented in Table 1. The mean (standard deviation) age of participants was 64.5 (9.7) years and 52.3% were women. Over a median follow-up time of 8 years, 1396 incident NCD events and 1422 deaths occurred. Both models yielded similar estimates, therefore we further report the results of the most adjusted model (Model 2).

Table 1. Baseline characteristics of 7644 participants

	Men	Women
Number	3647	3997
Age, years	64.2 (9.2)	64.8 (10.1)
Smoking, n (%)		
current	897 (24.6)	810 (20.3)
former	2143 (58.8)	1512 (37.8)
never	607 (16.6)	1675 (41.9)
Education, n (%)		
Elementary	332 (9.1)	588 (14.7)
Lower secondary	1041 (28.5)	2012 (50.3)
Higher secondary	1335 (36.6)	900 (22.5)
Tertiary	939 (25.7)	497 (12.4)
Marital status, n (%)		
Single	115 (3.2)	244 (6.1)
Married	3113 (85.4)	2474 (61.9)
Widowed	222 (6.1)	870 (21.8)
Divorced/Separated	197 (5.4)	409 (10.2)
BMI, kg/m^2	27.0 (3.5)	27.3 (4.6)
Systolic blood pressure, mm Hg	141.0 (20.3)	137.8 (21.5)
Use of antihypertensive medications, n (%)	811 (22.2)	927 (23.2)
Total cholesterol, mmol/l	5.4 (0.9)	5.9 (0.9)
Triglycerides, mmol/l	1.6 (0.9)	1.5 (0.7)
Use of lipid-lowering medications, n (%)	662 (18.2)	576 (14.4)
TSH, mIU/L, median (IQR)	1.8 (1.2-2.4)	1.9 (1.3-2.6)
FT_4, pmol/L	15.9 (2.0)	15.6 (1.9)

Data are mean (sd), unless otherwise specified. Abbreviations: sd, standard deviation; BMI, body-mass index; TSH, thyroid-stimulating hormone; IQR, interquartile range; FT_4, free thyroxine.

Association of thyroid function within the reference range with the risk of NCD and death

The association of TSH tertiles with the risk of incident NCD was not statistically significant (Table 2). Compared with the lowest tertile, the highest TSH tertile was associated with a lower risk of mortality among participants without NCD (HR, 0.67; 95% CI, 0.54 to 0.83) (Table 2). Also, there was a borderline statistically significant association between the highest TSH tertile and a lower risk of mortality among participants with NCD (HR, 0.88; 95% CI, 0.75 to 1.03) (Table 2).

The highest FT_4 tertile was associated with a 1.17 times higher risk of incident NCD than the lowest tertile (95% CI, 1.02 to 1.34) (Table 2). The highest FT_4 tertile was also associated with a 1.56 times higher risk of mortality among participants with NCD (95% CI, 1.32 to 1.85) and a 1.44 times higher risk of mortality among participants without NCD (95% CI, 1.15 to 1.79) (Table 2), compared with the lowest tertile.

Results for TSH and FT_4 analyses remained similar after excluding the events that occurred during the first 2 years of follow-up (Supplemental Table 1) and after excluding users of thyroid function-altering medications (Supplemental Table 1).

Table 2. HRs for incident NCD* and death among TSH and FT_4 tertiles

			TSH		FT_4	
Transition	**Cases/PY**	**TSH/FT_4 tertiles**	**Model 1 HR (95% CI)**	**Model 2 HR (95% CI)**	**Model 1 HR (95% CI)**	**Model 2 HR (95% CI)**
Incident NCD	1396/27705	Tertile 1	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)
		Tertile 2	0.98 (0.86; 1.11)	0.98 (0.86; 1.12)	**1.16 (1.01; 1.32)**	**1.17 (1.02; 1.33)**
		Tertile 3	1.05 (0.92; 1.19)	1.05 (0.92; 1.19)	**1.17 (1.02; 1.33)**	**1.17 (1.02; 1.34)**
Mortality among those without NCD	532/32828	Tertile 1	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)
		Tertile 2	**0.68 (0.55; 0.83)**	**0.70 (0.56; 0.85)**	1.21 (0.96; 1.51)	1.20 (0.95; 1.50)
		Tertile 3	**0.64 (0.52; 0.79)**	**0.67 (0.54; 0.83)**	**1.52 (1.22; 1.88)**	**1.44 (1.15; 1.79)**
Mortality among those with NCD	890/18456	Tertile 1	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)
		Tertile 2	0.90 (0.77; 1.06)	0.91 (0.78; 1.06)	**1.23 (1.03; 1.47)**	**1.24 (1.04; 1.47)**
		Tertile 3	**0.84 (0.72; 0.99)**	0.88 (0.75; 1.03)	**1.59 (1.35; 1.88)**	**1.56 (1.32; 1.85)**

*NCD includes cardiovascular disease, diabetes mellitus, and cancer. Model 1: age, sex, and cohort. Model 2: Model 1, smoking, alcohol intake, education level, marital status, body mass index, systolic blood pressure, total cholesterol, triglycerides, use of antihypertensive medications, and use of lipid-lowering medications. Abbreviations: HR, hazard ratio; TSH, thyroid-stimulating hormone; FT_4, free thyroxine; PY, person-years; CI, confidence interval; NCD, non-communicable diseases.

Association of thyroid function within the reference range with total LE and LE with and without NCD

Total LE increased significantly from the lowest to the middle TSH tertile and did not change substantially from the middle to the highest TSH tertile. Compared with those in the lowest tertile, men in the highest TSH tertile lived 1.5 (95% CI, 0.8 to 2.3) years longer overall, of which 0.1 (95% CI, -0.8 to 1.2) years longer without NCD and 1.4 (95% CI, 0.5 to 2.3) years longer with NCD (Table 3). Compared with those in the lowest tertile, women in the highest TSH tertile lived 1.5 (95% CI, 0.8 to 2.2) years longer overall, of which, 0.2 (95% CI, -0.8 to 1.3) years longer without NCD and 1.3 (95% CI, 0.3 to 2.1) years longer with NCD (Table 3).

Table 3. LE with and without NCD* at age 50 among TSH and FT_4 tertiles, in men and women

TSH/FT_4 tertiles	Differences in total LE†	Differences in LE free of NCD†	Differences in LE with NCD†
TSH tertiles			
Men			
Tertile 1	Reference	Reference	Reference
Tertile 2	**1.4 (0.5; 2.1)**	0.6 (-0.3; 1.4)	0.8 (-0.1; 1.8)
Tertile 3	**1.5 (0.8; 2.3)**	0.1 (-0.8; 1.2)	**1.4 (0.5; 2.3)**
Women			
Tertile 1	Reference	Reference	Reference
Tertile 2	**1.4 (0.6; 2.0)**	0.7 (-0.2; 1.6)	0.7 (-0.2; 1.5)
Tertile 3	**1.5 (0.8; 2.2)**	0.2 (-0.8; 1.3)	**1.3 (0.3; 2.1)**
FT_4 tertiles			
Men			
Tertile 1	Reference	Reference	Reference
Tertile 2	**-2.0 (-3.4;-0.7)**	**-1.6 (-2.8;-0.5)**	-0.4 (-1.6; 0.9)
Tertile 3	**-3.7 (-5.1;-2.2)**	**-1.8 (-3.1;-0.7)**	**-1.9 (-3.4;-0.3)**
Women			
Tertile 1	Reference	Reference	Reference
Tertile 2	**-1.8 (-3.0;-0.7)**	**-1.7 (-2.9;-0.6)**	-0.1 (-1.1; 1.2)
Tertile 3	**-3.3 (-4.7;-1.9)**	**-2.0 (-3.4;-0.7)**	-1.3 (-2.7; 0.2)

*NCD includes cardiovascular disease, diabetes mellitus, and cancer. Data are given as years (95% confidence intervals). †Differences in LE are calculated using the first tertile as reference. All life expectancies have been calculated with hazard ratios adjusted for age, cohort, smoking, alcohol intake, education level, marital status, body mass index, systolic blood pressure, total cholesterol, triglycerides, use of antihypertensive medications, and use of lipid-lowering medications. Abbreviations: LE, life expectancy; TSH, thyroid-stimulating hormone; FT_4, free thyroxine; NCD, non-communicable diseases.

Total LE decreased progressively with increasing FT_4 tertiles (Table 3). Compared with those in the lowest tertile, the difference in LE for men in the highest FT_4 tertile was -3.7 (95% CI, -5.1 to -2.2) years overall, of which -1.8 (95% CI, -3.1 to -0.7) years without NCD and -1.9 (95% CI, -3.4 to -0.3) years with NCD (Table 3). Compared with those in the lowest tertile, the difference in LE for women in the highest FT_4 tertile was -3.3 (95% CI, -4.7 to -1.9) years overall, of which -2.0 (95% CI, -3.4 to -0.7) years without NCD and -1.3 (95% CI, -2.7 to 0.2) years with NCD (Table 3). Results remained similar over the length of follow-up of 8 years (Supplemental Table 2) or after including CKD in the definition of NCD (Supplemental Table 3) or after including COPD in the definition of NCD (Supplemental Table 4). Compared with those in the lowest tertile, men and women in the highest FT_4 tertile lived less years free of CVD, diabetes, and cancer, respectively (Supplemental Tables 5-7).

DISCUSSION

In this large prospective population-based cohort study among middle-aged and older adults, we found meaningful differences in LE with and without NCD within the reference ranges of thyroid function. Participants with low-normal FT_4 levels lived up to 3.7 years longer overall, of which up to 1.9 years longer without NCD than those with high-normal FT_4 levels. Participants with high-normal TSH levels lived up to 1.5 years longer overall, of which up to 1.4 years longer with NCD than those with low-normal TSH levels. No meaningful sex differences throughout the TSH and FT_4 tertiles were observed.

Another study from our group previously reported meaningful differences in total LE and LE without CVD within the reference ranges of TSH and FT_4 levels.[14] Meanwhile, the present study provides a broader perspective by using the multidimensional measure of LE with and without NCD. Importantly, our findings reinforce the idea of qualitative and quantitative differences within the reference range of thyroid function. Furthermore, our results support a reevaluation of the reference ranges of thyroid function in middle-aged and older adults, implying the possibility of a downward shift in the FT_4 current limits.

Previous studies have suggested that the beneficial effects of high-normal thyroid function on metabolism can be counterbalanced by detrimental effects on other systems, such as the cardiovascular system.[11,14,17,18] In this context, our study sheds light onto the resultant of the system-specific effects of thyroid function,

suggesting that the overall risk of NCD increases in the high-normal range of FT_4 levels. However, the life expectancy estimates are not only attributable to the risk of developing the diseases, but also depend on the risk of dying before developing the diseases and the risk of dying after developing the diseases. In line with previous studies conducted in middle-aged and elderly, we showed that high-normal thyroid function is associated with an increased risk of mortality.[14,34,35] This was further translated into a decreased LE with and without NCD related to the high-normal range of thyroid function.

More specifically, *LE without NCD* is the resultant of 2 components: risk of incident NCD (transition 1) and risk of mortality among participants without NCD (transition 2). Compared with the lowest tertile, the highest FT_4 tertile was associated with a higher risk of incident NCD, meaning an earlier clinical manifestation of NCD and fewer years lived without NCD. The highest FT_4 tertile was also associated with an increased mortality risk among participants without NCD, resulting in a further decrease in total LE and LE without NCD. *LE with NCD* is the resultant of 2 components: risk of incident NCD (transition 1) and risk of mortality among participants with NCD (transition 3). Compared with the lowest tertile, the highest FT_4 tertile was associated with a 1.17 times higher risk of incident NCD, meaning an earlier clinical manifestation of NCD and more years lived with NCD. However, participants with NCD in the highest FT_4 tertile had an even higher risk of mortality (ie, 1.56 times higher), which explains the decrease in the number of years lived with NCD.

Our sensitivity analyses indicate that the estimates of LE with and without NCD reflect the combination of all NCD, and are not driven by one chronic disease alone. In a consistent manner, participants with low-normal thyroid function lived more years with CVD, diabetes, and cancer, respectively, than those with high-normal thyroid function. Besides, participants with low-normal thyroid function lived more years without CVD, diabetes, and cancer, respectively, than those with high-normal thyroid function. Together, these differences in the number of years lived with and without diseases contributed to the total differences in life expectancy within the reference range of thyroid function.

To the best of our knowledge, this is the first population-based cohort study that investigates the association between thyroid function and the risk of incident NCD. Also, this is the first study that examines the differences in LE with and without NCD within the reference range of thyroid function. We included a large number of participants with extensive and detailed information on covariates including exposures,

outcomes, and potential confounders. Events were adjudicated using standardized criteria. Another strength of our study is the prospective design with a long follow-up period. Thyroid function measurements were performed before the occurrence of incident NCD events. The possibility of reverse causation was taken into account by excluding the events that occurred during the first 2 years of follow-up. Multiple sensitivity analyses provided consistent findings.

Several limitations should also be considered. Due to the incomplete information on CKD, we could not include CKD in the primary outcome of NCD. The eGFR measurements were performed only twice, and the exact date of incident CKD was uncertain. However, we used the repeated measurements of eGFR to determine the slope of eGFR changes over time. Our results remained similar after including CKD in the definition of NCD. Moreover, we did not have data available on serum triiodothyronine levels. Nevertheless, TSH and FT_4 represent the most relevant measurements of thyroid function in clinical practice. Also, we lacked repeated measurements of thyroid function. However, it has been shown that studies using only one measurement of TSH and FT_4 can underestimate the association of thyroid function with morbidity and mortality by approximately one third, due to the regression dilution bias.[36] Thus, repeated measurements of thyroid function would be expected to strengthen our risk estimates. Besides, the possibility of residual confounding cannot be entirely ruled out due to the observational character of our study. Lastly, the Rotterdam Study includes predominantly white participants older than 45 years. Therefore, our findings need to be confirmed in other populations with similar characteristics to our population, as well as in other ethnicities and younger individuals.

Conclusions

In a population of middle-aged and older euthyroid subjects, we found that high-normal FT_4 levels were associated with an increased risk of incident NCD. Furthermore, we found meaningful differences in LE with and without NCD within the reference ranges of TSH and FT_4 levels. These results support a reevaluation of the current reference ranges of thyroid function. Future research is needed to replicate our findings, establish causality and elucidate the exact underlying mechanisms.

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SUPPLEMENTAL MATERIAL

Supplemental Table 1. HRs for incident NCD and death among TSH and FT_4 tertiles, after excluding the first 2 years of follow-up for NCD and death or after excluding the users of thyroid function-altering medications

		After excluding the first 2 years of follow-up for NCD and death			After excluding the users of thyroid function-altering medications		
Transition	**TSH/FT_4 tertiles**	**Cases/PY**	**TSH HR (95% CI)**	**FT_4 HR (95% CI)**	**Cases/PY**	**Model 1 HR (95% CI)**	**Model 2 HR (95% CI)**
Incident NCD	Tertile 1	1036/27312	1 (Reference)	1 (Reference)	1365/27334	1 (Reference)	1 (Reference)
	Tertile 2		0.92 (0.79; 1.06)	**1.18 (1.01; 1.37)**		**1.16 (1.00; 1.35)**	**1.18 (1.01; 1.37)**
	Tertile 3		1.02 (0.88; 1.18)	1.16 (1.00; 1.35)		1.16 (0.99; 1.34)	1.16 (1.00; 1.35)
Mortality among those without NCD	Tertile 1	385/30677	1 (Reference)	1 (Reference)	515/32341	1 (Reference)	1 (Reference)
	Tertile 2		**0.77 (0.60; 0.99)**	1.29 (0.99; 1.68)		1.29 (0.99; 1.68)	1.29 (0.99; 1.68)
	Tertile 3		**0.67 (0.51; 0.88)**	**1.42 (1.09; 1.84)**		**1.51 (1.18; 1.96)**	**1.42 (1.09; 1.84)**
Mortality among those with NCD	Tertile 1	682/16599	1 (Reference)	1 (Reference)	849/17991	1 (Reference)	1 (Reference)
	Tertile 2		0.90 (0.75; 1.07)	**1.27 (1.04; 1.56)**		**1.27 (1.04; 1.56)**	**1.27 (1.04; 1.56)**
	Tertile 3		**0.80 (0.66; 0.97)**	**1.55 (1.27; 1.87)**		**1.57 (1.30; 1.90)**	**1.55 (1.27; 1.87)**

Analyses were adjusted for age, sex, cohort, smoking, alcohol intake, education level, marital status, body mass index, systolic blood pressure, total cholesterol, triglycerides, use of antihypertensive medications, and use of lipid-lowering medications. Abbreviations: HR, hazard ratio; TSH, thyroid-stimulating hormone; FT_4, free thyroxine; PY, person-years; CI, confidence interval; NCD, non-communicable diseases.

Supplemental Table 2. LE at age 50 years, among TSH and FT_4 tertiles, in men and women over 8 years of follow-up

	Differences in total LE	Differences in LE free of NCD	Differences in LE with NCD
TSH tertiles			
Men			
Tertile 1	Reference	Reference	Reference
Tertile 2	1.8	0.3	1.5
Tertile 3	1.6	0	1.6
Women			
Tertile 1	Reference	Reference	Reference
Tertile 2	1.7	0.5	1.2
Tertile 3	1.5	0.1	1.4
FT_4 tertiles			
Men			
Tertile 1	Reference	Reference	Reference
Tertile 2	-1.6	-1.1	-0.4
Tertile 3	-3.5	-1.7	-1.8
Women			
Tertile 1	Reference	Reference	Reference
Tertile 2	-1.4	-1.2	-0.2
Tertile 3	-3.1	-1.9	-1.3

Data are given as years. Differences in LE are calculated using the first tertile as reference. All life expectancies have been calculated with hazard ratios adjusted for age, cohort, smoking, alcohol intake, education level, marital status, diabetes mellitus, body mass index, systolic blood pressure, total cholesterol, triglycerides, use of antihypertensive medications, and use of lipid-lowering medications. Abbreviations: LE, life expectancy; TSH, thyroid-stimulating hormone; FT_4, free thyroxine; NCD, non-communicable diseases.

Supplemental Table 3. LE with and without NCD* at age 50 years among TSH and FT_4 tertiles, in men and women

TSH/FT_4 tertiles	Differences in total LE	Differences in LE free of NCD	Differences in LE with NCD
TSH tertiles			
Men			
Tertile 1	Reference	Reference	Reference
Tertile 2	1.5	0.2	1.3
Tertile 3	1.8	0.1	1.6
Women			
Tertile 1	Reference	Reference	Reference
Tertile 2	1.5	0.3	1.1
Tertile 3	1.7	0.2	1.5
FT_4 tertiles			
Men			
Tertile 1	Reference	Reference	Reference
Tertile 2	-2.0	-1.3	-0.7
Tertile 3	-3.7	-1.7	-2.0
Women			
Tertile 1	Reference	Reference	Reference
Tertile 2	-1.7	-1.4	-0.2
Tertile 3	-3.3	-1.9	-1.4

*NCD includes cardiovascular disease, diabetes mellitus, cancer, and chronic kidney disease. Data are given as years. Differences in LE are calculated using the first tertile as reference. All life expectancies have been calculated with hazard ratios adjusted for age, cohort, smoking, alcohol intake, education level, marital status, body mass index, systolic blood pressure, total cholesterol, triglycerides, use of antihypertensive medications, and use of lipid-lowering medications. Abbreviations: LE, life expectancy; TSH, thyroid-stimulating hormone; FT_4, free thyroxine; NCD, non-communicable diseases.

Supplemental Table 4. LE with and without NCD* at age 50 years among TSH and FT_4 tertiles, in men and women

TSH/FT_4 tertiles	Differences in total LE	Differences in LE free of NCD	Differences in LE with NCD
TSH tertiles			
Men			
Tertile 1	Reference	Reference	Reference
Tertile 2	1.6	1.0	0.6
Tertile 3	1.5	0.1	1.4
Women			
Tertile 1	Reference	Reference	Reference
Tertile 2	1.5	1.2	0.4
Tertile 3	1.5	0.3	1.2
FT_4 tertiles			
Men			
Tertile 1	Reference	Reference	Reference
Tertile 2	-1.9	-1.3	-0.6
Tertile 3	-3.6	-1.7	-1.9
Women			
Tertile 1	Reference	Reference	Reference
Tertile 2	-1.7	-1.5	-0.2
Tertile 3	-3.1	-1.9	-1.2

*NCD includes cardiovascular disease, diabetes mellitus, chronic obstructive pulmonary disease, and cancer. Data are given as years (95% confidence intervals). †Differences in LE are calculated using the first tertile as reference. All life expectancies have been calculated with hazard ratios adjusted for age, cohort, smoking, alcohol intake, education level, marital status, body mass index, systolic blood pressure, total cholesterol, triglycerides, use of antihypertensive medications, and use of lipid-lowering medications. Abbreviations: LE, life expectancy; TSH, thyroid-stimulating hormone; FT_4, free thyroxine; NCD, non-communicable diseases.

Supplemental Table 5. LE with and without CVD at age 50 years among TSH and FT_4 tertiles, in men and women

TSH/FT_4 tertiles	Differences in total LE	Differences in LE free of CVD	Differences in LE with CVD
TSH tertiles			
Men			
Tertile 1	Reference	Reference	Reference
Tertile 2	1.8	2.1	-0.3
Tertile 3	2.0	1.3	0.7
Women			
Tertile 1	Reference	Reference	Reference
Tertile 2	1.6	1.9	-0.3
Tertile 3	1.7	1.2	0.5
FT_4 tertiles			
Men			
Tertile 1	Reference	Reference	Reference
Tertile 2	-2.1	-1.9	-0.2
Tertile 3	-3.9	-3.1	-0.8
Women			
Tertile 1	Reference	Reference	Reference
Tertile 2	-1.8	-1.7	-0.1
Tertile 3	-3.3	-2.8	-0.5

Data are given as years. Differences in LE are calculated using the first tertile as reference. All life expectancies have been calculated with hazard ratios adjusted for age, cohort, smoking, alcohol intake, education level, marital status, body mass index, systolic blood pressure, total cholesterol, triglycerides, use of antihypertensive medications, use of lipid-lowering medications, prevalent diabetes, and prevalent cancer. Abbreviations: LE, life expectancy; TSH, thyroid-stimulating hormone; FT_4, free thyroxine; CVD, cardiovascular disease.

Supplemental Table 6. LE with and without diabetes at age 50 years among TSH and FT_4 tertiles, in men and women

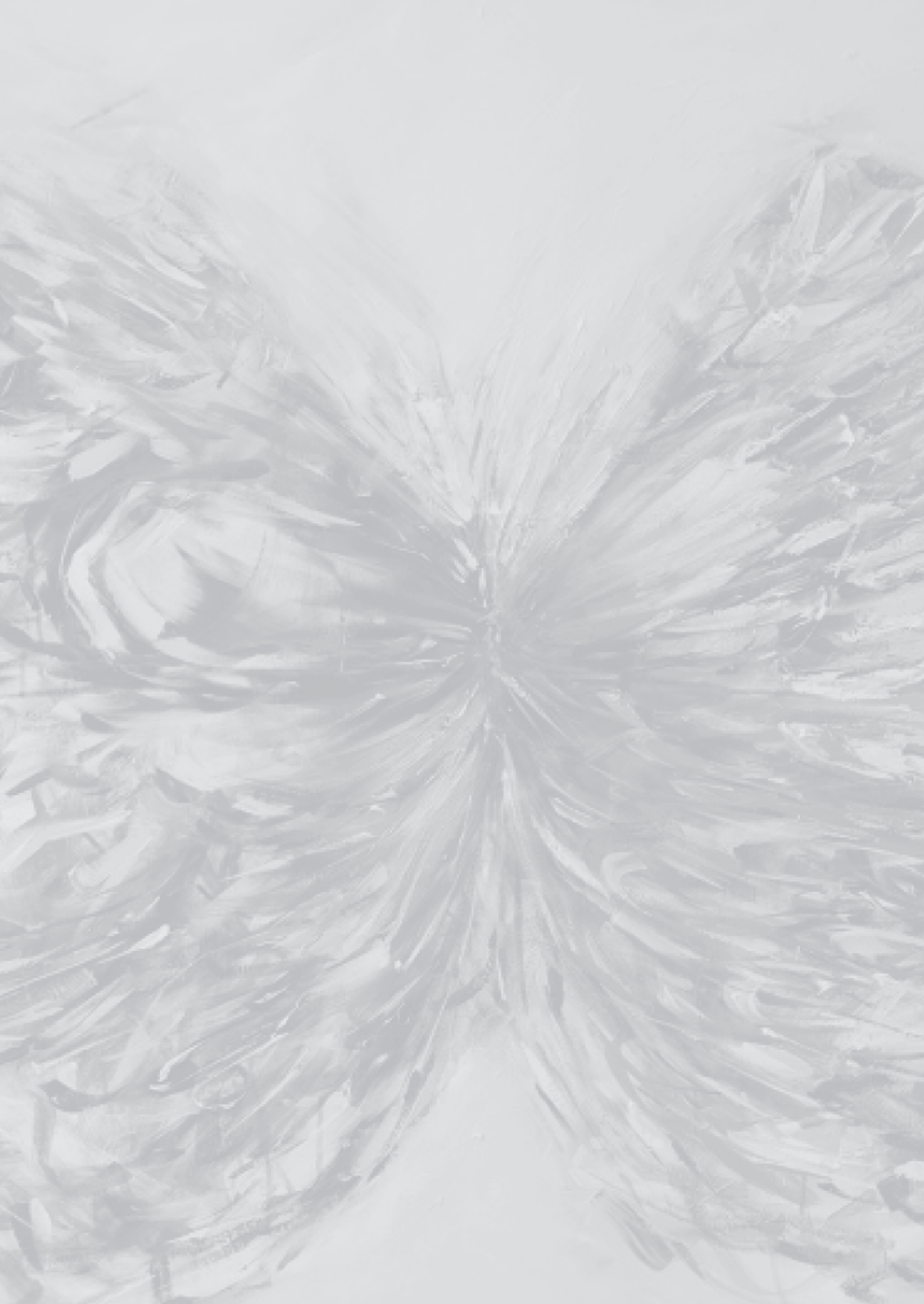
TSH/FT_4 tertiles	Differences in total LE	Differences in LE free of diabetes	Differences in LE with diabetes
TSH tertiles			
Men			
Tertile 1	Reference	Reference	Reference
Tertile 2	1.3	0.5	0.8
Tertile 3	1.8	1.3	0.5
Women			
Tertile 1	Reference	Reference	Reference
Tertile 2	1.1	0.3	0.8
Tertile 3	1.5	1.1	0.4
FT_4 tertiles			
Men			
Tertile 1	Reference	Reference	Reference
Tertile 2	-1.8	-1.1	-0.7
Tertile 3	-3.6	-2.5	-1.1
Women			
Tertile 1	Reference	Reference	Reference
Tertile 2	-1.5	-0.9	-0.6
Tertile 3	-3.1	-2.0	-1.1

Data are given as years. Differences in LE are calculated using the first tertile as reference. All life expectancies have been calculated with hazard ratios adjusted for age, cohort, smoking, alcohol intake, education level, marital status, body mass index, systolic blood pressure, total cholesterol, triglycerides, use of antihypertensive medications, use of lipid-lowering medications, prevalent cancer, and prevalent cardiovascular disease. Abbreviations: LE, life expectancy; TSH, thyroid-stimulating hormone; FT_4, free thyroxine.

Supplemental Table 7. LE with and without cancer at age 50 years among TSH and FT_4 tertiles, in men and women

TSH/FT_4 tertiles	Differences in total LE	Differences in LE free of cancer	Differences in LE with cancer
TSH tertiles			
Men			
Tertile 1	Reference	Reference	Reference
Tertile 2	1.6	1.3	0.3
Tertile 3	1.6	0.8	0.8
Women			
Tertile 1	Reference	Reference	Reference
Tertile 2	1.4	1.2	0.2
Tertile 3	1.4	0.7	0.7
FT_4 tertiles			
Men			
Tertile 1	Reference	Reference	Reference
Tertile 2	-1.8	-2.1	0.3
Tertile 3	-3.5	-2.7	-0.8
Women			
Tertile 1	Reference	Reference	Reference
Tertile 2	-1.7	-2.0	0.3
Tertile 3	-3.2	-2.6	-0.6

Data are given as years. Differences in LE are calculated using the first tertile as reference. All life expectancies have been calculated with hazard ratios adjusted for age, cohort, smoking, alcohol intake, education level, marital status, body mass index, systolic blood pressure, total cholesterol, triglycerides, use of antihypertensive medications, use of lipid-lowering medications, prevalent diabetes, and prevalent cardiovascular disease. Abbreviations: LE, life expectancy; TSH, thyroid-stimulating hormone; FT_4, free thyroxine.



CHAPTER 4

GENERAL DISCUSSION

REFLECTION ON THE MAIN FINDINGS

Chapter 2 of this thesis investigated the association of thyroid function with specific aspects of cardiometabolic health, including fatty liver, fibrosis, atherosclerosis, coagulation and epicardial adipose tissue (EAT). Chapter 3 investigated the association of thyroid function with general health, using several multidimensional measures that can reflect the pleiotropic effects of thyroid hormones. The main findings of this thesis are summarized in Table 1.

Thyroid function and specific aspects of cardiometabolic health

Thyroid function and the risk of nonalcoholic fatty liver disease: Nonalcoholic fatty liver disease (NAFLD) comprises a broad spectrum, ranging from steatosis to nonalcoholic steatohepatitis (NASH) with fibrosis.[1] NAFLD is the most common chronic liver condition with a global prevalence of more than 25%.[2,3] The prevalence of NAFLD is rapidly increasing, and NASH-related cirrhosis is becoming the leading cause of liver transplantation worldwide.[4] Therefore, the identification of novel modifiable risk factors of NAFLD is of major importance. Thyroid hormones can influence the intrahepatic lipid metabolism and the development of hepatic steatosis.[5] However, previous cross-sectional population-based studies investigating the association of thyroid function with hepatic steatosis have provided inconsistent results.[6,7] The only prospective study to date was performed by Xu et al, who showed that subclinical hypothyroidism is associated with an increased risk of developing NAFLD.[8] Still, the risk of NAFLD in the remaining categories of thyroid function, other than subclinical hypothyroidism, has not been prospectively explored. To address this gap, we investigated the association between the whole spectrum of thyroid function and the risk of NAFLD, in a large prospective population-based cohort study (Chapter 2.1).

Our results indicated that the association of thyroid function with NAFLD is not only limited to subclinical hypothyroidism, but is extended both within as well as outside the reference range of thyroid function. We found a negative linear association between free thyroxine (FT_4) levels and incident NAFLD, even among euthyroid subjects, as well as a positive linear association for thyroid-stimulating hormone (TSH) levels. The risk of NAFLD progressively decreased from a hypothyroid to a hyperthyroid state.

Aiming to provide some mechanistic insights, we further investigated whether and which cardiovascular risk factors could explain the association of thyroid function with NAFLD. Interestingly, our data indicated that circulating triglyceride levels

Table 1. Overview on the main findings of this thesis

	TSH	FT_4
Thyroid function and specific aspects of cardiometabolic health		
Nonalcoholic fatty liver disease (P)	↑/=	↓
Fibrotic diseases of the liver, lung and heart (C and P)	↑/=	=
Atherosclerosis (P)		
Coronary artery calcification	=	↑
Incident atherosclerotic CV events	=	↑
Atherosclerotic CV deaths	↓/=	↑
Coagulation factors (C)		
Fibrinogen	=	↑
VWF	=	↑
ADAMTS13	=	↓
Epicardial adipose tissue (C)	=	↑*
Potential mediating role (P)		
Fibrinogen & CV outcomes	=	M
VWF & CV outcomes	=	M
ADAMTS13 & CV outcomes	=	=
Epicardial adipose tissue & AF	=	=
Thyroid function and general health		
Frailty index changes (P)	=	↑
Gait aspects related to thyroid function (C)	Tandem, base of support, velocity	=
Life expectancy with and without NCD† (P)	↑	↓
Life expectancy with and without CV disease† (P)	↑	↓

Arrows represent the direction of the associations. Arrows pointing upwards represent positive associations, whereas arrows pointing downwards represent negative associations. Equal signs represent absence of statistically significant associations. *This finding applies only to subjects with large waist circumferences. †This study was performed among euthyroid participants. Abbreviations: TSH, thyroid-stimulating hormone (mIU/L); FT_4, free thyroxine (ng/dL); CV, cardiovascular; VWF, von Willebrand factor; ADAMTS13, a disintegrin and metalloprotease with thrombospondin motif repeats 13; AF, atrial fibrillation; M, mediator; NCD, non-communicable diseases; (P), prospective; (C), cross-sectional.

and body mass index may play an important role in the pathways linking thyroid function to NAFLD (ie, excess risk mediated by triglycerides and BMI, up to 22.2%).

Another novel aspect of our study was the utilization of liver elastography, which allowed us to additionally explore the risk of developing NAFLD with fibrosis. We showed that the risk of developing NAFLD with fibrosis progressively decreases from a hypothyroid to a hyperthyroid state. In line, lower thyroid function was associated with a higher risk of having NAFLD with fibrosis. Based on these findings, we hypothesize that low thyroid function may either accelerate the progression of liver steatosis to fibrosis or may directly stimulate the development of liver fibrosis, independent of steatosis. Further research will need to pinpoint the exact underlying mechanisms linking thyroid function to liver fibrosis. Moreover, future interventional studies in animals and humans are warranted to explore the potential beneficial effects of thyroid hormone supplementation on liver fibrosis.

Thyroid function and the risk of fibrosis: Current research is focused on the identification of novel determinants of fibrosis, which could be further translated into the development of effective antifibrotic drugs. Among other factors, low thyroid function has been implicated in the occurrence and progression of liver, pulmonary and myocardial fibrosis.[9-11] However, evidence is fragmented and inconclusive. In Chapter 2.2, we aimed to summarize the current evidence regarding the link between thyroid function and the risk of developing fibrosis in the human liver, lung and heart. After screening 1764 titles and abstracts, we identified 10 studies meeting the inclusion criteria.[6,12-20] Of the identified studies, 6 reported on liver fibrosis,[6,12-16] 2 on pulmonary fibrosis,[17,18] and 2 on myocardial fibrosis.[19,20] In the setting of diverse study populations, low thyroid function was consistently associated with increased odds of liver fibrosis, pulmonary fibrosis, and myocardial fibrosis. However, most of the evidence on this topic was based on cross-sectional data. In the future, adequately powered studies are needed to prospectively investigate the long-term effects of thyroid hormones on the occurrence and progression of fibrosis. Furthermore, future interventional studies in humans are needed to explore whether thyroid hormones or thyroid hormone analogues can prevent the progression of fibrosis. These investigations could lead to new avenues regarding the development of new therapies against fibrotic diseases. Lastly, future research is needed to elucidate the exact underlying mechanisms linking low thyroid function to fibrosis.

Thyroid function and the risk of atherosclerotic cardiovascular morbidity and mortality: Atherosclerosis is an accumulation of lipids and fibrous elements in the arterial walls, that can progress insidiously from an asymptomatic luminal narrowing of the arteries known as subclinical atherosclerosis, to the clinical manifestation of serious cardiovascular events to death.[21] Thyroid hormones have been linked to both proatherogenic[22,23] and antiatherogenic processes.[24] Epidemiological studies have so far investigated the association between specific ranges of thyroid function and distinct atherosclerosis events as coronary heart disease or stroke, with inconsistent results.[25-28] However, the role of thyroid function on the different stages of atherosclerosis progression has been unclear. Therefore, Chapter 2.3 examined the association between the full range of thyroid function and atherosclerosis throughout its spectrum, spanning from subclinical atherosclerosis (measured by coronary artery calcification) to clinical atherosclerotic cardiovascular (ASCV) events to ASCV mortality.

We found that FT_4 levels were positively and linearly associated with subclinical and clinical atherosclerosis. Increasing circulating FT_4 levels were associated with twice the odds of elevated coronary artery calcification scores, 87% greater risk of ASCV events, and double the risk of ASCV mortality. After restricting the analyses to euthyroid participants, these associations became even stronger. Specifically, the higher limit of the FT_4 reference range was associated with a 2.70 and a 4.15 times higher risk of incident ASCV events and ASCV mortality, respectively, compared with the lower reference limit. Also, the magnitude of the association for ASCV mortality was greater compared with non-ASCV mortality, which indicates that atherosclerosis plays an important role in the pathways linking high thyroid function to increased mortality risk.

Interestingly, our findings were independent of traditional cardiovascular risk factors such as hypertension or dyslipidemia. This suggests that the link between thyroid hormones and atherosclerosis can be explained by mechanisms that go beyond traditional cardiovascular risk factors. Hemodynamic changes, endothelial damage and increased thrombogenesis might play a role.

Thyroid function and coagulation: Large meta-analyses and systematic reviews of clinical-based studies have shown an increased risk of bleeding in hypothyroidism and an increased risk of thrombosis in hyperthyroidism.[23,29] Previous studies, however, did not investigate whether the anticoagulant effects of hypothyroidism and

the procoagulant effects of hyperthyroidism are extended even within the normal reference range of thyroid function. To address this, large studies in the general population are needed. Therefore, we performed a large population-based cohort study (Chapter 2.4), which investigated the association of thyroid function with several coagulation factors, including fibrinogen, von Willebrand factor (VWF) antigen and ADAMTS13 (a disintegrin and metalloprotease with thrombospondin motif repeats 13) activity.

We found that high and high-normal FT_4 levels are associated with increased fibrinogen and VWF levels, which indicate a procoagulant state. In line with our results, a direct role of thyroid hormones on the transcription of fibrinogen and VWF genes has been suggested.[30] Moreover, we found that high and high-normal FT_4 levels are associated with a decreased activity of ADAMTS13. This suggests that thyroid hormones can attenuate the role of ADAMTS13 in cleaving the procoagulant VWF multimers into less procoagulant forms. Future studies are warranted to explore the possibility of a direct effect of thyroid hormones on ADAMTS13.

Thyroid function and epicardial adipose tissue: The role of thyroid hormones on adiposity is complex and depends on the location and composition of adipose tissue. EAT, which surrounds the myocardium, possesses both white and brown adipose tissue properties.[31] A thyroid-hormone dependent gene, namely uncoupling protein 1 gene is recently shown to be highly expressed in EAT, thus suggesting a potential direct effect of thyroid hormones on mitochondrial uncoupling and EAT activation.[31] In line, several small studies (n<100) have suggested a potential role of thyroid dysfunction on EAT activation.[32-36] However, the association of thyroid function with EAT has not been explored in larger studies, nor has it been investigated throughout the full range of thyroid function in the general population. To address this, we performed a large population-based cohort study (Chapter 2.5), in which we evaluated EAT volumes by using well-standardized CT scan measurements. We found that higher FT_4 levels among participants with a large waist circumference are associated with larger EAT volumes. Hypothetically, increased thyroid hormone levels may contribute to the transdifferentiation of white adipocytes to brown adipocytes in the EAT of obese patients.

Mediators linking thyroid function to cardiovascular disease: In Chapters 2.4 and 2.5, we sought to provide mechanistic insights on the cardiovascular effects of thyroid hormones.

Previous evidence suggests that thyroid hormones regulate the synthesis of coagulation proteins, which in turn contribute to blood viscosity.[30] In Chapter 2.4, we investigated the potential mediating role of coagulation factors in the association of thyroid function with cardiovascular disease and mortality. VWF and fibrinogen (but not ADAMTS13) were identified as partial mediators linking FT_4 to cardiovascular events. The observed proportion of mediation was approximately 10%, which is quite considerable given the multiple mechanisms through which thyroid hormones affect cardiovascular health. Besides, it can be assumed that other coagulation factors, such as factors VII, VIII, IX, X, XII, can play an additional mediating role.[23,37,38] Unfortunately, data on these factors were not available in our study. If all the relevant coagulation factors could also be taken into account in our mediation analyses, the expected proportion of mediation related to coagulation may have been even higher than what we observed in our study.

Another explanation of the cardiovascular effects of thyroid hormones can be related to EAT. Thyroid hormones may affect the metabolism of EAT, which is a rapidly emerging risk factor for CVD, and particularly for atrial fibrillation (AF).[31,39] In Chapter 2.5, we investigated the potential mediating role of EAT in the association of thyroid function with AF. No relevant mediating effect of EAT was found, but our study indicated that the combined effects of thyroid hormones and EAT may increase the risk of AF synergistically.

Thyroid function and general health

Thyroid function associated with frailty index, a measure of frailty and general health: In Chapter 3.1, we investigated the association of thyroid function with frailty index, a well-established measure of frailty and general health. Interestingly, higher FT_4 levels were associated with increasing scores of frailty index, thus indicating an increased risk of health deterioration over time. These findings can reflect various deleterious effects of excess thyroid hormones, such as an increased risk of neurodegeneration, arrhythmias, hemodynamic changes, hypercoagulability, and reduction in bone mineral density.[40-43]

Identification of gait aspects related to thyroid function: Thyroid function in the general population has been linked to gait velocity.[44,45] However, it remains unknown whether other gait aspects are related to thyroid function. In Chapter 3.2, we identified tandem and base of support as novel gait domains related to thyroid function. The identification of thyroid-related gait domains may provide valuable hints on the pathways linking thyroid function to gait. Tandem, base of support and velocity have been so far associated with executive functioning, balance, and distinct brain structures (ie, prefrontal regions, parietal cortex, pallidum, putamen, and cerebellum) that may be targeted by thyroid hormones.[46-51]

Meaningful differences in life expectancy within the reference range of thyroid function: In view of the ongoing debate on redefining the reference ranges of TSH and FT_4 levels, several population-based studies have shown that variations in thyroid function within the reference range are associated with an increased risk of chronic diseases and mortality.[26,27,40,52-54] In Chapters 3.3 and 3.4, we extended the previous knowledge, by revealing meaningful differences in total life expectancy and life expectancy with and without diseases within the reference range of thyroid function. We found that subjects with low-normal thyroid function lived more years with and without non-communicable diseases than those with high-normal thyroid function. As a result, low-normal thyroid function was also associated with a prolonged life expectancy compared with high-normal thyroid function. These differences in life expectancy can reflect differences in the risk of adverse outcomes within the reference range of thyroid function. So far, low-normal TSH and high-normal FT_4 levels have been prospectively linked to an increased risk of AF, ASCV disease, heart failure, dementia and fractures, which are all associated with an increased risk of mortality.[27,55,56]

METHODOLOGICAL CONSIDERATIONS

Population-based cohort studies, strengths and pitfalls

Most studies included in this thesis were embedded within the framework of the Rotterdam Study, a prospective population-based cohort study in middle-aged and older adults.[57] Major strengths of our studies are the well-characterized population-based study sample, the large number of eligible participants, and the extensive

information on covariates including exposures, outcomes, potential confounders and mediators. Multiple sensitivity analyses provided consistent findings.

The following considerations should also be taken into account. Due to the observational character, population-based studies may be subject to bias including healthy volunteer bias or attrition bias. However, the possibility of these biases within the Rotterdam Study is minimized because of the random sampling of participants from the general population, the high response rate of participants and the nearly complete follow-up. Another type of bias in observational studies is the information bias, which can be either differential or non-differential. However, the possibility of information bias in our studies is minimized because of the utilization of standardized procedures of data collection, blinding of both participants and investigators to thyroid function measurements, and the adjudication of events in accordance with the current guidelines. Another concern in observational studies is related to the possibility of confounding. To address confounding, we adjusted our analyses for potential confounders, that were selected based on biological plausibility and previous literature.[58] However, the possibility of residual confounding cannot be ruled out. Potential confounders as smoking status, alcohol consumption and diet were self-reported and therefore could have been affected by measurement errors. Furthermore, the Rotterdam Study includes predominantly white Northern Europeans older than 45 years.[57] Therefore, we cannot ascertain the generalizability of our findings to other ethnicities or younger subjects.

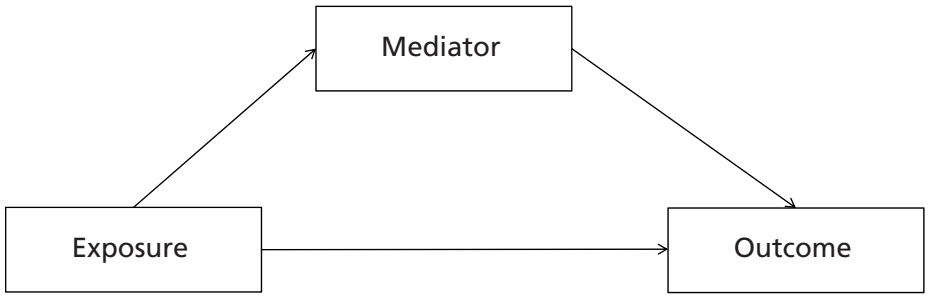
The studies of this thesis are mostly characterized by a prospective design with a long term follow-up time. Prospective designs provide evidence for a temporal relation between the exposure and outcome, thus reducing the possibility of reverse causation.[59] When applicable (eg, in Chapters 2.3, 3.3, 3.4), we additionally addressed reverse causation by excluding the events that occurred within the first two years of follow-up. However, some analyses in this thesis had a cross-sectional character, which does not provide evidence on the temporality of an association. This specifically applies to Chapter 3.2, in which we reported a nonlinear association of TSH levels with global gait. Similarly, the cross-sectional analysis in Chapter 3.1 revealed a nonlinear association of TSH levels with frailty index. Taken together, the cross-sectional analyses in Chapters 3.1 and 3.2 indicated that subjects with low and high thyroid function are more likely to have a worse heath state than those with normal thyroid function. On the other hand, the prospective analysis in Chapter 3.1 suggested that subjects with low thyroid function do not have an increased risk of

health deterioration over time. This discrepancy between the cross-sectional and prospective results can be attributed to the condition of "non-thyroidal illness", which is typically characterized by low thyroid function secondary to a poor health status.[60] The cross-sectional association between low thyroid function and poor health may reflect an alteration of thyroid parameters due to health-related issues rather than vice-versa. Therefore, the possibility of reverse causation due to the "non-thyroidal illness" needs to be taken into consideration when interpreting the results of cross-sectional studies on thyroid function.

Mediation analyses

Mediation analyses are helpful tools to explore and identify the underlying mechanisms of an association.[61] Besides the potential direct effect of the exposure on the outcome, the model proposes that the exposure influences a mediator, which in turn influences the outcome (Figure 1). In this thesis, mediation analyses were used to explore some of the pathways through which thyroid hormones affect cardiovascular health. In Chapter 2.4, we investigated the potential mediating role of several coagulation factors, including fibrinogen, VWF, and ADAMTS13 activity. We found evidence of partial mediation, implying that the coagulation factors account for some, but not all, of the association between FT_4 and cardiovascular events. In Chapter 2.5, we used the four-way decomposition approach, that unifies within a single framework the methods using mediation and interaction.[62] Although we did not describe a mediating role of EAT, we found evidence for a suggested interaction of FT_4 with EAT volumes on AF risk.

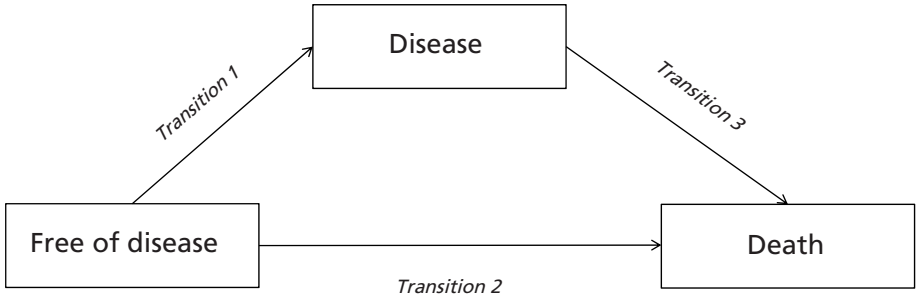
Figure 1. A statistical mediation model.



Multistate life tables

Multistate life tables is a demographic tool that is often used to estimate the total life expectancy and disease-specific life expectancies.[63] This tool combines the information of individuals in three possible health states, namely "free of disease", "disease" and "death". Potential transitions of participants are: (1) from free of disease to disease, (2) from free of disease to death, (3) from disease to death (Figure 2). The age of each individual is measured at the study entry, when the event occurs and at the end of the follow-up.[64] Age-specific rates of disease and mortality probabilities are used to calculate the total life expectancy and disease-specific life expectancies. A limitation of this approach is related to the arbitrary categorization of participants into health states, thus not accounting for the potential amelioration or aggravation of disease over time. Hence, the results derived from the multistate life table analysis are only an approximation of the real disease burden. In Chapters 3.3 and 3.4, the multistate life table analysis was used to investigate the association of thyroid function with total life expectancy and disease-specific life expectancies among euthyroid subjects. We found considerable differences in life expectancy within the reference range of thyroid function. These findings provide supporting evidence for a reevaluation of the current reference ranges of thyroid function.

Figure 2. Transitions in multistate life tables.



POTENTIAL IMPLICATIONS AND FUTURE DIRECTIONS

Causality

In this thesis, the biological plausibility of the research questions, the strength and consistency of the associations, the prospective designs of most studies and the various sensitivity analyses addressing reverse causation strongly suggest a causal

relation between exposures and outcomes.[65] Yet, the observational character of our studies does not allow us to establish causality.

In the future, methods of causal inference can be useful to gain further insights on the direction of the associations. Mendelian Randomization (MR) is a pragmatic method of causal inference which uses genetic variants in non-experimental data.[66,67] This approach is based on the principle that genetic variants are generally not associated with confounders owing to the random independent assortment of DNA at meiotic segregation of alleles.[68] Given that genetic variants are immune to reverse causation, MR can be useful to clarify the direction of an association. However, the following considerations need to be taken into account. First, MR studies are based on the critical assumption that the genetic variants should be associated with the exposure. To satisfy this assumption, a large proportion of TSH and FT_4 heritability needs to be explored and explained by future research. Second, genetic variants in MR can sometimes have multiple phenotypic effects, also known as pleiotropic effects.[69] Third, the effects of genetic variants may be buffered by compensatory developmental processes. Fourth, MR studies require very large sample sizes in order to obtain sufficient statistical power.

Randomized clinical trials (RCTs) can provide important evidence on the effects of treatment and can help overcome some limitations of the observational studies.[70] One of the major strengths of RCTs is randomization, which addresses the issue of confounding. However, the randomization of individuals to specific interventions can be unethical. Besides, RCTs are expensive and require a long time to be completed. Therefore, before designing new RCTs, data from previous RCTs and observational studies need to be carefully evaluated.[71] To date, guidelines for the treatment of subclinical thyroid dysfunction[72,73] are mainly based on the results of observational research and short-term small RCTs.[74-78] The long-term risks related to the treatment of subclinical thyroid dysfunction have not been comprehensively explored. Moreover, it remains unclear whether the optimal treatment thresholds for TSH and FT_4 levels are similar to the TSH and FT_4 reference ranges. As a result, thyroid patients often carry the risk of being overtreated or undertreated. In order to minimize this risk, adequately powered RCTs are warranted to explore the risks and benefits of the thyroid disease treatment and eventually establish the optimal treatment targets for the TSH and FT_4 levels.

Mechanisms

The mediation analyses in Chapters 2.1, 2.4, 2.5 of this thesis provide some novel insights on the pathways through which thyroid hormones affect cardiometabolic health. Specifically, our results in Chapter 2.1 suggest that even slight reductions in circulating thyroid hormone levels can increase the risk of NAFLD via affecting the metabolism of triglycerides and fat. Furthermore, our results in Chapter 2.4 indicate that high and high-normal thyroid hormones may increase the risk of cardiovascular diseases via altering the activity of circulating coagulation factors, such as VWF, ADAMTS13, and fibrinogen. Future observational studies are needed to replicate the results of our mediation analyses in other populations. In addition, *in vitro* and *in vivo* experimental studies are also needed to explore the exact mechanisms that can explain the role of thyroid function on cardiometabolic health and general health. Once identified, the modifiable mediators can be further targeted to eventually reduce the burden of the clinical implications of thyroid dysfunction.

Circulating thyroxine, a potential predictive marker?

Observational studies provide evidence for either the presence or absence of an association. Once an association is reported, additional research may further head towards new avenues regarding causation and underlying mechanisms. Regardless of whether an association is causal or not, it can still be very useful to make predictions.

The studies included in this thesis showed that variations in thyroid function are associated with specific aspects of cardiometabolic health (Chapter 2) and with several measures of general health (Chapter 3), independently of traditional cardiovascular risk factors. Remarkably, our observed associations among euthyroid participants were similar and sometimes even stronger than in the general population, indicating the robust consistency of our findings. Overall, our results suggest that thyroid function measurements among middle-aged and older adults can be considered as potential predictive markers for cardiometabolic health and general health.

FT_4 measurements may even represent a better predictive marker than TSH measurements. In line with other studies among middle-aged and older adults, we consistently showed that the association between FT_4 and clinical outcomes is generally stronger compared with the association between TSH and clinical outcomes (Table 1).[12,55,79] One potential explanation for these results is that the direct effects

of thyroid hormones on various organs may be independent from the effects of thyroid hormones on the pituitary gland. Another potential explanation is related to the ageing process, which may modify the TSH-FT_4 set point of the negative feedback mechanism.[80] In order to maintain the same FT_4 levels, older persons (such as the Rotterdam Study participants) may need different TSH levels compared with the younger persons.

Risk prediction modelling can become a new line of thyroid-related research. Presumably, the discrimination abilities of some current predictive models can be improved after adding FT_4 measurement as a potential predictor. Hence, future studies may aim to assess the potential role of FT_4 in predicting the risk of adverse outcomes. Besides, future studies may aim to identify potential subgroups at high risk for developing adverse outcomes. Once identified, subgroups at risk may further receive additional testing and care.

Reference ranges of TSH and FT_4 levels

Previous population-based studies have reported that variations in thyroid function within the reference range are associated with several diseases such as AF, stroke, and diabetes.[26,54,79] Studies included in this thesis extend the previous literature by showing that variations in thyroid function within the reference range are also associated with other clinical outcomes, such as NAFLD and atherosclerosis. These data, taken together, suggest that the effects of thyroid dysfunction are extended even within the reference ranges of TSH and FT_4 levels. Based on this evidence, future studies may need to perform a reevaluation of the current reference ranges of thyroid function.

The reevaluation of TSH and FT_4 reference ranges may have important clinical implications on the diagnosis and treatment of thyroid disease. Therefore, the criteria of reevaluation need to be carefully established. One approach of reevaluation can redefine the reference ranges of TSH and FT_4 levels based on the risk of developing adverse outcomes. This approach has two main challenges. First, an expert group will need to specify the relevant adverse outcomes that are related to thyroid function. To achieve this, inconsistent literature reports on the effects of thyroid function will need to be reconciliated. Second, risk estimates of all relevant adverse outcomes will need to be further combined into an integrated risk estimate. This is complex, because the beneficial effects of specific TSH and FT_4 levels on one system can be counterbalanced by their harmful effects on another system. Previ-

ous prospective population-based studies have reported that high-normal thyroid function constitutes a decreased risk of metabolic diseases (eg, diabetes), but an increased risk of cardiovascular diseases (eg, AF).[54,79] Additionally, we report that high-normal thyroid function constitutes a decreased risk of NAFLD, but an increased risk of ASCV events.[12,55] It is therefore challenging to determine the balance of overall benefits and risks for specific cutoffs of TSH and FT_4 levels. Moreover, the observed associations of thyroid function with various adverse events are generally linear, thus making it difficult to propose specific cutoffs.

Another approach for reevaluating the thyroid function reference ranges could be based on the measures of general health that reflect the pleiotropic effects of thyroid hormones. The major challenge of this approach is that a "golden standard" measure of general health does not exist. In these circumstances, future research can take into consideration our results in Chapters 3.3 and 3.4, which imply the possibility of a downward shift of FT_4 reference ranges.

Identification of novel thyroid hormone agonists

In line with previous studies,[54,79] our findings suggest that thyroid hormones exert either beneficial or harmful effects depending on the targeted organs (eg, Chapters 2.1, 2.3). Given the complexity of thyroid hormone action, researchers over the last years have been intrigued by the possibility of discovering novel thyroid hormone analogues that can maximize the beneficial effects of thyroid hormones and minimize their deleterious effects. Several interventional studies to date have suggested potential beneficial effects of thyroid hormone analogues in reducing the risk of metabolic diseases as hepatic steatosis, diabetes, hyperlipidemia, and obesity.[81-84] Unfortunately, detrimental effects of thyroid hormone analogues in various systems, including the cardiovascular system, have been reported.[85] The most promising thyroid hormone analogue, eprotirome, was not approved for clinical use in humans due to its adverse effects on the cartilage of animals.[85] In this context, one of the major challenges for future research remains the identification of novel effective thyroid hormone analogues. In particular, future studies may need to identify the thyroid hormone receptor genes that are responsible for the specific beneficial and deleterious effects of thyroid hormones.

Additional measurements

The Rotterdam Study is characterized by extensive and detailed information regarding the measurements of exposures, outcomes, potential confounders and mediators. Future studies may consider performing several additional measurements. First, repeated measurements of TSH and FT_4 levels could provide additional information on the strength and consistency of the associations. However, studies using only one measurement of TSH and FT_4 can underestimate the association of thyroid function with morbidity and mortality by approximately one third, due to the regression dilution bias.[86] Hence, repeated measurements of thyroid function would be expected to strengthen even more our risk estimates. Second, although TSH and FT_4 concentrations are currently considered as the most relevant measurements of thyroid function in clinical practice, measurements of free triiodothyronine (FT_3) levels could provide more comprehensive information on the action and bioavailability of thyroid hormones. Available FT_3 measurements allow the calculation of the FT_3/FT_4 ratio, which is a marker of peripheral thyroxine deiodination. Third, longitudinal measurements of EAT, coagulation factors and gait patterns would be expected to minimize the possibility of reverse causation that derives from cross-sectional designs.

CONCLUSIONS

This thesis extends the current knowledge on the association of thyroid function with specific aspects of cardiometabolic health. Adopting a broader perspective, we further present novel insights on the association of thyroid function with markers of general health. Our results suggest that the clinical consequences of thyroid dysfunction are extended even within the reference ranges of TSH and FT_4 levels, thus providing supporting evidence for a reevaluation of the current reference ranges of thyroid function. Further studies may consider incorporating thyroid function measurements in models for predicting the risk of adverse outcomes. Moreover, future research is needed to replicate our results, establish causality, and explore additional underlying mechanisms.

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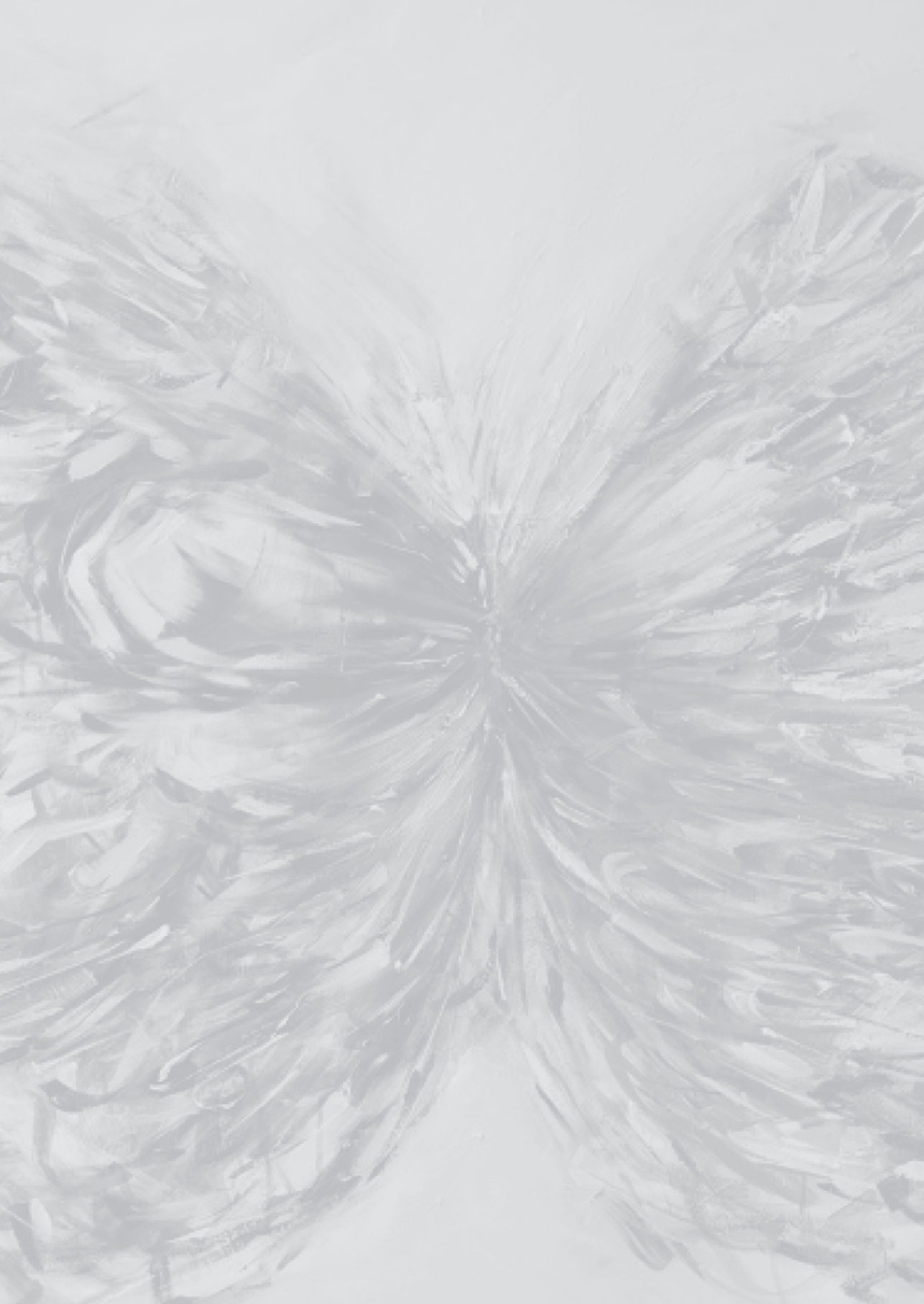


CHAPTER 5

SUMMARY/SAMENVATTING

SUMMARY

In **Chapter 1** of this thesis, we provide a general background on the pleiotropic effects of thyroid hormones, with a particular focus on cardiometabolic health. Furthermore, we describe the objectives, rationale and outline of this thesis. One study in this thesis is a systematic review. The other studies of this thesis are embedded within the Rotterdam Study, a large prospective population-based cohort study among middle-aged and older adults.

In **Chapter 2**, we aim to expand the current knowledge about the specific effects of thyroid function on cardiometabolic health. We therefore investigate the association of thyroid function with several aspects of cardiometabolic health, including nonalcoholic fatty liver disease (NAFLD), atherosclerosis, coagulation, and EAT (epicardial adipose tissue). In addition, we provide some novel mechanistic insights about the effects of thyroid hormones on cardiovascular system. In Chapter 2.1, we prospectively investigate the association of thyroid function with the risk of NAFLD and liver fibrosis. We show that low and low-normal thyroid function are associated with an increased risk of NAFLD and liver fibrosis. In Chapter 2.2, we systematically appraise the evidence regarding the role of thyroid function on fibrosis of the liver, lung, and heart in humans. The systematic review suggests that low thyroid function is consistently associated with an increased risk of fibrotic diseases in the liver, lung and heart. In Chapter 2.3, we examine the association of thyroid function with atherosclerosis throughout its spectrum, spanning from subclinical atherosclerosis (measured by coronary artery calcification) to clinical atherosclerotic cardiovascular events to atherosclerotic cardiovascular mortality. We show that FT_4 levels, even within the normal reference range, are positively and linearly associated with high coronary artery calcification score, and increased risk of atherosclerotic cardiovascular morbidity and mortality. Interestingly, these results are independent of traditional cardiovascular risk factors. In Chapter 2.4, we investigate the association of thyroid function with several coagulation factors, including procoagulant (ie, fibrinogen and von Willebrand factor antigen) and anticoagulant (ie, ADAMTS13 activity) factors. We show that higher FT_4 levels are associated with higher fibrinogen, higher von Willebrand factor antigen and lower ADAMTS13 activity, which indicate a procoagulant state. Fibrinogen and von Willebrand factor antigen (but not ADAMTS13) are further identified as partial mediators linking FT_4 to cardiovascular disease. In Chapter 2.5, we examine the association of thyroid function with EAT measured by

computed tomography. We show that higher FT_4 levels among participants with a large waist circumference are associated with larger EAT volumes. Furthermore, we investigate the potential mediating role of EAT in the association of thyroid function with atrial fibrillation. We do not find a mediating effect of EAT, though our results suggest that the synergic effects of thyroid hormones and EAT may increase the risk of atrial fibrillation.

In **Chapter 3**, we aim to yield novel insights on the qualitative and quantitative impact of thyroid function on health. We therefore investigate the association of thyroid function with general health, using several multidimensional measures that can reflect the pleiotropic effects of thyroid hormones, such as frailty index, gait patterns, total life expectancy, and disease-specific life expectancy. In Chapter 3.1, we investigate the association of thyroid function with frailty index. We show that higher FT_4 levels are associated with increasing scores of frailty index, thus indicating an increased risk of health deterioration over time. In Chapter 3.2, we present the association of thyroid function with gait patterns. Tandem and base of support are identified as novel gait domains related to thyroid function. In view of the ongoing debate on redefining the reference ranges of TSH and FT_4 levels, Chapters 3.3 and 3.4 investigate potential differences in total and disease-specific life expectancy, within the reference range of thyroid function. We show that subjects with low-normal thyroid function live longer overall, and also live more years with and without non-communicable diseases than those with high-normal thyroid function.

In **Chapter 4,** we provide a reflection on the main findings of this thesis and highlight several methodological considerations in epidemiological research. Moreover, we describe the potential clinical implications of our findings and future perspectives.

SAMENVATTING

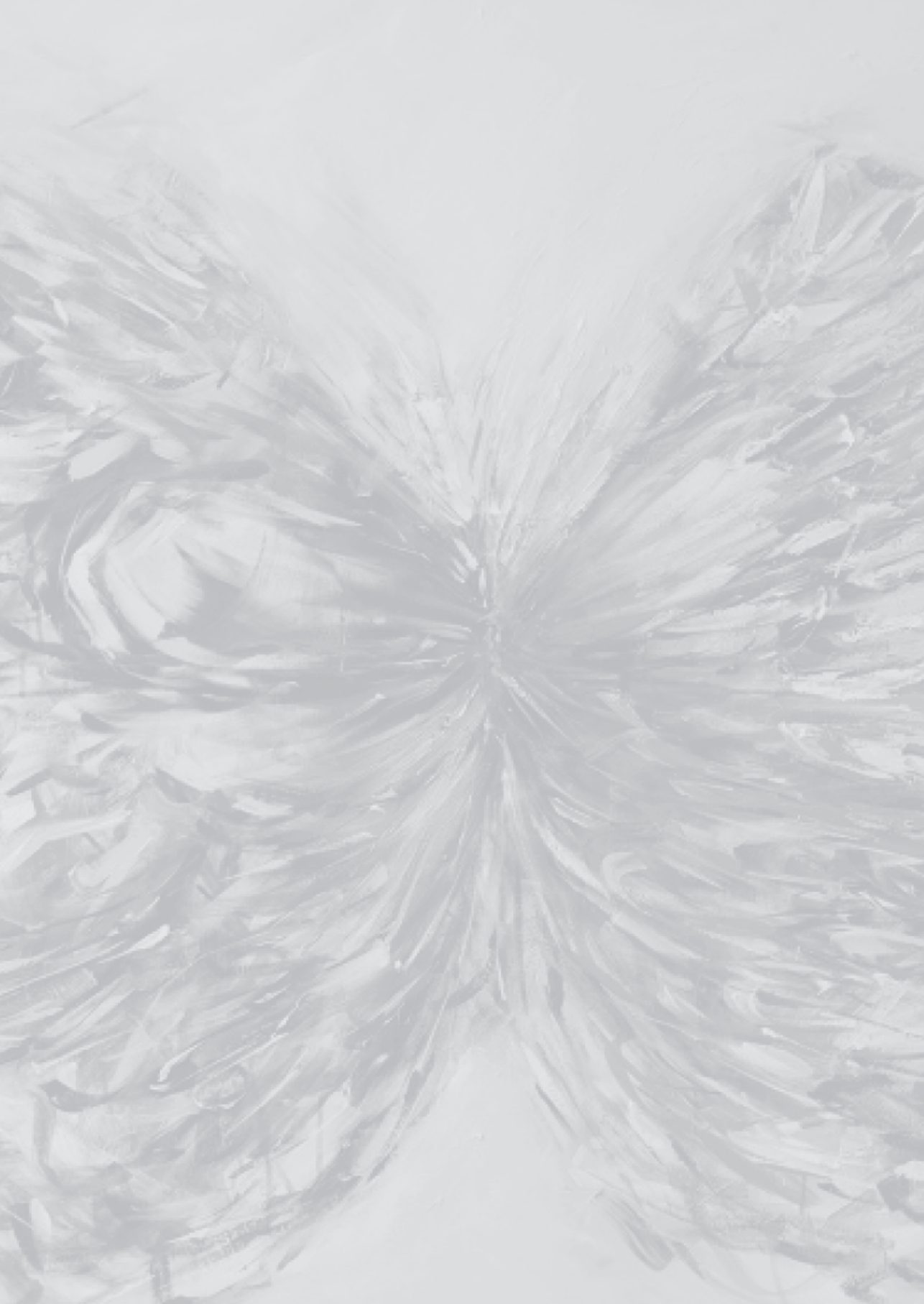
In **Hoofdstuk 1** van dit proefschrift beschrijven wij de pleiotrope effecten van schildklierhormonen, met bijzondere aandacht voor de effecten op de cardiometabole gezondheid. Verder beschrijven we de doelstellingen, beweegredenen en de hoofdlijnen van dit proefschrift. Eén onderzoek in dit proefschrift is een systematische review. De andere studies van dit proefschrift zijn onderdeel van de Rotterdam Study, een grote prospectieve populatie-gebaseerde cohortstudievan middelbare en oudere volwassenen.

In **Hoofdstuk 2** proberen we de huidige kennis over de specifieke effecten van de schildklierfunctie op cardiometabole gezondheid uit te breiden. We onderzoeken daarom de associatie van de schildklierfunctie met verschillende aspecten van de cardiometabole gezondheid, waaronder niet-alcoholische leververvetting (NAFLD), atherosclerose, coagulatie en epicardiaal vetweefsel. Daarnaast bieden we enkele nieuwe mechanistische inzichten over de effecten van schildklierhormonen op het cardiovasculaire systeem. In hoofdstuk 2.1 onderzoeken we prospectief de associatie van de schildklierfunctie met het risico op NAFLD en leverfibrose. We laten zien dat lage en laag-normale schildklierfunctie geassocieerd zijn met een verhoogd risico op NAFLD en leverfibrose. In hoofdstuk 2.2 beoordelen we systematisch het bewijsmateriaal met betrekking tot de rol van de schildklierfunctie op fibrose van de lever, de longen en het hart in de mens. De resultaten van de systematische review suggereren dat een lage schildklierfunctie gepaard gaat met een verhoogd risico op fibrotische aandoeningen van de lever, de longen en het hart. In Hoofdstuk 2.3 onderzoeken we de associatie van de schildklierfunctie met atherosclerose over het gehele spectrum, van subklinische atherosclerose (gemeten door coronaire arteriële calcificatie) tot klinische atherosclerotische cardiovasculaire uitkomsten tot atherosclerotische cardiovasculaire mortaliteit. We laten zien dat FT_4waarden, zelfs binnen het normale referentiegebied, positief en lineair geassocieerd zijn met een hoge score op de coronaire aderverkalking, en een verhoogd risico op atherosclerotische cardiovasculaire morbiditeit en mortaliteit. Interessant is dat deze resultaten onafhankelijk zijn van de traditionele cardiovasculaire risicofactoren. In hoofdstuk 2.4 onderzoeken we de associatie van de schildklierfunctie met verschillende stollingsfactoren, waaronder procoagulant (dwz fibrinogeen en von Willebrand factorantigeen) en anticoagulant (ie ADAMTS13-activiteit) factoren. We laten zien dat hogere FT_4-niveaus geassocieerd zijn met een hoger fibrinogeen,

hoger von Willebrand-factorantigeen en lagere ADAMTS13-activiteit, wat wijst op een procoagulante toestand. Fibrinogeen en von Willebrand factorantigeen (maar niet ADAMTS13) worden verder geïdentificeerd als gedeeltelijke bemiddelaars die FT_4 koppelen aan cardiovasculaire ziekte. In hoofdstuk 2.5 onderzoeken we de associatie van de schildklierfunctie met EAT gemeten met computertomografie. We laten zien dat hogere FT_4-niveaus bij deelnemers met een grote middelomtrek worden geassocieerd met grotere epicardiaal vetweefsel volumes. We vinden echter geen bemiddelend effect van epicardiaal vetweefsel in de asscociatie tussen schildklierfunctie en atrium fibrilleren.Wel suggereert onze studie dat de synergetische effecten van schildklierhormonen en epicardiaal vetweefsel het risico op atrium fibrilleren kunnen verhogen.

In **Hoofdstuk 3** verschaffen we nieuwe inzichten over de kwalitatieve en kwantitatieve impact van de schildklierfunctie op de gezondheid. We onderzoeken daarom de associatie van de schildklierfunctie met de algemene gezondheid, met behulp van verschillende multidimensionale maten die de pleiotrope effecten van schildklierhormonen kunnen weerspiegelen, zoals de frailty index (ie, kwetsbaarheidindex), looppatronen, de totale levensverwachting en de ziekte-specifieke levensverwachting. In hoofdstuk 3.1 onderzoeken we de associatie van de schildklierfunctie met de frailty index. We laten zien dat hogere FT_4-niveaus geassocieerd zijn met toenemende scores van de frailty index, wat dus wijst op een verhoogd risico op verslechtering van de gezondheid in de loop van de tijd. In hoofdstuk 3.2 presenteren we de associatie van de schildklierfunctie met looppatronen. Tandem en basis van ondersteuning worden geïdentificeerd als nieuwe loopdomeinen gerelateerd aan de schildklierfunctie. Met het oog op het lopende debat betreft het herdefiniëren van de referentiewaarden van TSH- en FT_4-waardes, onderzoeken de hoofdstukken 3.3 en 3.4 mogelijke verschillen in totale en ziekte-specifieke levensverwachting, binnen het referentiegebied van de schildklierfunctie. We laten zien dat personen met een laag-normale schildklierfunctie langer leven en ook meer jaren leven zowel met als zonder chronische aandoeningen dan mensen met een hoog-normale schildklierfunctie.

In **Hoofdstuk 4** geven we een reflectie op de belangrijkste bevindingen van dit proefschrift en benadrukken we verschillende methodologische overwegingen in epidemiologisch onderzoek. Bovendien beschrijven we de potentiële klinische implicaties van onze bevindingen en toekomstperspectieven.



CHAPTER 6

APPENDICES

LETTER TO THE EDITOR

RESPONSE REGARDING ARTICLE:"THYROID FUNCTION AND THE RISK OF ATHEROSCLEROTIC CARDIOVASCULAR MORBIDITY AND MORTALITY: THE ROTTERDAM STUDY"

Arjola Bano, Robin P. Peeters, Maryam Kavousi
Adapted from Circ Res. 2018;122(3):e18

We appreciate the comments by Drs. Zhao and Schooling regarding our recent publication.[1] In this study, we showed that higher circulating free thyroxine levels are associated with an increased risk of atherosclerosis throughout its full spectrum.

Zhao and Schooling argue that our observed associations are not supported by a Mendelian Randomization (MR) study, which found no evidence of an association between thyroid function and ischemic heart disease.[2] The following considerations need to be taken into account with regard to this study. First, the MR study focused on coronary artery disease. However, there are no MR studies, to our knowledge, on thyroid function and atherosclerotic cardiovascular disease. Second, the MR approach assumes that genetic variants determine the exposure. Still, only a limited number of genetic variants for free thyroxine have been identified, while a large proportion of thyroid function heritability remains unexplained. Third, the possibility of developmental compensation (ie, canalization) and pleiotropic effects of genetic variants cannot be excluded. Taken together, the current lack of genetic evidence does not rule out a potential effect of thyroid function on atherosclerotic cardiovascular disease.

We agree with the authors that we cannot prove a causal relationship due to the observational character of our study. However, the biological plausibility of our findings, the temporal relationship of the exposure with atherosclerotic events, and the various sensitivity analyses accounting for reverse causation strongly suggest an effect of thyroid function on atherosclerotic cardiovascular morbidity and mortality.[1] Moreover, our findings are consistent with the results of the randomized controlled trial cited by Zhao and Schooling.[3] This trial investigated the effects of dextrothyroxine treatment in patients with a history of myocardial infarction. The proportions of all-cause deaths, deaths from cardiovascular disease, deaths from coronary heart

disease and non-fatal recurrent myocardial infarctions were higher in the treatment arm than in the placebo arm of the trial, leading to a discontinuation of the trial after 36 months. In line, our study shows that higher free thyroxine levels are associated with an increased risk of atherosclerotic cardiovascular mortality, particularly among subjects with preexisting atherosclerotic cardiovascular disease.

Furthermore, Zhao and Schooling hypothesize that androgens can confound or mediate the association of thyroid function with atherosclerotic cardiovascular outcomes. This is an intriguing hypothesis, though the association of testosterone with atherosclerotic cardiovascular outcomes remains largely unclear.[4] To date, randomized controlled trials investigating the effects of testosterone treatment on major cardiovascular events have yielded conflicting results.[4] We had data available on testosterone concentrations in more than 99% of participants. After adding testosterone to our models, the association of thyroid function with atherosclerotic cardiovascular outcomes remained unchanged or became slightly stronger. Sex-specific analyses provided consistent findings before and after additional adjustments for testosterone. These data suggest that the association of thyroid function with atherosclerosis is independent of testosterone concentrations.

In the future, large MR studies are warranted to examine the association of genetically predicted thyroid function with atherosclerotic cardiovascular outcomes. Further investigations are also needed to elucidate the exact mechanisms linking thyroid function to atherosclerosis.

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LETTER TO THE EDITOR
LIFE-EXPECTANCY OF LOW-NORMAL THYROID FUNCTION: REPLY

Arjola Bano, Robin P. Peeters, Oscar H. Franco
Adapted from JAMA Intern Med. 2018;178(3):437-438

We would like to thank Dr. Inoue and colleagues for their interest in our recent publication.[1] As suggested by Inoue and colleagues, we now provide some additional information about our analyses. Our life expectancy calculations utilized age-adjusted hazards and age-specific mortality rates, based on the data of individuals at different ages and different health states. In the third transition (ie, mortality among those with cardiovascular disease [CVD]), stratified analyses among participants who acquired CVD during the study period and participants who already had a history of CVD at baseline consistently showed that high-normal thyroid function is linked to a higher mortality risk than low-normal thyroid function. In addition, sensitivity analyses restricting the follow-up time to different lengths (ie, 6, 8, 10 years of follow-up) yielded similar results. These data point towards a persistent effect of thyroid hormones on mortality risk across time.

Both the first and the second transition included participants who were free of CVD at baseline. Participants in the first transition (ie, incident CVD) were followed up until the occurrence of CVD events, whereas those in the second transition (ie, mortality among those without CVD) were followed up until they died. As a consequence, person years at risk were different between the two transitions.

We agree with Dr. Inoue and colleagues that low thyroid function has a negative impact on cardiovascular health. According to a large meta-analysis from the Thyroid Studies Collaboration, patients with subclinical hypothyroidism and thyrotropin levels above 10 mIU/L have an increased risk of coronary heart disease.[2] However, it is unclear to what extent these deleterious effects can be extended to lower thyrotropin levels. Future studies aiming to define the optimal reference ranges of thyrotropin and free thyroxine are warranted. Also, adequately powered randomized clinical trials focusing on the treatment of subclinical hypothyroidism in relation to CVD need to provide more robust evidence.

Our study showed that at the age of 50 years, individuals with low-normal thyroid function live longer than those with high-normal thyroid function. This is in line with other studies performed in middle-aged and older adults, suggesting that the risk of CVD and mortality increases from low-normal to high-normal thyroid function.[3,4] Such findings, however, may not be generalizable to younger populations.

We concur with Dr. Inoue and colleagues that low-normal thyroid function has been linked to metabolic syndrome. Our results, however, did not materially change after accounting for metabolic syndrome components, including diabetes mellitus, blood pressure, body mass index and lipid levels. Other factors beyond metabolic syndrome and its components therefore likely explain our findings. Within the reference range of thyroid function, differences in longevity can reflect differences in the risk of adverse outcomes. So far, lower thyrotropin and higher free thyroxine levels within the euthyroid range have been prospectively linked to an increased risk of atrial fibrillation, atherosclerotic CVD, heart failure and dementia, which are all associated with an increased risk of mortality.[4-6]

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LIST OF PUBLICATIONS AND MANUSCRIPTS

Bano A*, Chaker L*, Plompen EP, Hofman A, Dehghan A, Franco OH, Janssen HL, Darwish Murad S, Peeters RP. Thyroid function and the risk of nonalcoholic fatty liver disease: The Rotterdam Study. *J Clin Endocrinol Metab.* 2016;101(8):3204-3211.

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Bano A, Chaker L, de Maat MPM, Atiq F, Kavousi M, Franco OH, Mattace-Raso FUS, Leebeek FWG, Peeters RP. Thyroid function and cardiovascular disease: the mediating role of coagulation factors. *Submitted*

Bano A*, Gan E*, Addison C, Narayanan K, Weaver JU, Tsatlidis V, Razvi S. Age may influence the impact of TRAbs on thyroid function and relapse-risk in patients with Graves' disease. *Submitted*

Nano J, Pulido T, **Bano A**, Brahimaj A, Alferink LJM, Kraja B, Darwish Murad S, Dehghan A, Franco OH, Muka T. Fatty liver index and risk of diabetes, cardiovascular disease and mortality: The Rotterdam Study. *Submitted*

Bano A, Chaker L, Mattace-Raso FUS, Peeters RP, Franco OH. Association of thyroid function with life expectancy with and without non-communicable diseases: The Rotterdam Study. *Manuscript in preparation*

Bano A, et al. Thyroid function and the risk of fibrosis of the liver, lung, and heart: A systematic review of human studies. *Manuscript in preparation*

** Denotes equal contribution within a manuscript*

ABOUT THE AUTHOR

Arjola Bano was born on 21 October, 1985 in Kucove, Albania. In 2004, she graduated cum laude from the General High School in her home town. She started studying medicine at the University of Tirana in the same year and obtained her medical degree in 2010. Furthermore, she completed 4 years of residency in the Department of Internal Medicine, University Hospital Centre "Mother Teresa" of Tirana, and obtained the degree "Specialist in Internal Medicine" from the University of Tirana. As part of her medical training in Internal Medicine, Arjola was awarded a scholarship from the "Agence Universitaire de la Francophonie" to study Endocrinology at the University Hospital Center of Bicetre in Paris. In August 2014, Arjola was awarded a scholarship from "Erasmus Western Balkans" to pursue a Master of Science program in Clinical Epidemiology at the Netherlands Institute of Health Sciences, Erasmus University, Rotterdam (2014-2015). After obtaining her master degree, Arjola completed a Doctor of Science program in Clinical Epidemiology at the Netherlands Institute of Health Sciences (2015-2016). These studies in Clinical Epidemiology were combined with a PhD program at the Departments of Internal Medicine and Epidemiology of Erasmus Medical Center, under the supervision of Prof. Robin Peeters, Prof. Oscar Franco, Prof. Francesco Mattace-Raso and Dr. Layal Chaker. During her PhD training, Arjola performed multiple research projects that were focused on the role of thyroid function on cardiometabolic health and general health. Her research work is encompassed in this PhD thesis entitled "Thyroid function, cardiometabolic health and general health". In 2018, Arjola was awarded a fellowship grant from the European Thyroid Association to perform further research at the Cardiovascular Research Centre, Institute of Genetic Medicine, Newcastle University.

PHD PORTFOLIO

PhD student	**Arjola Bano**
Erasmus MC Department	Internal Medicine, Academic Center for Thyroid Diseases, Epidemiology
Promotors	Prof. Dr. Robin P. Peeters Prof. Dr. Oscar H. Franco Prof. Dr. Francesco U.S. Mattace-Raso
Co-promotor	Dr. Layal Chaker

Training	Year	ECTS
Master of Science in Clinical Epidemiology, NIHES, Erasmus Medical Center, Rotterdam, the Netherlands		
Research period Master of Science	2014-2015	33.5
General courses		
Study Design	2014	4.3
Biostatistical Methods I: Basic Principles	2014	5.7
Clinical Epidemiology	2014	5.7
Methodologic Topics in Epidemiologic Research	2014	1.4
Biostatistical Methods II: Classical Regression Models	2014	4.3
Principles of Research in Medicine	2014	0.7
Cohort studies	2014	0.7
Case-control studies	2014	0.7
Logistic Regression	2015	1.4
Causal Mediation Analysis	2015	0.7
Primary and Secondary Prevention Research	2015	0.7
Methods of Public Health Research	2014	0.7
Markers and Prediction Research	2015	0.7
Health Economics	2014	0.7
Introduction to Global Public Health	2015	0.7
The Practice of Epidemiologic Analyses	2015	0.7
Fundamentals of Medical Decision Making	2015	0.7
Advanced courses		
Planning and Evaluation of Screening	2015	1.4
Public Health in Low and Middle Income Countries	2015	3.0
Skill courses		
English Language	2014	1.4
Introduction to Medical Writing	2015	1.1
Courses for the Quantitative Researcher	2015	1.4

Doctor of Science in Clinical Epidemiology, NIHES, Erasmus Medical Center, Rotterdam, the Netherlands

Research period Doctor of Science	2015-2016	62.3
Bayesian Statistics	2016	1.4
Conceptual Foundation of Epidemiologic Study Design	2016	0.7
Causal Inference	2016	0.7
History of Epidemiologic Ideas	2016	0.7
Advances in Epidemiologic Analysis	2016	0.4
Causal Mediation Analysis	2016	0.7
Principles of Epidemiologic Data-analysis	2016	0.7
Missing Values in Clinical Research	2016	0.7
Women's Health	2016	0.9
Health Services: Research and Practice	2016	0.9
Introduction to Psychology in Medicine	2016	1.4

Academic Courses

Research Integrity	2017	0.3

Conferences – Oral presentations

Dutch Endocrine Meeting, Noordwijk, the Netherlands *Thyroid function and the risk of nonalcoholic fatty liver disease*	2016	0.7
Research Meeting of Internal Medicine, Rotterdam, the Netherlands *Low thyroid function linked to nonalcoholic fatty liver disease*	2016	0.7
Dutch Endocrine Meeting, Noordwijk, the Netherlands *Low-normal thyroid function associated with increased life expectancy: The Rotterdam Study*	2017	0.7
Endocrine Society Annual Meeting, Orlando, Florida (press released) *People with higher thyroid hormone levels may be at greater risk for atherosclerosis*	2017	0.7
Research Meeting of Internal Medicine, Rotterdam, the Netherlands *Low-normal thyroid function linked to an increased life expectancy*	2017	0.7
European Thyroid Association Annual Meeting, Belgrade, Serbia (Topic Highlights Session) *Association of thyroid function with life expectancy with and without cardiovascular disease*	2017	0.7
Dutch Endocrine Meeting, Noordwijk, the Netherlands *Thyroid function and cardiovascular outcomes: Is there a mediating role of coagulation?*	2018	0.7

Conferences – Poster presentations

Science Days Internal Medicine, Antwerp, Belgium *Thyroid function and the risk of nonalcoholic fatty liver disease*	2016	0.7
Science Days Internal Medicine, Antwerp, Belgium *Low-normal thyroid function associated with increased life expectancy*	2017	0.7
Science Days Internal Medicine, Antwerp, Belgium *Thyroid function and cardiovascular outcomes: Is there a mediating role of coagulation?*	2018	0.7

Seminars and meetings

Thyroid Lab Meetings	2015-2018	1.0
Cardiovascular group Meetings	2015-2018	1.0
Seminars at the Department of Epidemiology	2015-2018	1.0
2020 Epidemiology Meetings	2015-2018	1.0
Dutch Thyroid Club Annual Meetings, Amsterdam	2015-2017	0.7

Other Activities

Peer Reviews for JAMA, European Journal of Epidemiology	2017-2018	0.5

Grants

ERAWEB Scholarship for a Master of Science in Clinical Epidemiology	2014
Travel Grant "Erasmus Trustfonds" for ENDO 2017	2017
European Thyroid Association "Exchange fellowship grant"	2018

"The more one is able to leave one's cultural home, the more easily is one able to judge it, and the whole world as well, with the spiritual detachment and generosity necessary for true vision. The more easily, too, does one assess oneself and alien cultures with the same combination of intimacy and distance"

Edward Said

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To my friends and family

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