GLANDULA THYREOIDEA ET SENESCENS

THYROID AND AGING

Layal Chaker

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To my mom, who is no longer with me

To Wouter, who is always beside me

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**Denotes equal contribution*

CHAPTER 1

INTRODUCTION

CHAPTER 1.1

GENERAL INTRODUCTION AND OUTLINE

GENERAL INTRODUCTION

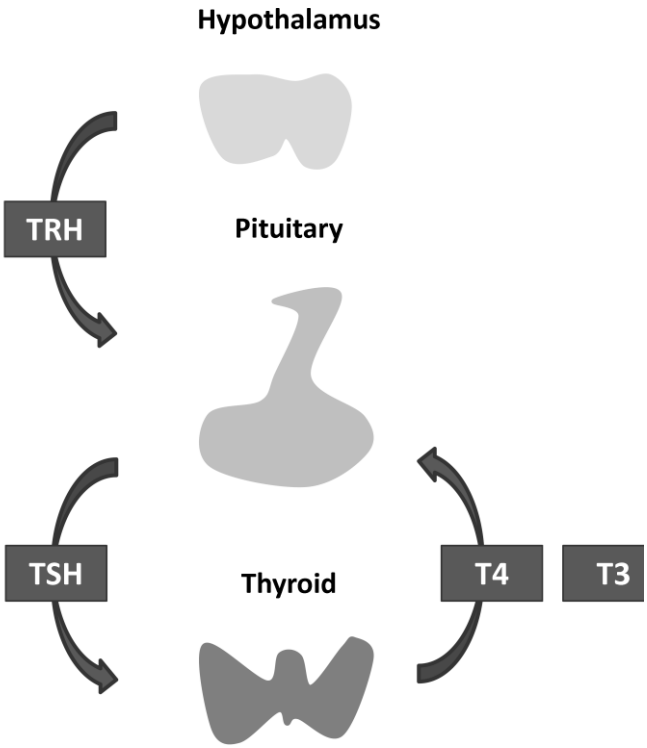
The thyroid gland is a butterfly-shaped organ in the base of neck and produces the thyroid hormones thyroxine (T4) and triiodothyronine (T3) in approximately a 14:1 ratio.[1] Circulating thyroid hormone levels are tightly regulated by the negative feedback mechanism of the hypothalamic-pituitary-thyroid axis (Figure 1); through thyrotropin-releasing hormone secreted from the hypothalamus and thyroid-stimulating hormone (TSH) from the pituitary. The set-point for this axis is individually determined, meaning that the TSH level needed to achieve similar levels of circulating thyroid hormones is different between individuals.[2] This is reflected in a smaller intra-individual variability than inter-individual variability. Main determinant of this set-point seems to be genetic variability[3,4] but other existential and environmental factors have been proposed to contribute, such as older age.[5] Serum T4 is predominantly bound to protein and thus not available for use in the end-target organs and tissues.[6] Therefore, free thyroxine (FT4) is used in both clinical and research settings as serum marker for thyroid function, next to TSH.

Thyroid hormone action is crucial for the function of virtually all organs and tissues, but transport and metabolism of thyroid hormone is organ- and cell specific.[7,8] The pleiotropic effects of thyroid hormone are also apparent in the signs and symptoms of overt thyroid disorders, hyperthyroidism (thyroid hormone excess) and hypothyroidism (thyroid hormone deficiency). For example; signs and complaints of hyperthyroidism include but are not restricted to palpitations, weight loss, diarrhea, heat intolerance, emotional liability, atrial fibrillation and low bone mineral density. However, due to the large variation in clinical presentation and general absence of symptom specificity[9,10], the definition of thyroid dysfunction is predominantly biochemical. Overt or clinical hypothyroidism is defined by serum TSH concentrations above the reference range and serum FT4 concentrations below the reference range, whilst with subclinical hypothyroidism FT4 is still within the reference range.[11] In the case of mild or subclinical hyperthyroidism TSH levels are below the reference range, and serum FT4 levels are above the reference range, whilst with subclinical hyperthyroidism FT4 is still within the reference range.[11]

Thyroid disorders have been long recognized disease entities. Seaweed, containing iodine, was described as a remedy for patients with goiter back in 2700 BCE. The first injection with extract of sheep thyroid for treatment of hypothyroidism was administered in 1891 Nevertheless; many unresolved issues concerning thyroid function and dysfunction still exist to date. Thyroid dysfunction, especially subclinical thyroid disorders, is common in the general population. The prevalence of clinical and subclinical hypothyroidism is 0.2-5.3 % and 4-15% of the adult population, respectively.[12-15] For overt and subclinical hyperthyroidism the prevalence is 0.8-1.3% and 0.6-9.8%, respectively.[16,17] These numbers depend on the definitions and assays used and populations studied, amongst others. Because clinical thyroid disease is generally treated, long-term consequences of thyroid dysfunction have been mainly studied in the context of subclinical hyper- and hypothyroidism. The most studied target of thyroid hormone in scientific literature has been the cardiovascular system, where both subclinical hyperthyroidism and subclinical hypothyroidism have been associated with coronary heart disease and heart failure in large individual participant data meta-analyses.[18-20] Some of these associations have also shown to extend within the currently defined reference ranges.[21] Therefore a debate concerning the accuracy and usefulness in terms of clinical care and prevention of the reference ranges has emerged.

The relation between TSH and FT4 has been described as log-linear, and TSH is therefore perceived as the most sensitive marker in subjects with thyroid disease.[22] Most studies to date investigating the association of thyroid function with clinical outcomes have incorporated FT4 serum concentrations only in the context of TSH (e.g. clinical versus subclinical hypothyroidism). However, in euthyroid subjects, TSH predominantly reflects the pituitary-thyroid axis set point[23] while, independent of TSH, circulating FT4 (and subsequently T3 acting intracellular) represents the bioavailable thyroid hormone that can be taken up by cells and thereby could lead to clinical consequences of thyroid hormones peripherally. It is unclear to what extent FT4 solely could be a sufficient marker of thyroid function, dysfunction and thyroid-related diseases and whether TSH and FT4 could be indicating complementary perspectives into thyroid health.

Figure 1 The negative feedback mechanism



T3 = Triiodothyronine; T4 = Thyroxine; TRH = Thyrotropin-releasing hormone; TSH = Thyroid-stimulating hormone.

AIM OF THIS THESIS

The aim of this thesis was to study the association of thyroid function, both TSH and FT4, with cardiovascular, neurological and other diseases of older age, beyond the current categorization for thyroid function and dysfunction. We hypothesized that risk of disease is not restricted to statistically defined reference ranges of thyroid function but represent a continuum of risk and as such also extends within these reference ranges.

OUTLINE OF THIS THESIS

In **Chapter 2** we focus on the outcome that to date has proven most relevant for thyroid function and dysfunction: cardiovascular disease and mortality. ***Chapters 2.1*** and ***2.2*** describe the association of thyroid function variations with sudden cardiac death and atrial fibrillation, respectively. The focus of the chapters is quantifying the association of thyroid function within the currently applied reference range with the increased risk of disease. In ***Chapter 2.3*** we examine the association of thyroid function with QT variability as a potential mediator of deleterious thyroid hormone effects on cardiac repolarization**.** Atherosclerosis is of specific interest due to the consistent finding in previous studies that the association of thyroid function with cardiovascular disease seems independent of cardiovascular risk factors and therefore perhaps not through atherosclerotic mediators. ***Chapter 2.4*** is devoted to the link of thyroid function with not only clinical makers of atherosclerosis (e.g. myocardial infarction), but also subclinical markers (i.e. coronary artery calcification score). ***Chapter 2.5*** aims to create a proof of concept for identifying the optimal health range for thyroid function based on the risk of cardiovascular disease i.e. a threshold for the laboratory markers of thyroid function based on the risk of cardiovascular mortality, coronary heart disease and stroke.

Chapter 3 describes the important implications of thyroid hormone and thyroid function for brain health. In ***Chapters 3.1, 3.2*** and ***3.3*** describes the association of subclinical thyroid dysfunction and variations of thyroid function within the normal range and the risk of stroke in a meta-analysis design. ***Chapter 3.1*** summarizes evidence from literature, while ***Chapters 3.2 and 3.3*** are conducted using an individual participant data meta-analysis method. ***Chapter 3.4*** investigates the association of thyroid function with dementia and hypothesizes that the association could be through vascular pathways, including markers of vascular disease on MRI. In ***Chapter 3.5*** we study potential age-dependent effects of thyroid function on measures of brain morphology and microstructure, as possible alternative mediators of the association between thyroid dysfunction and cognitive decline.

Chapter 4 examines metabolism, one of the other major targets of thyroid hormone action. Type II diabetes, together with thyroid disease the most common

endocrine disorder, is a large contributor to morbidity and mortality and a strong risk factor of cardiovascular disease amongst others. ***Chapter 4.1*** estimates the absolute risk of developing diabetes in the upcoming years in participants without diabetes at baseline in general and in persons with prediabetes specifically. Non-alcoholic fatty liver disease (NAFLD), which is strongly correlated with metabolic syndrome, is the most common chronic liver condition worldwide. Hypothyroidism has been implicated with NAFLD in previous studies. In ***Chapter 4.2*** we assess the association of thyroid function parameters and thyroid status with NAFLD cross-sectionally and longitudinally. Additionally we assessed the risk of liver fibrosis in those with NAFLD according to thyroid function.

In **Chapter 5** we determine the association of thyroid function with cancer risk (***Chapter 5.1***), chronic kidney disease (***Chapter 5.2***), gait (***Chapter 5.3***), and AMD (***Chapter 5.4***), all outcomes in or markers of advancing age, and examine changes of thyroid function with aging (in ***Chapter 5.5***).

Finally, in **Chapter 6**, we summarize the main findings of this thesis, define the implications of the results and discuss future perspectives into thyroid research and care.

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CHAPTER 1.2

HYPOTHYROIDISM

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ABSTRACT

Hypothyroidism is a common condition of thyroid hormone deficiency, which is readily diagnosed and treated but potentially fatal in untreated severe cases. The definition of hypothyroidism is based on the statistical reference ranges of the relevant biochemical parameters and is increasingly a matter of discussion. Clinical manifestations of hypothyroidism range from life threatening to no signs or symptoms. The most common symptoms of hypothyroidism in adults are fatigue, lethargy, cold intolerance, weight gain, constipation, change in voice and dry skin, but the clinical presentation can differ with age and gender, amongst other factors. Standard care of hypothyroidism is thyroid hormone replacement therapy with levothyroxine. However, a substantial proportion of patients reach biochemical treatment targets but remain unsatisfied due to persistent complaints. In this *Seminar* we discuss the epidemiology, etiology and symptomatology, summarize evidence on diagnosis, long-term risk, treatment modalities and management and highlight future directions for research into hypothyroidism.

INTRODUCTION

Hypothyroidism refers to the common pathological condition of thyroid hormone deficiency. When untreated, it can lead to serious adverse health effects and ultimately death. Due to large variation in clinical presentation and a general lack of symptom specificity, the definition of hypothyroidism is predominantly biochemical. Overt or clinical primary hypothyroidism is defined as thyroid-stimulating hormone (TSH) levels above the reference range with free thyroxine (FT4) levels below the reference range. Mild or subclinical hypothyroidism is defined by TSH levels above the reference range while FT4 levels are within the normal range and is commonly regarded as a sign of early thyroidal failure. Subclinical hypothyroidism, reviewed in a previous *Lancet* Seminar, is not the focus of this seminar.[1]

Whether the current reference ranges of TSH and FT4, defining thyroid dysfunction, are appropriate is a matter of debate. This is of clinical importance because these reference ranges are generally used as a threshold for treatment. Thyroid hormone replacement with levothyroxine (L-T4) is the standard of care in patients with hypothyroidism. However, a substantial proportion of L-T4-treated patients have persistent complaints despite reaching the biochemical therapy targets. This has prompted the question of whether L-T4 treatment is sufficient for all patients or whether alternative therapies (e.g. combination with liothyronine (L-T3) preparations) could be adopted.

EPIDEMIOLOGY

Prevalence and risk factors

The prevalence of overt hypothyroidism in the general population varies between 0.3 and 3.7% in the US and between 0.2 to 5.3% in Europe[2-6], depending on the definition used. A meta-analysis from studies across 9 European countries estimated the prevalence of undiagnosed hypothyroidism, including both overt and mild cases, around 5%.[5] Differences in iodine status affect the prevalence of hypothyroidism, which occurs more frequently both in populations with a relatively high iodine intake and in severely iodine-deficient populations.[7,8] Hypothyroidism occurs more frequently in women, at older age and in Caucasian populations, although data on ethnic differences are sparse.[3,9,10] Hypothyroidism is more

common in patients with auto-immune diseases, such as diabetes type 1, autoimmune gastric atrophy and celiac disease and can occur as part of multiple autoimmune endocrinopathies. Individuals with Downs' or Turners' syndrome have a higher risk of hypothyroidism. In contrast, tobacco smoking and moderate alcohol are associated with a lower risk of hypothyroidism.[11,12]

Genetic epidemiology

The heritability of TSH and FT4 is estimated to be 65% and 23-65% respectively.[13,14] Genome-wide association studies (GWAS), have so far only explained a small proportion of thyroid function variability[15] To date, only three GWAS have focused on hypothyroidism specifically.[16-18] The loci most consistently implicated in hypothyroidism include auto-immunity related genes and general and thyroid-specific regulatory genes (Table 1). Most of these are also related to serum TSH within the reference range.[15] Monogenetic disorders leading to (congenital) hypothyroidism are rare and include TSH resistance (due to an inactivating mutation in TSHR), thyroid dysgenesis and thyroid dyshormonogenesis.[19]

ETIOLOGY

Hypothyroidism can be classified as primary (due to thyroid hormone deficiency), secondary (e.g. due to TSH deficiency), tertiary (e.g. due to thyrotropin-releasing hormone [TRH] deficiency) and peripheral (extra-thyroidal) (Table 1). Central hypothyroidism (including both secondary and tertiary) and perhipheral hypothyroidism are rare and include less than 1% of hypothyroid patients.[20]

Primary hypothyroidism

Chronic autoimmune thyroiditis

In iodine sufficient areas, the most common etiology of hypothyroidism is chronic autoimmune (Hashimoto) thyroiditis. High antithyroid antibodies (predominantly thyroid peroxidase antibodies [TPOAb] and/or anti-thyroglobulin antibodies [TgAb]) are present in nearly all patients with auto-immune thyroiditis. High TPOAbs are also detected in approximately 11% of the general population.[6] In patients with subclinical hypothyroidism, TPOAbs measurements help to predict progression to overt disease.[21,22] The exact mechanisms underlying autoimmune thyroiditis are not known, but both genetic and environmental factors are involved. A higher

genetic risk score using 5 genetic variants identified by GWAS for TPOAb's showed a graded association with higher TSH levels and clinical hypothyroidism. [23] [24] Smokers have lower TPOAbs levels than non-smokers, and incidence of auto-immune thyroiditis increases after smoking cessation.[25,26] Other environmental factors implicated in autoimmune thyroiditis are vitamin D and selenium deficiency as well as moderate alcohol intake.[27]

Iodine

Iodine is an essential component of thyroid hormone. Iodine deficiency can result in goiter, thyroid nodules and hypothyroidism. The most severe consequence of iodine deficiency is cretinism (restricted mental and physical development in utero and childhood). Iodine fortification programs are one of the safest and cheapest public health interventions in the prevention of cognitive and physical impairment.[28,29] Despite efforts, suboptimal iodine status still affects large parts of Africa and Asia, but also several high-income countries in specific subpopulations, most notably pregnant women (e.g. some areas of Italy, US and UK).[29-31] In populations that shift from severe to mild iodine deficiency the prevalence of hypothyroidism decreases. In populations shifting from mild deficiency to optimum or excessive intake of iodine, the prevalence of autoimmune hypothyroidism increases.[32,33]

Drugs

Iodine-containing drugs, most infamously amiodarone, can restrict thyroid hormone production through iodine overload, immediately blocking thyroid hormone synthesis (Wolff-Chaikoff effect). Approximately 14% of amiodarone-treated patients develops hypothyroidism.[34] Lithium is also causes hypothyroidism via effects on thyroid hormone synthesis and release.[35] In one study 6% of lithium-treated patients needed L-T4 therapy within 18-months.[36]

Tyrosine kinase inhibitors (TKI) are targeted therapy for several cancers. In a meta-analysis of randomized controlled trials (RCT's), 14% of patients receiving sunitinib, developed hypothyroidism. Other TKI (e.g. sorafenib) are also, although less frequently, associated with risk of developing hypothyroidism.[37] Several other drugs, including interferon-alfa, thalidomide, certain monoclonal antibodies, anti-epileptic drugs and drugs for second-line treatment of multidrug resistant tuberculosis can also cause primary hypothyroidism (Table 1).

Other etiologies of primary hypothyroidism

Hypothyroidism is common after radioiodine treatment, after hemithyroidectomy or after neck radiation for cancer therapy.[38-42] In the long-term, ~80% of radioiodine treated Graves patients will become hypothyroid, even when low doses are used. Hypothyroidism is reported to occur in 55% of patients treated for toxic nodular goiter [38] and approximately 8% of patients treated for solitary toxic nodules.[43] In a meta-analysis of 32 studies, 20% of patients developed hypothyroidism after hemithyroidectomy.[41] Other causes of primary hypothyroidism include transient thyroiditis and infiltrative disease (Table 1).

Central hypothyroidism

Central hypothyroidism is rare and affects both sexes equally. It is more often related to pituitary than hypothalamic disorders but frequently involves both.[20] Biochemically, central hypothyroidism is defined by a low or low-normal TSH with an inappropriately low FT4. Occasionally, TSH is mildly elevated, likely due to a decreased bioactivity.[44] More than half of cases of central hypothyroidism are due to pituitary adenomas.[20] Other causes of central hypothyroidism include pituitary or hypothalamic dysfunction due to head trauma, pituitary apoplexy, Sheehan's syndrome, surgery, radiotherapy, genetic and, infiltrative disease. Several drugs are known to intervene on the hypothalamic-pituitary-thyroid axis (Table 1).[45,46]

Peripheral hypothyroidism

Consumptive hypothyroidism is caused by aberrant expression of the thyroid hormone inactivating deiodinase (Dio) 3 enzyme in tumor tissue. Although very rare, it can induce severe hypothyroidism. It was first described in a newborn with infantile hepatic hemangiomatosis but can also occur in patients with vascular and fibrotic tumors and gastrointestinal stromal tumors.[47] Patients with rare genetic syndromes leading to a reduced sensitivity to thyroid hormone (Table 1) usually have normal TSH values, but can also present with tissue-specific hypothyroidism.[48]

Table 1 Etiology of hypothyroidism

Primary hypothyroidism
Chronic autoimmune thyroiditis (Hashimoto)
Iodine
Severe iodine deficiency
Mild and severe iodine excess
Drugs such as amiodarone, lithium, TKI, interferon-alfa, thalidomide, monoclonal antibodies(e.g. ipilimumab and nivolumab), anti-epileptics(e.g. valproate)
Iatrogenic
Post radio-iodine treatment (e.g. for Graves' disease or toxic nodular disease)
Post (hemi)thyroidectomy
Post radiotherapy or surgery in neck/head region
Transient thyroiditis
Viral (De Quervains's)
Post-partum
Silent thyroiditis
Destructive thyroiditis
Thyroid gland infiltration*
Infectious (e.g. Mycoplasma)
Malignant (e.g. Thyroid malignancy, lymphoma, metastasis of malignancy elsewhere)
Autoimmune (e.g. Sarcoidosis)
Inflammatory (e.g. Riedels's)
Genetic*
Auto-immunity related genes (e.g. HLA class I region, PTPN22, SH2B3, and VAV3)
General and thyroid-specfic (e.g. FOXE1, ATXN2 and PDE8B)
Central hypothyroidism
Pituitary tumors (secreting or non-secreting)
Pituitary dysfunction (e.g. Sheehan's)
Hypothalamic dysfunction (e.g. posttraumatic)
TSH or TRH resistance
Drugs (e.g. dopamine, somatostatins, glucocorticosteroids, and RXR selective ligands).
Increased TSH due to leptin stimulation**
Peripheral (extra-thyroidal) hypothyroidism
Consumptive Hypothyroidism
Tissue-specific hypothyroidism due to decreased sensitivity to TH (e.g. mutations in *MCT8, SECISBP2, TRalpha* and *TRbeta*)

* rare causes of primary hypothyroidism, ** evidence mainly from animal models.
Abbreviations: TH thyroid hormone, TKI Tyrosine kinase inhibitors, TRH thyrotropin releasing hormone, TSH thyroid-stimulating hormone

CLINICAL PRESENTATION AND IMPLICATIONS

Myxedema coma and severe hypothyroidism

The clinical manifestations of hypothyroidism range from life threatening – in the case of myxedema coma – to no signs or symptoms. Myxedema coma, first described in the late 1900s when it was an inevitable outcome of longstanding untreated and severe hypothyroidism has become a rare condition. Nevertheless, due to its dramatic course with mortality rates of 40% despite treatment, early recognition is vital.[49] Myxedema coma leads to an altered mental status, hypothermia, progressive lethargy, and bradycardia and can eventually lead to multiple organ dysfunction syndrome and death. Early start of thyroid hormone therapy and other supportive measures is pivotal.[50]

Although very rare, severe primary hypothyroidism can lead to pituitary hyperplasia with concomitant pituitary pathology (e.g. secondary adrenal insufficiency) and symptoms (e.g. amenorrhea).[51]

Signs and symptoms

The most common symptoms of hypothyroidism in adults are fatigue, lethargy, cold intolerance, weight gain, constipation, change in voice and dry skin, but the clinical presentation can include a wide variety of symptoms and differs with age, gender and time between onset and diagnosis (Table 2).[52,53] Unfortunately, the specificity of these symptoms to diagnose hypothyroidism is low, especially in the elderly who present with fewer and less classical signs and symptoms.[53] Change of symptoms may predict hypothyroidism, since change in 7 or more symptoms in the last year increases the likelihood of hypothyroidism (likelihood ratio 8.7).[54] However, in a case-control study, none of 34 hypothyroidism-related symptoms could be used to identify patients with hypothyroidism.[55] Furthermore, 15% of patients with auto-immune hypothyroidism do not report any or only one hypothyroidism related symptom, while 70%of euthyroid controls have one or more thyroid-related complaints.[55]

Table 2 Clinical presentation and implications of hypothyroidism

Organ/System	Presentation	Signs and implications
General/ Metabolism	Weight gain Cold intolerance Fatigue	Increase in BMI Low metabolic rate *Myxedema* *Hypothermia*
Cardiovascular	Fatigue on exertion Shortness of breath	Dyslipidemia Bradycardia Hypertension, *Endothelial dysfunction/increased IMT* *Diastolic Dysfunction* *Pericardial effusion* *Hyperhomocystenaemia* *ECG changes*
Neurosensory	Hoarseness of voice Decreased taste Decreased vision Decreased hearing	Neuropathy Cochlear dysfunction Decreased olfactory and gustatory sensitivity
Neurological/ Psychiatric	Impaired memory Paresthesia Mood impairment	Impaired cognitive function Delayed relaxation of tendon reflexes *Depression* *Dementia* *Ataxia* *CTS & other nerve entrapment syndromes* *Myxedema Coma*
Gastrointestinal	Constipation	Reduced esophageal motility *NAFLD* *Ascites (very rare)*

Table 2 Clinical presentation and implications of hypothyroidism (continued)

Endocrinological	Infertility and subfertility Menstrual Disturbance Galactorrhoea	Goiter Glucose metabolism dysregulation Infertility Sexual dysfunction Increase prolactin *Pituitary hyperplasia*
Musculoskeletal	Muscle weakness, Muscle cramps, Arthralgia	Creatine phosphokinase elevation *Hoffman's syndrome,* *Osteoporotic fracture* *
Hemostatsis & Haematological	Bleeding Fatigue	Mild anemia *Acquired von Willebrand Disease* *Decreased Protein C and S* *Increased RDW* *Increased mean platelet volume*
Skin and hair	Dry skin Hair loss	Coarse skin *Loss of lateral eyebrows* *Yellow palms of the hand* *Alopecia areata*
Electrolytes and kidney function		Decreased eGFR *Hyponatremia*

* most probably overtreatment, Italic = uncommon presentation

Abbreviations: BMI Body-mass index; CTS Carpal Tunnel Syndrome; eGFR estimated glomerular filtration rate; IMT intima media thickness, NAFLD non-alcoholic fatty liver disease; RDW, red cell distribution width.

Hypothyroidism has clinical implications related to virtually all end-organs (Table 2), but the cardiovascular system is the most robustly studied. Hypothyroidism results in an increased vascular resistance, decreased cardiac output, decreased left ventricular function and changes in several other markers of cardiovascular contractility. Patients with hypothyroidism more often exhibit myocardial injuries and pericardial effusions than matched euthyroid controls.56 Furthermore, they have a higher prevalence of cardiovascular risk factors and often exhibit features of the metabolic syndrome including hypertension, increased waist circumference and dyslipidemia. 57 Hypothyroidism also increases total cholesterol, low-density lipoprotein and homocysteine levels.

Patients with acute hypothyroidism, in the context of thyroid cancer treatment, display a decline in mood and quality of life.[58] Hypothyroidism is considered a cause of reversible dementia, although unclear how often this occurs and in what proportion truly reversible.[59] Other manifestations include neurosensory, musculoskeletal and gastrointestinal signs and symptoms (Table 2). Due to the pleiotropic effects of thyroid hormone, hypothyroidism can also affect the course of other disorders. For example, statin intolerance is more prevalent in hypothyroid individuals than in controls.[60]

Long-term outcomes

Most long-term consequences of hypothyroidism have been studied in the context of subclinical hypothyroidism, as overt hypothyroidism is generally treated. Only few studies investigated the association of hypothyroidism with all-cause mortality and results are mainly available for subclinical hypothyroidism.[61-63] A study in 599 participants (all 85 years of age) suggested that (subclinical) hypothyroidism in the oldest old might be associated with better survival.[63] This could however not be confirmed in a large individual participant based meta-analyses, which included over 2500 participants above 80.[61] The same meta-analysis did show an increased risk of coronary heart disease (CHD) events and CHD mortality in those with higher TSH levels, particularly with TSH levels above 10 mIU/L. The relation between hypothyroidism and coronary artery disease however has been long recognized.[64] Subclinical hypothyroidism with TSH levels above 10 mIU/L have also been associated with an increased risk of heart failure.[61,65] Patients with hypothyroidism undergoing percutaneous coronary intervention had more major adverse

cardiovascular and cerebral events compared to those with normal thyroid function and to those with adequately treated hypothyroidism.[66] The association with stroke is less evident and might only be apparent in younger individuals.[67] Interestingly, patients with hypothyroidism have less neurological deficits post-stroke than controls[68]; normally after a stroke there is localized hypothyroidism due to induction of Dio3 in the ischemic brain area.[69,70] The risk of CHD in patients with subclinical hypothyroidism does not differ by TPOAb levels, suggesting that auto-immunity per se is not a contributing factor to the association.[71] Hypothyroidism can present with cognitive impairments but the association of hypothyroidism with development of cognitive impairment and dementia is controversial, since a recent population-based cohort study showed a protective effect of higher TSH levels on the risk of dementia.[72-75] Studies have also shown an association of hypothyroidism with NAFLD, cancer mortality, arthritis, kidney dysfunction and diabetes and in most cases causality is suggested but not proven.[76-79 80]

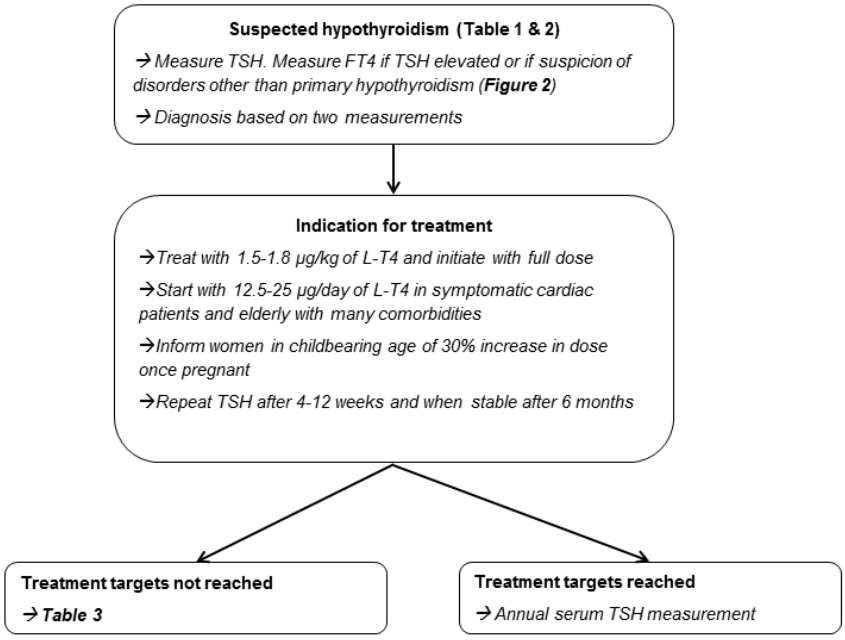
DIAGNOSIS

As previously mentioned, primary hypothyroidism is defined as TSH levels above the reference range (most commonly used 0.4-4.0 mIU/L) with FT4 levels below the reference range (Figure 1). The U.S. Preventive Service Task Force has suggested reserving the term “overt hypothyroidism” for cases where patients experience symptoms.[81] This will however be challenging to assess due to the large variability in presentation of even severe hypothyroidism. In addition, patients may only recognize prior symptoms after the initiation of L-T4 treatment.

TSH shows circadian fluctuations, with higher concentrations towards the evening. Patients with severe hypothyroidism show irregularity of TSH secretion.[82] Seasonal variations have also been described, with higher TSH levels in winter and spring.[83] There are no indications for routine measurement of total triiodothyronine (T3), total T4 or free T3. TPOAb measurement is not strictly necessary to diagnose hypothyroidism but is useful to affirm the diagnosis of autoimmune primary hypothyroidism. Hypothyroidism is often characterized by a hypo-echogenic pattern seen on thyroid sonography, even in the absence of raised TPOAb. However, in

absence of additional clinical indications, such as abnormal thyroid palpation, an ultrasound is not required.

Figure 1 Diagnosis and treatment of primary hypothyroidism



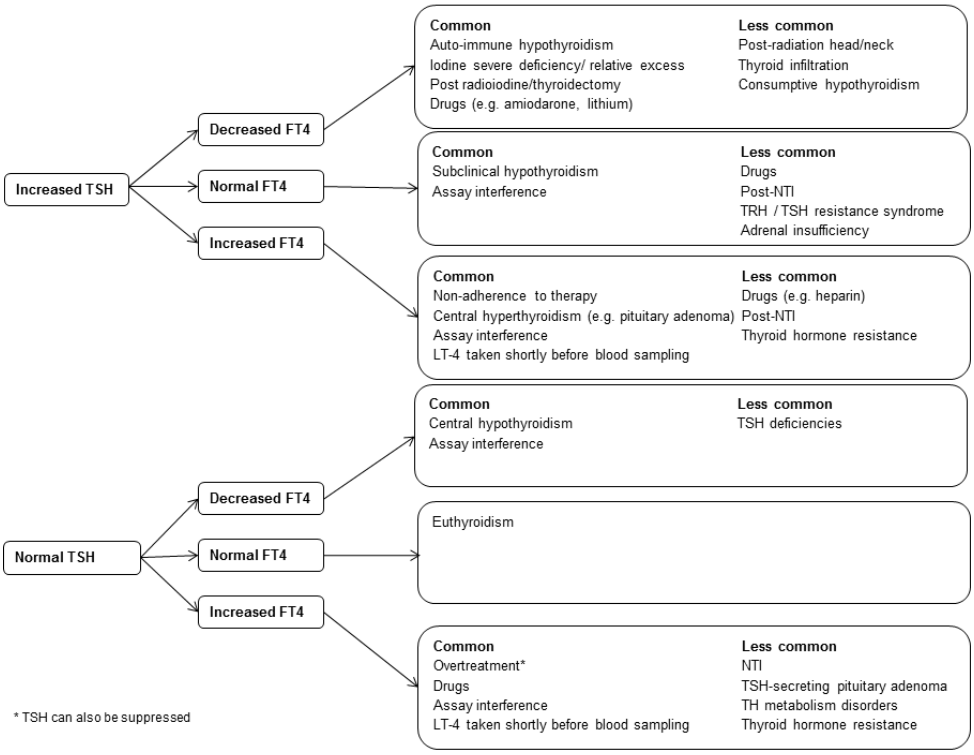
TSH; thryoid-stimulating hormone.

Reference ranges of thyroid function tests

Most commercially available TSH and FT4 assays are immunoassays and their reference ranges are statistically defined taking the 2.5th and 97.5th percentile in an apparently healthy population. The reference ranges therefore inherently do not consider symptomatology or risk of adverse events or disease. This is demonstrated by recent studies showing an increased risk of adverse events with thyroid function variations even within these reference ranges.[74,84-87] Furthermore, the reference ranges differ with age, gender and ethnic background.[88] The currently applied reference ranges for thyroid function have been a matter of debate in recent years, [89,90] Applying age-specific reference ranges, typically with a higher upper limit of TSH in older individuals, showed conflicting results concerning

younger individuals in studies from the UK and Australia.[91,92] Nevertheless, both showed a reclassification from abnormal to normal thyroid function predominantly in older individuals.[91,92] Due to lack of information on consequences of treatment, there are currently no convincing arguments to change the applied reference ranges.

Figure 2 Interpretation of thyroid function tests associated with hypothyroidism



NTI; non-thyroidal illness, TSH; thryoid-stimulating hormone.TRH; thyrotropin-releasing hormone.

Conditions interfering with diagnosis

There are several conditions that can interfere with the laboratory measurements of thyroid analytes. This should be suspected when thyroid function tests do not match the clinical presentation. Human anti-animal antibodies in patient's serum can cause falsely high TSH and can interfere with FT4 equilibrium dialysis platform assays. Heparin, including low molecular weight heparin, can lead to falsely

elevated levels of FT4.[93] High biotin intake, a popular over-the-counter supplement, can interfere with biotin-based hormone assays leading to false high or false low thyroid function tests.

FT4 measurement is important to diagnose hypothyroidism (e.g. central hypothyroidism) and during follow-up and treatment. The accuracy of FT4 immunoassays has been questioned in conditions that affect binding protein concentrations (i.e. albumin or thyroxine-binding globulin) such as pregnancy or acute illness. However, FT4 assays generally perform well in daily clinical practice. FT4 measured by liquid chromatography–tandem mass spectrometry (LC-MS/MS) seems to perform better in certain clinical conditions,[94] but is not available in the vast majority of centers.

Independent of measurement artifacts, severe illness is characterized by low thyroid hormone status, but the TSH is generally within the normal range, although it can be transiently increased during recovery of non-thyroidal illness (NTI).[95] Changes in thyroid hormone metabolism, thyroid hormone transporters, and thyroid hormone receptors all play a role in the pathofysiology. NTI occurs in different disease states, but is present in almost all critically ill patients.[95] Thyroid function testing should therefore not be performed in these patients, unless there is a clear suspicion of thyroid disease or central hypothyroidism. To date, there is no evidence that thyroid hormone replacement therapy is beneficial in critically ill patients.

Screening

Despite the high prevalence of hypothyroidism, easy diagnostics and cheap treatment, there is no consensus about TSH screening in specific subgroups of the general population. Several organizations, including the American Thyroid Association (ATA), American Association of Clinical Endocrinologists and the Latin American Thyroid Society recommend screening above a particular age (ranging from >35 year old individuals every 5 years to those ≥ 60 years once), especially in women.[96,97] The U.S. Preventive Services Task Force found no evidence for or against screening while the Royal College of Physicians London states that screening of the general population is unjustified.[81,98] However, evidence does support case finding of hypothyroidism in patients with dementia, infertility, auto-immune diseases, hypercholesterolemia, dysmenorrhea, family history of auto-

immune hypothyroidism, patients taking amiodarone or lithium or when iatrogenic hypothyroidism could be suspected (e.g. after neck radiation).

TREATMENT

L-T4 monotherapy in solid formulation, taken on an empty stomach, is the treatment of choice for hypothyroidism. Presence of clinical features of hypothyroidism with biochemical confirmation of overt hypothyroidism is the indication for treatment initiation. There is no rationale for not prescribing generic preparations, but switches between L-T4 products in patients that are stable are not recommended.[99] The optimal daily L-T4 dose in overt hypothyroidism is typically between 1.5–1.8 µg/actual body weight (kg) per day.[99-101] In patients with coronary artery disease the starting dose is generally 12.5-25 µg per day and should be gradually increased based on symptoms and TSH levels.[99] This regimen is often also preferred in the elderly, especially when many comorbidities exist.[99,100] In younger patients without comorbidities, the full dose can usually be given from the start with adequate monitoring to avoid overtreatment. After initial start of therapy, TSH measurement is repeated after 4-12 weeks, and subsequently every 6 months and, once stabilized, annually. Adjustment according to laboratory findings should be made, keeping in mind that in some patients (i.e. low body weight, higher age) small changes in dose can have substantial effects on serum TSH concentrations. The clinical significance of low T3 levels in some patients despite reaching normal TSH levels is unknown. T3 should not routinely be measured to evaluate treatment.[102]

Women of childbearing age

It is of pivotal importance to inform women of childbearing age with L-T4-treated hypothyroidism to increase their levothyroxine dosage in case of pregnancy. Due to several physiologic changes during pregnancy an increase in L-T4 dose is required to maintain euthyroidism.[103] Women with hypothyroidism should therefore increase the L-T4 dose by 30% once pregnant, and directly contact their physician for further guidance.[104] Screening, definition of (subclinical) hypothyroidism and potential treatment during pregnancy are beyond the scope of this seminar.[105,106]

Treatment targets

Treatment targets include normalization of TSH and resolution of physical and mental complaints, while avoiding under- or overtreatment.[99] Nevertheless, there is a considerable proportion of treated hypothyroid patients that do not reach these goals. An estimated 35-60% of patients treated with L-T4 are not within the target range of TSH (either over- or undertreated).[107,108] A study from the UK showed that after 5 years of L-T4 therapy almost 6% of patients have TSH levels below 0.1mIU/L and over 10% have TSH levels above 10.0mIU/L.[107] Overtreatment (i.e. iatrogenic subclinical or overt hyperthyroidism) bears the risk of deleterious health effects such as atrial fibrillation and osteoporosis and should always be avoided, especially in elderly and postmenopausal women. Under-treatment (i.e. persistent thyroid hormone deficiency) can result in increased risk of cardiovascular disease and persistent signs and symptoms. Treatment targets for central hypothyroidism are different from primary hypothyroidism because clinicians cannot rely on the "reflex TSH strategy". Further information on treatment of central hypothyroidism can be found elsewhere.[20]

Causes of not reaching treatment targets

Causes of not reaching therapy targets include inadequate dosage prescription or intake, interaction with supplements or medication, concurrent medical conditions and non-adherence to therapy (Table 3). Lower LT4doses are needed to suppress TSH secretion in the elderly and higher doses are needed after thyroidectomy. LT4 treatment and therapy targets in the context of thyroid malignancy are beyond the scope of this seminar.

L-T4 is absorbed in the small intestine and intake is advised in the morning 30-60 minutes before breakfast. Intake before bedtime (2 to 3 hours after last meal) may improve absorption, and can be considered to increase compliance.[109] Multiple drugs can interfere with absorption, availability or metabolism of L-T4, although evidence for some of these preparations comes from N=1 trials. Gastrointestinal conditions that reduce L-T4 absorption include H. Pylori gastritis, celiac disease, and, autoimmune atrophic gastritis. Some studies suggest that liquid and soft gel formulations of L-T4 do not depend on gastric pH for their absorption, and could provide a solution for patients with difficulties ingesting L-T4 30-60 minutes prior to breakfast.[110,111] A recent double-blind randomized cross-over trial of liquid

thyroxine in 77 treatment-naive hypothyroidism patients demonstrated no significant differences in thyroid function tests when the liquid preparation was ingested at or 30 minutes before breakfast.[112] However, no studies to date have compared liquid gel formulations of L-T4 with solid formulations in relation to clinical outcomes.

If high TSH persists and other etiologies have been excluded, the possibility of non-adherence, a common cause of therapy failure, should be considered and discussed with the patient. High TSH with normal or high-normal FT4 values can be a result of L-T4 tablets taken shortly before blood sampling. A supervised thyroxine absorption test to distinguish non-adherence from other reasons for undertreatment should be considered. A protocol that combines both acute and longer-term supervised administration has been suggested and is presented in Figure 3.[113,114]

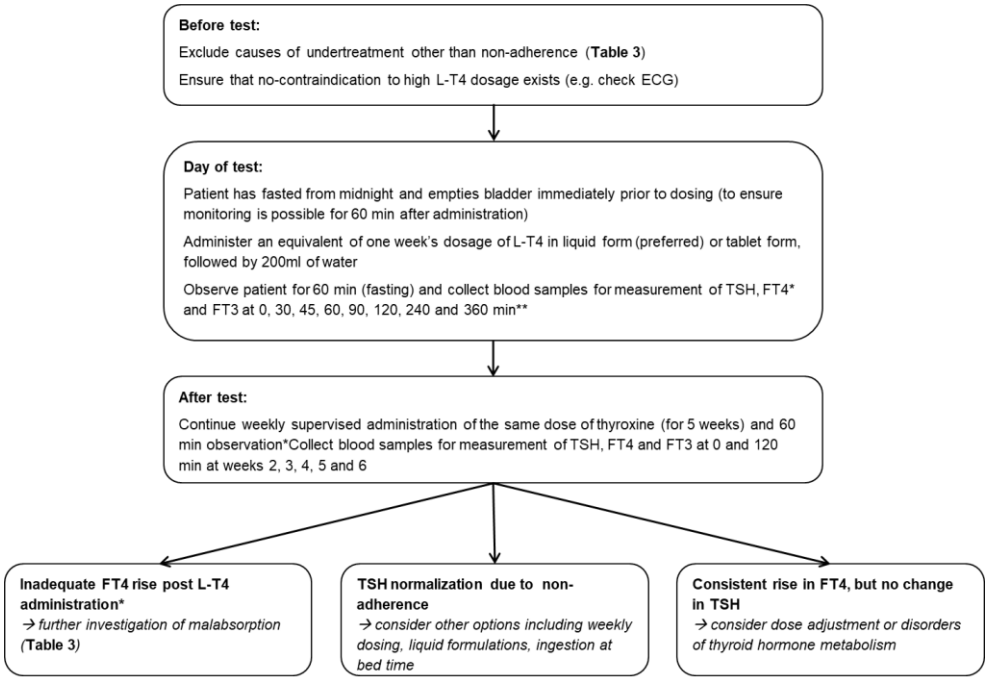
Persistent complaints despite biochemical normalization of TSH

About 5-10% of biochemically well-controlled patients with L-T4 treated hypothyroidism experience persistent symptoms such as depression and impaired mental well-being.[115] First of all, concurrent diseases or perimenopausal status may cause these complaints and should be excluded. Patients with hypothyroidism have a higher prevalence of autoimmune pathologies that could give rise to similar symptoms. Some studies suggest that auto-immunity per se could lead to persistent symptoms.[116] Circulating thyroid hormone levels are regulated by the hypothalamic-pituitary-thyroid axis with an individually determined set-point, reflected in a smaller intra-individual variability than inter-individual variability.[117] This means that the TSH level needed to achieve similar levels of circulating thyroid hormones differs between individuals. Differences in individual set-point may explain why patients with similar TSH levels under treatment respond differently. However, the individual set-point prior to hypothyroidism is rarely known. Also, studies that targeted different TSH goals in hypothyroid patients generally do not show alterations in well-being or other clinical parameters.[118,119]

An alternative explanation for persistent complaints in specific patients may be the imperfections of L-T4 monotherapy itself. It is generally accepted that L-T4 can ensure adequate concentrations of circuating T4 which can then be converted into T3 by deiodination (by Dio1 and Dio2). However, in euthyroid patients, about 20%

of circulating T3 is derived from direct thyroidal secretion, whereas in patients on L-T4 monotherapy all T3 is derived from peripheral T4 to T3 conversion. As a consequence, patients on L-T4 monotherapy have higher FT4 to FT3 ratios than euthyroid individuals. Some patients with normalized TSH have serum T3 concentrations below the reference range, while FT4 serum levels are high.[120-122] The clinical significance of this is unknown. However, L-T4 monotherapy cannot restore physiological T3 concentrations or thyroid hormone-dependent biological effects in all tissues of hypothyroid rats.[123,124] Although a normal TSH reflects euthyroidism at the level of the pituitary, this may not necessarily reflect euthyroidism in all tissues. Tissue-specific differences in inactivation of Dio2 may play a role, resulting in normalization of T3 levels in the hypothalamus before T3 levels have fully normalized in the rest of the body.[123]

Figure 3 Thyroxine absorption test



Protocol for supervised thyroxine absorption test, followed by weekly supervised thyroxine administration. Adapted from reference 115, by permission of Elsevier. TSH=thryoid-stimulating hormone. *Laboratory test values, especially an increase in free thyroxine, can be interpreted already at this stage. †Adequate free thyroxine rise is estimated to be roughly 50% from baseline at 120 min[116]

Table 3 Reasons for failure to reach L-T4 treatment goals and recommendations

Elevated TSH with or without (persistent) symptoms	**Normal TSH and (persistent) symptoms**
Inadequate dosage ➔ *Consider higher dosage; especially in patients with no remaining functional thyroid capacity (e.g. after total thyroidectomy or radio ablation therapy in Graves' disease)*	Concurrent (autoimmune) disease/ etiologies ➔ *The following diseases could be considered: Autoimmune atrophic gastritis with pernicious anemia, Addison disease, Diabetes, rheumatoid arthritis.*
Simultaneous intake of L-T4 with food can impair L-T4 absorption ➔ *L-T4 intake 30-60 min before breakfast or at bedtime (2-3 hours after evening meal); discuss patient preference*	Inadequate thyroid hormone concentrations on tissue level. ➔ *Acknowledgement of patients' symptoms. Check if the patient feels better at a different TSH level in the normal range (individual set-point). A trial of LT4-LT3 combination therapy can subsequently be considered in adherent patients with long-lasting steady state of TSH serum*
Medications affecting L-T4 absorption include calcium carbonate*, ferrous sulfate*, PPI, aluminum containing antacid, sucralfate, orlistat ➔ *Separate intake of L-T4 from interfering medications and supplements (e.g. 4 hours)*	**Low TSH with or without (persistent) symptoms**
Medications affecting L-T4 availability and requirement include estrogens, androgens, sertraline, phenobartbital**, carbamazepine, phenytoin, rifampicin. ➔ *Monitoring of TSH at initiation and adjustment of L-T4 dosage if required*	Overtreatment due to high dosage ➔ *Consider lower dosage in elderly and subclinical hypothyroidism. Ask if patient takes any over-the-counter preparations that might contain thyroid hormone*
Malabsorption due to gastro-intestinal disease and conditions ➔ *H. Pylori gastritis, celiac disease, auto-immune atrophic gastritis, diabetic gastropathy should be considered and possibly treated*	Medical Conditions ➔ *Certain drugs (e.g. metformin) and loss of weight can decrease TSH levels*
Non-adherence ➔ *Common cause, but should be suspected after other etiologies have been excluded. Consider thyroxine absorption test (Figure 3)*	

* Evidence from prospective trials ** Anti-epileptics drugs accelerate T4 and T3 conjugation but serum levels of TSH do not necessarily increase.
Abbreviations: L-T4 levohyroxine; PPI proton pump inhibitors; T3 triiodothyronine; T4 thyroxine; TSH thyroid-stimulating hormone.

L-T4/LT-3 Combination Therapy

This has prompted several trials in the past 15 years with combined L-T4 and L-T3.[125] Although some studies show some beneficial effect, such as patient preference for combination therapy or an improved metabolic profile, [126-129] all studies together do not demonstrate that patients on combination therapy fare better than those taking L-T4 monotherapy.[130] Possible explanations why combination therapy has failed to show superiority include inadequate L-T4 and L-T3 dosages or frequency of administration.[131] L-T3 has a short half-life and none of the above mentioned studies used a slow-release T3. Most trials were also of relatively short duration, and the instruments used (questionnaires) may not have been sufficiently targeted or sensitive enough to detect the symptoms experienced by patients.

Alternatively, trials so far may have failed to identify the appropriate subgroups that would benefit. Most trials did not specifically recruit patients who do not feel well on L-T4 or those with particularly low serum T3 levels. Individuals with genetic variations in thyroid hormone metabolism have not been specifically targeted.[131] A particular subgroup concerns individuals with common genetic variations in the Dio2 enzyme, responsible for local conversion of T4 to T3 in several tissues, including the brain.[132] A genetic variation in Dio2 (THr92Ala), with a longer-half life than wildtype and ectopic localization in the Golgi apparatus, has shown to alter expression profiles in the cerebral cortex in a similar pattern as seen in neurodegenerative disease, without evidence of changed thyroid hormone signaling.[133] In a study of 552 people, the Thr92Ala polymorphism in the Dio2 gene was associated with lower baseline psychological well-being in patients on L-T4 replacement and with better response to combination therapy.[134] However, after appropriate multiple testing correction, the results failed to reach significance. Also, a recent population-based cohort study showed no effect of the Thr92Ala polymorphism quality of life or cognitive function measures.[135] Sufficiently powered prospective RCT's are therefore required before conclusions can be drawn.

While both the ATA and ETA guidelines generally recommend against the routine use of combination therapy in hypothyroid patients, the recommendations concerning trials in patients with persistent symptoms slightly differ. The ETA states that a 3-months trial of L-T4 and L-T3 combination might be considered

experimentally in adherent biochemically well-controlled L-T4-treated hypothyroid patients with persistent complaints.[136] and provides methods for calculating L-T4 and L-T3 dosages.[136] Treatment should however be initiated only by accredited internists/endocrinologists, closely monitored and discontinued if no improvement is experienced. The ATA however, recommends against any routine use of such trials outside of formal research and clinical trials, mainly due to uncertainty regarding benefit and long-term safety.[99] Both the ETA and ATA do agree on the need for long-term RCT's to assess risk-benefit ratios. Such trials would need to incorporate investigation of the ideal thyroid parameters to monitor during combination therapy, and whether T3 levels would be an important parameter. The timing of phlebotomy is also important, particularly if L-T3 is being administered more than once daily.

There is lack of evidence to support other therapies for hypothyroidism. The use of thyroid extracts or L-T3 monotherapy is generally not recommended because of potential safety concerns related to the presence of supraphysiologic serum T3 levels and a paucity of long-term safety outcome data. The use of compounded thyroid hormones, dietary supplements, and any over-the counter drug for treatment of hypothyroidism is discouraged.

DIRECTIONS FOR FUTURE RESEARCH

Although great advances have been made in the identification of etiology, knowledge of clinical implications, diagnosis and treatment of hypothyroidism, several unanswered questions remain, especially regarding diagnosis and treatment of hypothyroidism.

Etiology of hypothyroidism

Many risk factors have been identified for abnormal TSH levels, FT4 levels and thyroid disease, but only a small proportion of the variability is explained.[137] Therefore, identification of novel risk factors is important. There is increasing evidence for endocrine disrupting chemicals as possible etiological factors for endocrine diseases. Thyroid-disrupting chemical (TDC) exposure can come from different sources ranging from environmental (e.g. flame retardants,) to dietary

(e.g. food packaging material).[138] A recent transatlantic call for action has been made to answer these questions in a collaborative effort.[139]

Clinical implications

The association of hypothyroidism with cardiovascular disease has been established and replicated in several studies.[61,65] However, the mechanisms behind this association remain unclear. The link between hypothyroidism and several cardiovascular diseases seems independent of traditional cardiovascular risk factors.[61,65,67] Further research focused on novel cardiovascular risk factors or other pathways could shed a light on the exact mechanisms. This is crucial to support treatment decisions and monitoring strategies in patients with (subclinical) hypothyroidism.

Diagnosis

The diagnosis of hypothyroidism is currently based on statistically defined reference ranges for TSH and FT4. As previously mentioned, these reference ranges do not consider whether patients are at risk to develop disease. Due to the arbitrary nature of the cut-offs defining mild and overt hypothyroidism, an alternative grading system has been proposed according to the thyroid function tests. The arbitrary nature of these cut-offs were also highlighted by the U.S. Preventive Service Task Force as one of the important factors hampering decision making on screening of thyroid dysfunction in asymptomatic patients.[81] This also holds true for treatment decisions in asymptomatic patients with hypothyroidism. Further research is needed to identify which adverse health events occur after long-term thyroid dysfunction. Furthermore, it needs to be established which levels of TSH and FT4 are accompanied by increased risk of disease. This information can come from collaborative efforts of observational cohort studies with sufficiently long follow-up. Only after this information is available, RCT's can assess if treatment of thyroid function beyond these thyroid function test levels reduces excess risk and assess the risk-benefit ratio of treatment.

Treatment

As mentioned, L-T4 monotherapy is the standard of care. However, there are several unresolved issues concerning patients that are biochemically well-controlled but unsatisfied. Future studies should addresswhether alternative regimens could provide a solution for at least a proportion of patients with residual

symptoms. Concerning treatment of hypothyroidism, the research areas most urgently in need of progress include 1) unravelling the etiology of persistent symptoms in biochemically well-controlled hypothyroid patients 2) investigating if a more adequate dosage (e.g. tailored to patient serum T3 or to a patient's own particular TSH set-point, if this could be identified) produces more satisfactory therapy, 3) investigation if new formulations (e.g. slow release L-T3) or more frequent administration of L-T4/L-T3 combination therapy (e.g. L-T3 thrice daily) can ameliorate patient symptoms 4) identification of subgroups that could benefit from therapies other than L-T4 monotherapy, for example by identifying additional genetic polymorphisms that could provide information on the individual thyroid set point. GWAS studies including larger number of individuals with more detailed genotyping could provide such information.

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CHAPTER 2

THE HEART

CHAPTER 2.1

THYROID FUNCTION AND SUDDEN CARDIAC DEATH

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ABSTRACT

BACKGROUND The association between thyroid function and cardiovascular disease is well established, but no study to date has assessed whether it is a risk factor for sudden cardiac death (SCD).
Therefore, we studied the association of thyroid function with SCD in a prospective population-based cohort.

METHODS Participants from the Rotterdam Study ≥45 years with thyroid-stimulating hormone or free thyroxine (FT4) measurements and clinical follow-up were eligible. We assessed the association of thyroid-stimulating hormone and FT4 with the risk of SCD by using an age- and sex-adjusted Cox proportional-hazards model, in all participants and also after restricting the analysis to euthyroid participants (defined by thyroid-stimulating hormone 0.4–4.0 mIU/L). Additional adjustment included cardiovascular risk factors, notably hypertension, serum cholesterol, and smoking. We stratified by age and sex and performed sensitivity analyses by excluding participants with abnormal FT4 values (reference range of 0.85–1.95 ng/dL) and including only witnessed SCDs as outcome. Absolute risks were calculated in a competing risk model by taking death by other causes into account.

RESULTS We included 10 318 participants with 261 incident SCDs (median follow-up, 9.1 years). Higher levels of FT4 were associated with an increased SCD risk, even in the normal range of thyroid function (hazard ratio, 2.28 per 1 ng/dL FT4; 95% confidence interval, 1.31–3.97). Stratification by age or sex and sensitivity analyses did not change the risk estimates substantially. The absolute 10-year risk of SCD increased in euthyroid participants from 1% to 4% with increasing FT4 levels.

CONCLUSIONS Higher FT4 levels are associated with an increased risk of SCD, even in euthyroid participants.

INTRODUCTION

Thyroid hormone is critical for the development and function of nearly all organs and tissues, with the cardiovascular system being one of the major targets. Thyroid hormone is known to increase heart rate, increase cardiac contractility, alter systolic and diastolic function and decrease systematic vascular resistance[1]. Thyroid dysfunction, even in the subclinical range, is associated with an increased incidence of cardiovascular risk factors and disease[2-4]. Both overt and subclinical hypothyroidism are associated with hypertension, dyslipidemia and coronary heart disease (CHD)[4,5], whereas excess of thyroid hormone, subclinical and overt hyperthyroidism, increases the risk of atrial fibrillation (AF), CHD and heart failure (HF)[2,6]. However, little is known about the association between thyroid (dys)function and the risk of sudden cardiac death (SCD).

SCD is defined as unexpected natural death from a cardiac cause within a short time period, generally <1 hour from the onset of symptoms, in a person without any prior condition that would appear fatal[7]. SCD accounts for over 50% of cardiovascular deaths and 15% to 20% of total mortality[8]. As much as 75 percent of SCDs have been attributed to CHD (known or unknown) and the risk factors for CHD and SCD are therefore very similar and include older age, male sex, hypertension, HF, smoking and dyslipidemia[7,9,10]. Additional risk factors include non-ischemic cardiac disease (e.g. congenital heart disease) and non-cardiac disorders (e.g. drug-induced)[10]. However, the predictability of SCD in the general population remains poor, as almost half of the SCD cases are the first presentation of cardiac disease[11]. It is therefore of crucial importance to identify additional risk factors other than well-established cardiovascular risk factors. We hypothesized that thyroid function is associated with an increased risk of SCD and set to determine this association and possible subgroups at risk in a population-based cohort study.

METHODS

Setting

All analyses were performed in 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[12]. We included participants from three independent cohorts within the Rotterdam Study. The RS Cohort 1 (RSI) includes participants aged 55 years and older and baseline data were collected during 1990-1993. RS Cohort II (RSII) includes participants aged 55 years and older and baseline data were collected from 2000-2001. For the RS Cohort 3 (RSIII), all residents of Ommoord aged 45 years and over who had not been invited before, were asked to participate and baseline data were collected from 2006 to 2008. The Medical Ethics Committee of the Erasmus University approved the study protocols 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.

Study population

Participants were eligible for inclusion if they had thyroid-stimulating hormone (TSH) or free thyroxine (FT4) measurements made at baseline visit of the study cohorts RSI (RSI-1), RSII (RSII-1) and RSIII (RSIII-1). Since a number of participants from RSI did not have thyroid measurements at the first visit, they were included in the analyses using data from their third visit 3 (RSI-3) and were follow-up since the date of their laboratory measurement. A total of 10,318 participants from the three cohorts were included in our analyses. All study participants were followed up from the day of baseline laboratory testing to date of SCD, to death from other causes, or to December 12th, 2010, whichever came first.

Assessment of thyroid function

For RSI-1, serum TSH (TSH Lumitest; Henning, Berlin, Germany) and F T4 levels (FT4; Vitros, ECI Immunodiagnostic System; Ortho-Clinical Diagnostics, Amersham, UK) were determined in a random subset of the baseline serum samples (n=1855). For RSI-3, RSII-1 and RSIII-1, thyroid function assessment was also performed in baseline serum samples for TSH and FT4 (The electrochemiluminescence immunoassay for thyroxine and thyrotropine, "ECLIA", Roche). The two tests' TSH reference ranges did not differ substantially and had a

good Spearman correlation co-efficient (0.96 for TSH, p < 0.0001and 0.81 for FT4, p < 0.0001). We determined the cut-off values for normal range TSH as 0.4-4.0 mIU/L according to national guidelines and our previous studies[13]. The reference range for FT4 was 0.85-1.95 ng/dL (=11-25 pmol/L)[14]. Euthyroidism was defined as a TSH value within the reference range. Hypothyroidism was defined by TSH mIU/L > 4.0 and FT4 <0.85 ng/dL whilst with subclinical hypothyroidism FT4 was still within the reference range. Hyperthyroidism was defined by TSH mIU/L < 0.4 and FT4 > 1.95 ng/dL whilst with subclinical hyperthyroidism FT4 was still within the reference range.

Sudden cardiac death definition and case ascertainment

nformation on SCD was obtained from medical records and death certificates. Deaths were considered from study entry through December 12th 2010. SCD was defined in accordance with the Myerburg definition[15], which is endorsed by the European Society of Cardiology[16], as “a natural death due to cardiac causes, heralded by abrupt loss of consciousness within one hour from onset of acute symptoms. Pre-existing heart disease may have been known to be present, but the time and mode of death are unexpected. We included cases of unwitnessed death if the person was seen in a stable medical condition 24 hours preceding death and if there was no evidence of a non-cardiac cause of death. We considered both witnessed and unwitnessed SCD cases for the main outcome, and conducted a sensitivity analysis excluding participants with unwitnessed SCD. In addition, in case of sparse information, cases were labelled as SCD when treating physicians labelled death as sudden or unexpected [17]. Case validation and definition of SCD has been described earlier in previous publications from the Rotterdam Study[17,18]. In short, SCD cases were adjudicated by two independent reviewers. SCD cases were coded as “possible” if there was insufficient information in the medical file regarding the period prior to and until death to code an SCD as “certain”. We conducted a sensitivity analysis excluding SCD cases coded as possible. If there was disagreement, this was resolved by consensus meetings. All cases were ultimately reviewed by a senior cardiologist.

Baseline and other measurements

Blood pressure was measured twice using a random-zero sphygmomanometer and averaged. Hypertension was defined as having a systolic blood pressure > 140

mmHg or a diastolic blood pressure > 90 mmHg or using antihypertensives at baseline (diuretics, anti-adrenergic agents, β blockers, calcium channel blockers and RAAS inhibitors). Smoking was categorized in current or non-current smokers. QT-interval was measured on a resting ECG. Heart-rate variability was automatically determined by MEANS. We assessed the standard deviation of the normal-to-normal RR intervals (SDNN)[19]: a time-domain HRV marker, based on all the RR intervals on the 10-second ECGs. For the analyses with heart-rate variability, we removed ECGs with excessive noise, excessive baseline wander, premature ventricular beats, and premature supraventricular beats. Outliers of heart-rate variability values were visually checked and discarded if related to poor signal quality. Pulse rate was measured twice with a pulse oximeter and averaged. Serum total cholesterol was measured using standard laboratory techniques. Diabetes was defined by an impaired fasting glucose ≥ 7 mmol/L, non-fasting glucose use ≥ 11.1 mmol/L or use of glucose lowering medication. Body-mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Ascertainment of AF in the Rotterdam Study has been described in detail previously [20]. In short, it was ascertained using three methods 1) ECG's at baseline and during follow-up, ascertained by a cardiologist, 2) medical information obtained from the General Practitioners, after ascertainment of ECG and 3) national registry of hospital discharge diagnosis. Cases of incident HF were obtained by continuous monitoring of participants during follow-up through automated linkage with files from general practitioners [12,21]. All available data on HF, such as hospital discharge letters and notes from general practitioners, were copied from the medical records. HF was adjudicated in accordance with in accordance with the guidelines of the European Society of Cardiology [22] and included typical signs and symptoms of heart failure confirmed by objective evidence of cardiac dysfunction.

Statistical analysis

We assessed the association of TSH or FT4 at baseline, in separate models, with the risk of SCD both in the full and the euthyroid range of thyroid function (defined by TSH 0.4-4.0 mIU/L), using Cox-proportional hazards model. We also evaluated the risk of SCD according to thyroid state with euthyroidism as reference. All primary analyses were sex and age-adjusted. Multivariable models additionally adjusted for cohort, pulse rate, hypertension, serum cholesterol, diabetes mellitus,

BMI, smoking and QT-interval, after applying multiple imputation for missing data of these covariates (missingness < 3% for all covariates). Variables in the multivariable models represent the most common confounding or mediating factors of the association between thyroid function and SCD. Absolute 10 year risk-probabilities were estimated, given the covariates used in the primary Cox-proportional hazards model, according to the Fine and Gray model [23]. This model takes the competing risks of death due to all other (non-SCD) causes into account. We also derived the subdistribution HRs (SHR) for included variables from this model.

Pre-defined stratification by sex, age categories and cohort were performed. The cut-off of the age categories was 65 years, which is close to the median age of our population. Sensitivity analyses included 1) restricting to the analyses euthyroid individuals with witnessed SCDs as outcome 2) excluding possible SCD events 3) restricting the analyses to euthyroid subjects with normal FT4 values and excluding individuals using thyroid medication defined as hormone replacement therapy or anti-thyroid drugs (e.g. Methimazole), 4) additionally censoring participants at thyroid medication use during follow-up 5) excluding abnormal FT4 values and thyroid function altering medication (defined as thyroid medication, amiodarone or corticosteroids) and 6) additionally excluding participants with CHD and HF at baseline 7) adjusting the analyses additionally for HRV.

We explored the possible role of incident AF or HF in the association between thyroid function and SCD by censoring the analyses at time of AF or HF. Follow-up data on HF incidence was available in 6893 participants. Furthermore, we compared the hazard ratios of SCD with those of total mortality, total cardiovascular mortality as well as non-sudden cardiovascular mortality. The proportional hazards assumption was assessed with plots and test of Schoenfeld residuals. The proportional hazards assumption was met for all analyses. We also performed a goodness-of-fit test for the Fine and Gray model for the absolute risk estimation, using the Zou Laird Fine test, and this revealed no linear, quadratic or log time varying effects of ft4 in the full or euthyroid range ($p > 0.35$ for all analyses). Non-linearity was tested using fractional polynomials and adding quadratic terms to the model. The best fit was determined to be linear. TSH was log transformed for all continuous analyses to approximate normality while FT4

levels were normally distributed. Fractional polynomials were performed using STATA (StataCorp. 2013. Stata Statistical Software: Release 13. College Station, TX: StataCorp LP). Schoenfeld plot, Schoenfeld test and competing risk calculations were performed in R (survival and cmprsk packages R-project, Institute for Statistics and Mathematics, R Core Team (2013), Vienna, Austria, version 3.0.2). All other statistical analyses were performed using SPSS version 21 (SPSS IBM, New York, U.S.A). Results of this study are reported according to the STROBE statement guidelines [24].

RESULTS

We included 10,318 participants with a maximum follow-up of 21.2 years and a median of 9.2 years (interquartile range 3.8-11.1). A total of 261 SCD events occurred during follow-up (incidence rate = 3.1 per 1000 person-years) and completeness of follow-up was 97.9% [25]. When restricting to euthyroid subjects, there were 231 cases. Of the total number of participants, 10,314 had TSH measurements and 10,225 had FT4 measurements. Baseline characteristics are shown in Table 1.

Table 1 Baseline characteristics of included participants

Variable	Mean (SD)*
Number of individuals in the study	10,318
Age, in years	64.7 (9.5)
Age range, years	46-106
Women N (%)	5886 (57.0)
Diabetes mellitus N (%)	1042 (10.1)
BMI	27.2 (4.2)
Cholesterol	5.89 (1.40)
Smoking current N (%)	2372 (23.0)
Hypertension N (%)	6142 (59.5)
Pulse rate	71 (11)
Median TSH (IQR) mIU/L	1.85 (1.23-2.72)
FT4 ng/dL	1.23 (0.20)
Thyroid hormone replacement therapy use N (%)	298 (2.9)

*unless specified otherwise

Abbreviations: BMI Body-Mass Index, IQR interquartile range, FT4 free thyroxine, SCD Sudden Cardiac Death, SD standard deviation, TSH Thyroid-Stimulating Hormone, N number, TSH is missing in 4 participants, FT4 is missing in 93 participants

Thyroid function and SCD

Higher levels of FT4 were associated with an increased risk of SCD with a hazard ratio (HR) of 1.87 per 1 ng/dL increase of FT4 (95% Confidence Interval [CI], 1.09-2.86) (Table 2). The log relative hazard between FT4 and SCD as well as the distribution of FT4 in the population are plotted in Figure 1. When restricting to euthyroid subjects, there was an increased risk of SCD events with a HR of 2.26 (95% CI, 1.30-3.94). In line with an increased risk for higher FT4 levels, higher TSH levels were associated with a concomitant decreased risk but failed to reach statistical significance (HR 0.92, 95% CI, 0.80-1.04, per one unit increase of natural log transformed TSH).

The highest compared to the lowest tertile of FT4 had a higher risk of SCD in the full range (HR 1.35, 95% CI, 1.01-1.83, p for trend 0.022) (Table 3). Sensitivity analyses did not alter risk estimates substantially (Table 4).

Table 2 Association between thyroid function and the risk of SCD

Thyroid function	SCD Events/ total N	HR (95% CI), Model 1	HR (95% CI), Model 2	HR (95% CI), Model 3
Full range of measurement				
TSH mIU/L	261/10,314	0.91 (0.80-1.04)	0.91 (0.80-1.03)	0.92 (0.80-1.04)
FT4 ng/dL	249/10,225	1.87 (1.18-2.96)	1.76 (1.10-2.86)	1.77 (1.09-2.86)
Euthyroid participants*				
TSH mIU/L	231/8953	0.81 (0.63-1.04)	0.80 (0.62-1.03)	0.80 (0.62-1.04)
FT4 ng/dL	222/8881	2.54 (1.48-4.40)	2.24 (1.31-4.40)	2.26 (1.30-3.94)

Model 1: adjusted for sex and age. Model 2: Model 1 + cohort, pulse rate, hypertension, cholesterol, diabetes, body-mass index and smoking. Model 3: Model 2 + and QT-interval. *Euthyroidism is defined by TSH 0.4-4.0 mIU/LResults for TSH are per one unit increase of the natural logarithm of TSH and results for FT4 per 1 ng/dL.Abbreviations: CI confidence interval, FT4 free thyroxine, HR hazard ratio, SCD sudden cardiac death, TSH thyroid-stimulating hormone.

When restricting to euthyroid subjects with normal FT4 values, the absolute 10 year risks increased from 1% to almost 4% with increasing FT4 values (Figure 2).

There seemed to be a differential risk of SCD in different age groups (Supplemental Table 1). However, the number of cases in the younger age category (≤ 65 years) was small and the p for interaction (p >0.30) insignificant. Stratification analyses for age, sex and cohort did not show differential risks (p for interaction for all analyses > 0.40) (Supplemental Table 1). Participants with

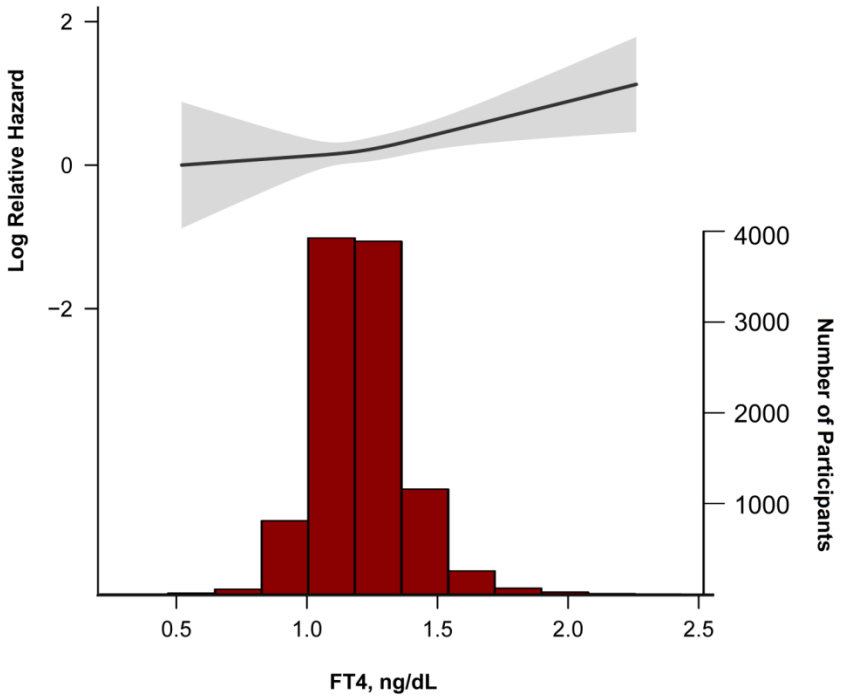
subclinical or overt hypo- or hyperthyroidism did not have a higher risk of SCD compared to euthyroid participants (Supplemental Table 2), but the number of events per category was small. Censoring the analyses at time of incident AF or HF did not alter risk estimates meaningfully (Supplemental Table 3 and 4), neither did censoring the analyses for both AF and HF (data not shown). The SHRs obtained from the competing risk model were slightly more attenuated for all variables compared to the HRs obtained from the conventional Cox-proportional hazard model, but remained qualitatively similar for the FT4 analyses (Supplemental Table 5). As compared to the SCD analyses the risk estimates were slightly higher for non-sudden cardiovascular mortality and
slightly lower for total mortality (Supplemental Table 6).

Table 3 Associations of tertiles of TSH and FT4 with SCD

	SCD Events/ Total N	**HR (95% CI), Model 1**	**HR (95% CI), Model 2**	**HR (95% CI), Model 3**
TSH tertiles				
0.01-1.43	113/3436	REFERENCE	REFERENCE	REFERENCE
1.44-2.36	81/3442	0.88 (0.66-1.17)	0.87 (0.65-1.15)	0.86 (0.65-1.15)
2.37-80.64	67/3436	0.78 (0.57-1.05)	0.79 (0.58-1.07)	0.78 (0.58-1.06)
P for trend		*0.17*	*0.18*	*0.18*
FT4 tertiles				
0.12-1.14	72/3420	REFERENCE	REFERENCE	REFERENCE
1.14-1.29	71/3404	1.03 (0.74-1.43)	1.03 (0.74-1.44)	1.03 (0.74-1.44)
1.29-4.73	106/3401	1.39 (1.03-1.87)	1.35 (1.01-1.83)	1.35 (1.01-1.83)
P for trend		*0.008*	*0.026*	*0.022*

Model 1: adjusted for sex and age. Model 2: Model 1 + cohort, pulse rate, hypertension, cholesterol, diabetes mellitus, body-mass index and smoking. Model 3: Model 2 + QT-interval.
*Euthyroidism is defined by TSH 0.4-4.0 mIU/L. FT4 is in ng/dL.
Abbreviations: CI confidence interval, FT4 free thyroxine, HR hazard ratio, SCD sudden cardiac death, TSH thyroid-stimulating hormone.

Figure 1 Relative risk of sudden cardiac death according to FT4 serum values



Log relative hazard plotted against absolute FT4 ng/dL values with historgram of FT4 distribution in the population. Estimates for the relative hazard were derived using 3 restricted cubic splines with a p for non-linearity = 0.49

DISCUSSION

In the current study, higher FT4 levels were associated with an increased relative and absolute risk of SCD, even in the normal range of thyroid function. The relative risk estimates were similar in the analyses with only witnessed SCDs as outcome or when excluding prevalent cases of cardiac diseases and those using thyroid-function altering medication.

This is the first study addressing the association between thyroid function and SCD in the general population. Two earlier studies that evaluated the relation between thyroid function and SCD were conducted in specific patient populations (HF

patients and diabetic hemodialysis patients). These studies did not investigate the association of FT4 with SCD and had conflicting results[26,27]. The SCD-HeFT study, conducted in HF patients, found no difference in SCD risk between TSH categories[27]. The 4D study, investigating 1000 diabetic hemodialysis patients, reported a higher risk of SCD in subjects with subclinical hyperthyroidism[26]. In the current study, additional adjustment for diabetes mellitus at baseline and exclusion of participants with prevalent HF did not alter risk estimates substantially. In addition, stratification by age and sex showed no differential risk. This suggests that the association of higher FT4 levels with an increased risk of SCD is not driven by a certain subgroup.

Thyroid hormone is an important overall regulator of the cardiovascular system[1]. The pathways through which thyroid hormone can interfere with the cardiovascular system are both direct as well as indirect (e.g. cardiovascular risk factors). Thyroid hormone directly influences the adrenergic system where it has a stimulatory effect on beta-adrenergic signaling leading to positive chronotopic, dromotropic and inotropic effects [28]. This hyperdynamic state, which causes many of the symptoms in hyperthyroid patients, could be one of the mechanisms explaining the relation between thyroid hormone and SCD. Furthermore, thyroid hormones have been shown to lead to QT-interval prolongation [29,30], which in turn is related to cardiovascular disease in general [31] and SCD in particular [18]. Another pathway could be through various cardiovascular risk factors related to thyroid dysfunction, and thus leading to ischemic heart disease, in turn a large contributor in SCD. Subclinical thyroid dysfunction has also been related to CHD and HF In large collaborative individual participant efforts[2-4]. In the current study we did not find evidence for either hypotheses as including pulse rate, reflecting a chronotropic effect of adrenergic system stimulation, and various cardiovascular risk factors, including QT-interval duration and HRV, did not change risk estimates in the multivariable analyses. The exact mechanism for the association between thyroid hormone and SCD therefore still needs to be determined. Alternative pathways could be via the effects of thyroid hormone on the activity and availability of several cation transporters such as cardiac Na-K-ATPase [32,33] or via the transcription and translation of several cardiac genes (e.g. α- and β-myosin heavy chain genes) [34].

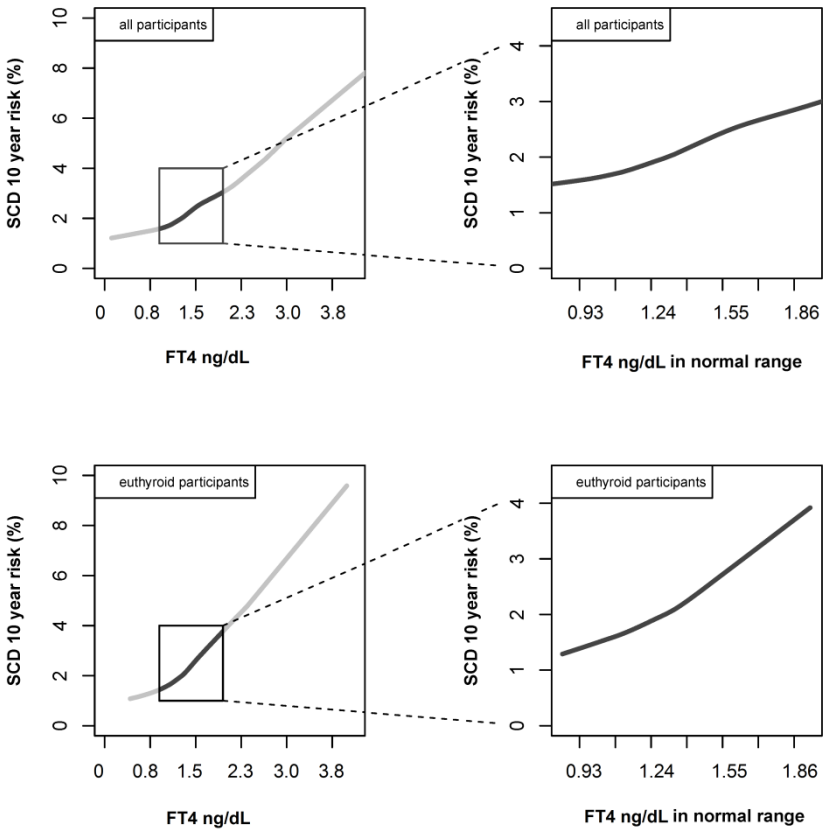
Table 4 Sensitivity analyses for association between thyroid function and SCD risk in euthyroid subjects*

Sensitivity analysis	SCD Events/ Total N	HR (95% CI), Model 1	HR (95% CI), Model 2
Restricting to witnessed SCD's			
TSH mIU/L	133/8953	0.74 (0.53-1.04)	0.74 (0.53-1.03)
FT4 ng/dL	126/8881	3.36 (1.69-6.66)	3.39 (1.68-6.81)
Excluding possible SCD's			
TSH mIU/L	195/8953	0.85 (0.65-1.21)	0.84 (0.64-1.11)
FT4 ng/dL	186/8881	2.20 (1.17-4.15)	2.02 (1.07-3.82)
Excluding abnormal FT4 values† & thyroid medication at baseline ‡			
TSH mIU/L	216/8642	0.83 (0.64-1.08)	0.83 (0.64-1.08)
FT4 ng/dL	216/8642	3.00 (1.44-6.18)	2.79 (1.34-5.71)
Excluding abnormal FT4 values† & thyroid medication at baseline and censoring participants with thyroid medication use at during follow-up‡			
TSH mIU/L	216/8642	0.83 (0.64-1.08)	0.83 (0.64-1.08)
FT4 ng/dL	216/8642	2.99 (1.45-6.17)	2.80 (1.34-5.82)
Excluding abnormal FT4 values† and thyroid function altering medication at baseline§			
TSH mIU/L	206/8519	0.81 (0.62-1.06)	0.81 (0.62-1.06)
FT4 ng/dL	206/8519	2.42 (1.14-5.16)	2.25 (1.05-4.82)
Excluding abnormal FT4 values†, thyroid function altering medication§ & prevalent HF and CHD			
TSH mIU/L	145/7746	0.80 (0.58-1.09)	0.80 (0.58-1.10)
FT4 ng/dL	145/7746	2.48 (1.00-6.11)	2.22 (0.95-5.52)
Additionally adjusting for HRV			
TSH mIU/L	135/7146	0.91 (0.65-1.28)	0.91 (0.65-1.28)
FT4 ng/dL	133/7131	3.38 (1.41-8.14)	3.04 (1.27-7.03)

Model 1: adjusted for sex and age. Model 2: Model 1+ cohort, pulse rate, hypertension, cholesterol, diabetes mellitus, body-mass index and smoking.* Euthyroidism is defined by TSH 0.4-4.0 mIU/L. †Normal range of FT4 0.85-1.95 ng/dL. ‡Thyroid medication is use of thyroid hormone replacement therapy or any anti-thyroid drug. §Thyroid function altering medication is defined as use of thyroid medication, amiodarone or corticosteroids. Results are per one unit increase of the natural logarithm of TSH, are per 1 unit increase for FT4.

Abbreviations: CHD coronary heart disease, CI confidence interval, FT4 free thyroxine, HF heart failure, HR hazard ratio, HRV heart rate variability, SCD sudden cardiac death, TSH thyroid-stimulating hormone.

Figure 2 Absolute 10-year risk of SCD by FT4 values



Absolute 10-years risks of SCD were calculated taking competing risk of death by other causes into account, and are plotted against FT4 values in all participants in the two upper figures and in euthyroid participants in the lower two. The normal range of FT4 is highlighted in the two right figures. FT4 free thyroxine, SCD Sudden Cardiac Death.

We find an effect of FT4 on the risk of SCD, while the association with TSH is less evident. These findings seem to be in line with previous literature investigating the risk of thyroid function on several clinical endpoints[14,35-37]. Thyroid hormone levels are regulated by the hypothalamus-pituitary-thyroid axis, which has a unique set point for each individual[38]. There is a wide variety of factors that can modulate this set point, including illness and aging. A change in set point over time might be an

explanation why the association with FT4 is stronger than for TSH. We do not have repeated measures of thyroid function and can therefore not investigate if changes in TSH over time could explain the apparent discrepant findings between FT4 and TSH.

We find roughly similar estimates for SCD, CVD mortality, and total mortality. This was not surprising, as the association between thyroid dysfunction and CVD mortality (mainly consisting of CHD mortality) has been described in several previous studies and is well established[4,39]. Guidelines recommend treatment of subclinical thyroid disease from a certain TSH value cut-off mainly based on data regarding CHD outcomes from large collaborative studies. Cardiovascular diseases are the leading cause of burden of disease and mortality in elderly worldwide[40]. In high-income countries, cancer mortality is the second leading cause. Although the underlying mechanisms are probably different to CVD mortality, the association of high thyroid dysfunction and cancer has been previously described and could therefore contribute to the association found with total mortality. The associations of thyroid function with CVD mortality and of thyroid function and SCD seem to both be independent of cardiovascular risk factors. However, bigger sample size and more detailed data are needed to determine whether these associations share the same or have distinct pathways.

SCD develops within a short timeframe and there is limited time to start intervention and cardiopulmonary resuscitation. Therefore, identification of modifiable risk factors is crucial in the setting of identification of certain populations or subgroups at risk, as well as screening and prevention. In the current study, we were not able to demonstrate differential risks by age and sex, but the association between thyroid function and SCD was more pronounced within the normal range of thyroid function. This can likely be explained by the fact that participants with a thyroid function outside the reference range have a higher probability of being treated, which will alter their risk. In contrast, people with FT4 levels in the normal range have no indication for treatment and are usually stable with very little intra-individual variation, reflecting the individual set point. When we exclude those using levothyroxine or anti-thyroid drugs at baseline or during follow-up, risk estimates only slightly shift towards the risk in euthyroid participants. However in our study,

no conclusions on the benefits or risks of thyroid medication can be drawn. Ideally, this should be investigated in a randomized controlled trial.

Strengths and limitations

Major strengths of our study are the number of participants and covariates included in the analyses. All data were collected irrespective of the current hypothesis. Furthermore,, we were able to phenotype SCD in detail and make a distinction between witnessed and unwitnessed types. The setting of a population-based cohort and the long follow-up allowed for estimation of long-term absolute risks. Limitations include possible residual confounding and the availability of one baseline measurement of thyroid function, not allowing for assessment of temporal changes of TSH and FT4. Furthermore, there were a limited number of participants with FT4 values outside the reference range (190 participants). The vast majority of participants in the study were of Caucasian descent and therefore our results might not be generalizable to other populations.

Conclusions

In summary, we describe an increased risk of SCD with increasing FT4 levels, even when restricting to euthyroid participants.

Online supplemental material

http://circ.ahajournals.org/content/134/10/713.long

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CHAPTER 2.2

THE ASSOCIATION OF NORMAL THYROID FUNCTION WITH ATRIAL FIBRILLATION

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ABSTRACT

BACKGROUND Hyperthyroidism is an established risk factor for atrial fibrillation (AF), but information concerning the association with variations within the normal range of thyroid function and subgroups at risk is lacking. We therefore aimed to investigate the association between normal thyroid function and AF prospectively and explore potential differential risk patterns.

METHODS From the Rotterdam Study we included 9166 participants ≥45 years with thyroid stimulating hormone (TSH) and/or free thyroxine (FT4) measurements and AF assessment (1997-2012, median follow-up 6.8 years), with 399 prevalent and 403 incident AF cases. Outcome measures were threefold 1) Hazard Ratio's (HRs) for the risk of incident AF by Cox Proportional-Hazards models 2) Ten-year absolute risks taking competing risk of death into account. 3) Discrimination ability of adding FT4 to the CHARGE-AF Simple Model, an established prediction model for AF.

RESULTS Higher FT4 levels were associated with higher risks of AF (HR 1.63, 95% Confidence Interval 1.19-2.22), when comparing those in the highest quartile to those in lowest quartile. Absolute 10-year risks increased with higher FT4 in participants ≤65 years from 1% to 9% and from 6% to 12% in subjects ≥65 years. Discrimination of the prediction model improved when adding FT4 to the Simple Model (c-statistic 0.722 vs 0.729, p=0.039). TSH levels were not associated with AF.

CONCLUSIONS There is an increased risk of AF with higher FT4 levels within the normal range, especially in younger subjects. Adding FT4 to the Simple Model slightly improved discrimination of risk prediction.

INTRODUCTION

Atrial fibrillation (AF) is the most common cardiac arrhythmia and a leading cause of cardiovascular disease, particularly stroke [1]. Lifetime risks for developing AF in Europe are calculated to be up to 25% [2]. Despite efforts improving management of major classical risk factors of AF (e.g. hypertension), prevalence and costs of AF are expected to increase in upcoming years[3,4]. This highlights the need to improve prevention of AF and identification of additional risk factors. Overt and subclinical hyperthyroidism are well-documented risk factors for AF, with a prevalence of AF of over 10% in patients with hyperthyroidism[5-7]. Furthermore, higher thyroid-stimulating hormone (TSH) levels within the normal range have been associated with increased cardiovascular mortality and an unfavorable metabolic profile [8], while low-normal TSH levels are associated with increased risks of fractures and depressive disorders [8,9]. Nevertheless, only two studies have specifically investigated the association between thyroid function in the normal range and AF[10,11] with both specific limitations. Neither addressed possible differential risks in specific subgroups (e.g. gender) or possible clinical implications. Identification of specific populations at risk is a crucial requirement in a primary prevention setting or with a targeted screening approach. Therefore, we aimed to investigate the association between variations in normal thyroid function and AF in a longitudinal prospective cohort study, to explore possible subgroups at risk and calculate absolute 10-year risk of AF. Furthermore, we assessed the potential role of thyroid function in a risk prediction model for AF.

METHODS

Study population

The Rotterdam Study is a prospective cohort study ongoing since 1990 in Ommoord, a suburb of the city of Rotterdam, The Netherlands. The study targets cardiovascular, endocrine, hepatic, neurologic, ophthalmic, psychiatric, dermatological, oncological and respiratory disease. Until now 14,926 subjects of 45 years and older are included in the Rotterdam Study. The participants interviewed at home and extensively examined at the research center at baseline. These examinations focused on possible causes of diseases in the middle-aged

and elderly. Participants of the Rotterdam Study are followed for several older age related diseases. The aims and design of the Rotterdam Study have been described in detail elsewhere [12]. For this study, we included participants from three independent cohorts within RS: RS Cohort 1 (RSI), including 7,983 participants aged ≥55 (baseline 1990-1993), RS Cohort II (RSII), including 3,011 participants aged ≥55 (baseline 2000-2001) and RS Cohort 3 (RSIII), including 3,932 participants aged ≥45 (baseline 2006-2008). Participants from the three cohorts were eligible for the study if TSH or FT4 measurements were performed and if information on AF was available. All study participants were followed up from the day of baseline laboratory testing to date of onset of AF, to death, or to June 1, 2012, whichever came first. The Medical Ethics Committee of the Erasmus University has approved study protocols, and written informed consent was obtained from all study participants.

Assessment of thyroid function

Thyroid function was measured using the same methods and assay in all samples, which were collected between 1997 and 2008, depending on the cohort. TSH, free thyroxine (FT4) and Thyroid Peroxidase Antibodies (TPOAb) measurements were performed in serum samples stored at -80°C (The electrochemiluminescence immunoassay for thyroxine, thyrotropin and thyroid perioxidase antibodies, "ECLIA", Roche). Date and time of blood drawing was recorded and regarded as baseline. We determined cut-off values for normal range TSH as 0.4-4.0 mIU/L and FT4 as 11-25 pmol/L (0.86-1.94 ng/dL) according to guidelines and previous studies [10]. TPOAb levels greater than 35 kU/mL were regarded as positive, according to manufacturer recommendation.

Diagnosis of atrial fibrillation

Ascertainment of AF cases within RS has been reported elsewhere [2]. In short, cases of AF, including paroxysmal AF, were ascertained using 3 methods. ECG's were recorded at baseline and during follow-up examinations, stored digitally, and analyzed by the Modular ECG Analysis System (MEANS)[13,14]. As verification, all ECGs with a diagnosis of AF, atrial flutter, or any other rhythm disorders by the MEANS program were recoded by 2 independent research physicians who were blinded to MEANS diagnosis. The judgment of a cardiologist was sought and taken as decisive in case disagreement persisted between the coding physicians.

Additionally, information on AF was obtained for all participants from general practitioners records, which included their own results as well as results from physicians practicing in hospitals and outpatient clinics. Finally, information was obtained from a national registry of all hospital discharge diagnoses. Those patients who developed AF during a serious disease, resulting in death shortly after the detection of AF, which was not the cause of the serious disease, were not considered as having AF and were censored on date of detection of AF. Furthermore, subjects with transitory atrial fibrillation during myocardial infarction or during cardiac operative procedures were not included among AF cases. We did not distinguish between AF and atrial flutter when identifying cases because both conditions are very similar with respect to risk factors and consequences[15,16].

Baseline measurements

Information on smoking and history of thyroid disease was derived from baseline questionnaires. Systolic and diastolic blood pressure were calculated as the average of two consecutive measurements. Hypertension was defined as systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90mmHg or using anti-hypertensive medication. Cholesterol was measured using standard laboratory techniques. History of diabetes was defined by a repeated impaired fasting glucose ≥ 7 or use of anti-glycemic medication. Coronary heart disease (CHD) was defined as a history of myocardial infarction (MI), percutaneous coronary intervention or coronary artery bypass graft[17]. Assessment of heart failure was done using a validated score[18], according to the definition of heart failure of the European Society of Cardiology[19].This score was based on the presence of at least two signs or symptoms suggestive of heart failure (shortness of breath, ankle swelling and pulmonary crepitations) or use of medication for the indication of heart failure, in combination with objective evidence of cardiovascular disease including angina pectoris, myocardial infarction, and left ventricular hypertrophy. Furthermore, heart failure cases were identified through hospital and medical records.

Statistical analysis

Details of the statistical analyses performed are included in the Supplemental Material. In short we performed logistic regression to assess the association between thyroid function and prevalent AF and cox-proportional hazards models were used to assess the association between thyroid function and incident AF

prospectively. TSH and FT4 were examined as continuous measures and quartiles. Primary models adjusted for age and gender and multivariable models additionally adjusted for smoking, hypertension, cholesterol, diabetes and BMI. We performed pre-defined stratification by age categories - cutoff at 65 years of age-, gender, prevalent CHD and TPOAb positivity. Absolute 10 year risk-probabilities were estimated taking competing risk of death into account [20]. To investigate the robustness of our findings, we conducted sensitivity analyses 1) including only those participants included in the previous report on thyroid function and AF from RS [10] 2) including only subjects using thyroid hormone medication, 3) excluding participants with thyroid function altering medication, including thyroid hormone replacement therapy, anti-thyroid drugs, amiodarone and corticosteroids at baseline 4) excluding participants with thyroid function altering medication at baseline and follow-up and 5) excluding the first two and first four years of follow-up to examine possible reverse causality. Furthermore, we compared discrimination of two prediction models by using C-statistic and Net Reclassification Index (NRI). The first model - proposed in 2013 by the CHARGE-AF Consortium and will be referred to as the Simple Model [21] (Supplemental Material). The second model additionally included FT4 in the full and normal range. For these analyses we excluded all non-white participants (n=357). The reclassification tables classified participants in AF risk categories of low (<2.5%), intermediate (2.5% to 5%), and high (>5%)[21]. Statistical analyses were performed using SPSS version 21 (SPSS IBM, New York, U.S.A), R statistical software (survival, cmprsk and nricens packages, R-project, Institute for Statistics and Mathematics, R Core Team (2013), Vienna, Austria, version 3.0.2) or Stata (StataCorp. 2013. Stata Statistical Software: Release 13. College Station, TX: StataCorp LP).

RESULTS

In total, 9166 participants were included (mean age 65 years) in the cross-sectional and 8740 in the longitudinal analyses (399 participants with AF at baseline and 27 with missing follow-up data were excluded) (Table 1). Among included participants, five missed TSH measurement and six FT4 measurement. Only participants with both TSH and FT4 measurements available were included in the analyses

evaluating the associations within normal ranges. During a median follow-up of 6.8 years (interquartile range 3.9-10.9 years) a total of 403 AF events occurred, with an incidence rate of 6.2 per 1000 person-years. There were 256 participants using levothyroxine replacement therapy, which was prescribed by their own GP or specialist and within the context of regular treatment and blinded to measurements of the Rotterdam Study. Of these, 208 reported a history of hypothyroidism and 40 reported a history another thyroid disease.

Table 1 Baseline characteristics of included participants (n=9166)*

Variable	**Number (%)**[a]
Age, years, mean (SD)	65.0 (9.9)
Gender, female	5200 (56.7)
History of Diabetes	773 (8.4)
BMI kg/m^2, mean (SD)	27.1 (4.1)
Cholesterol mmol/L, mean (SD)	5.7 (1.0)
Smoking,	
current	1857 (20.3)
past	4376 (47.7)
never	2855 (31.1)
Hypertension	4743 (51.7)
TSH mIU/L median (IQR)	1.91 (1.29-2.77)
FT4 pmol/L	15.7 (2.3)
TPOAb positive (>35 kU/L)	1200 (13.1)
Use of thyroid medication	256 (2.8)
History of CHD	638 (6.9)

* For the survival analyses, 399 participants had prevalent atrial fibrillation and 27 participant did not have follow-up times or data on atrial fibrillation and were excluded, making the total number 8740 participants.

[a] Values are number of participants and percentage unless otherwise specified

BMI, body-mass index; TSH, thyroid-stimulating hormone, FT4, free thyroxine; SD, Standard deviation; IQR, inter-quartile range; TPOAb, thyroid peroxidase antibodies; CHD, coronary heart disease, including myocardial infarction, coronary artery bypass graft or percutaneous coronary intervention

Thyroid function and atrial fibrillation

The cross-sectional analyses are shown in Table S1. Increased levels of FT4 were associated with an increased risk of AF for the continuous analysis (OR 1.21, 95% CI, 1.15-1.28) and for FT4 quartiles (OR 2.24, 95% CI, 1.59-3.16, lowest vs highest quartile; *P* for trend, <0.001). The longitudinal analyses were conducted after excluding subjects with prevalent AF, and comprised 8740 participants (Table 2). The risk of AF was significantly higher with higher levels of FT4, both outside (HR

1.07, 95% CI 1.03-1.12,) as within the normal range of thyroid function (HR 1.10, 95% CI 1.04-1.15) (Table 2). After excluding subjects with TSH and/or FT4 values outside the reference range and thyroid hormone medication users (n=1333), participants in the highest quartile compared with the lowest quartile of FT4 had an increased risk of AF in the multivariable analysis (HR 1.63, 95% CI, 1.19-2.22, p for trend of 0.005) (Table 2). Additionally adjusting for time of blood drawing, fasting state at blood drawing, prevalent CHD at baseline or cohort did not alter risk estimates. There was no association between TSH and AF in the cross-sectional or survival analyses (Table S1, Table 2).

Table 2 Association between thyroid function and incident atrial fibrillation (AF) *

	Incident AF *N* / *Total N*	**Total *N***	**HR (95% CI) adjusted for age and gender**	**HR (95% CI) multivariable adjustment [a]**
TSH mIU/L	402	8736	0.94 (0.84-1.06)	0.91 (0.81-1.03)
FT4 pmol/L	403	8734	1.06 (1.02-1.10)	1.07 (1.03-1.12)
Within normal function of TSH and FT4[b], excluding thyroid medication users				
TSH mIU/L	334	7409	0.91 (0.73-1.13)	0.91 (0.73-1.09)
FT4 pmol/L	334	7409	1.09 (1.03-1.15)	1.10 (1.04-1.15)
TSH quartiles				
0.41-1.28	94	1850	1 (REFERENCE)	1 (REFERENCE)
1.29-1.80	88	1858	1.03 (0.77-1.38)	1.04 (0.81-1.39)
1.81-2.48	76	1858	0.90 (0.66-1.22)	0.93 (0.69-1.26)
2.49-3.99	76	1843	0.99 (0.73-1.33)	0.95 (0.70-1.29)
p for trend			0.71	0.60
FT4 quartiles				
11.01-14.33	64	1862	1 (REFERENCE)	1 (REFERENCE)
14.34-15.59	84	1861	1.22 (0.88-1.70)	1.28 (0.93-1.78)
15.60-16.96	80	1861	1.18 (0.85-1.63)	1.20 (0.86-1.66)
16.97-24.69	106	1825	1.56 (1.14-2.12)	1.63 (1.19-2.22)
p for trend			0.008	0.005

* TSH was log transformed for the continuous analyses, results are per one increase of the natural logarithm of TSH. Five participants had missing TSH and six had missing FT4 values. No thyroid function measurements were missing in the normal range analyses.
[a] adjusted for age, gender, body mass index, smoking, hypertension, diabetes and cholesterol
[b] normal range of TSH 0.4-4.0 mIU/L and of FT4 11-25 pmol/L (Conversion 1 pmol/L=0.0777 ng/dL)
AF, atrial fibrillation; FT4, free thyroxine; TSH, thyroid-stimulating hormone; HR, hazard ratio; CI, confidence interval

Stratified and sensitivity analyses

There was a differential risk by age (p for interaction 0.040) (Table 3). Comparing the highest quartile of FT4 to the reference quartile in participants below and above 65 years of age there was an increased risk of AF, with HRs of 2.23 (95% CI, 1.18-4.22) and 1.45 (95% CI, 1.01-2.08) respectively (Figure 1, Table S2). No differences were found when stratifying for gender, history of CHD and TPOAb positivity (Table S3). Including only the participants analyzed previously in RS [10], risk estimates are more comparable to our current results, with an association between FT4 (p for trend 0.001) and AF but not for TSH (p for trend 0.32). Including only thyroid hormone users with thyroid function in normal range (n=720, events=12), also yielded higher risks for FT4, but did not reach statistical significance (HR 1.07, 95% CI, 0.88-1.31).

Table 3 Stratified analysis by age for TSH and FT4 within normal range and incident atrial fibrillation *

	Age	**AF *N/ Total N***	**HR (95% CI) adjusted for age & gender**	**P-value**	**HR (95% CI) multivariable adjustment [a]**	**P-value**
TSH	<65	82/4273	0.80 (0.51-1.26)	0.34	0.83 (0.53-1.31)	0.43
	≥65	252/3136	0.94 (0.73-1.20)	0.59	0.93 (0.72-1.19)	0.55
P int			*0.54*		*0.46*	
FT4	< 65	82/4273	1.19 (1.08-1.32)	<0.001	1.19 (1.07-1.32)	0.001
	≥ 65	252/3136	1.06 (1.00-1.12)	0.071	1.06 (1.00-1.13)	0.046
P int			*0.059*		*0.040*	

* normal range of TSH 0.4-4.0 mIU/L and of FT4 11-25 pmol/L (Conversion 1 pmol/L=0.0777 ng/dL), excluding thyroid hormone users. TSH was log transformed for the continuous analyses, results are per one increase of the natural logarithm of TSH

[a] adjusted for age, gender, body mass index, smoking, hypertension, diabetes and cholesterol. AF, atrial fibrillation; FT4, free thyroxine; TSH, thyroid-stimulating hormone; HR, hazard ratio; CI, confidence interval; p int, p for interaction.

Absolute risk calculation and prediction models

Absolute 10 year risks of AF were plotted against FT4 serum values within the normal range in Figure 2 for both age groups (<65 years and ≥65years). The 10 year absolute risk for those older than 65 years gradually increased from 6% up to 12%. For younger participants, risk increased from 1% to almost 9%. Adding FT4 to the Simple Model slightly improved discrimination of the model (c-statistic 0.722 vs 0.729, p=0.039, Table 4). Tables S5 and S6 summarize the NRI results when

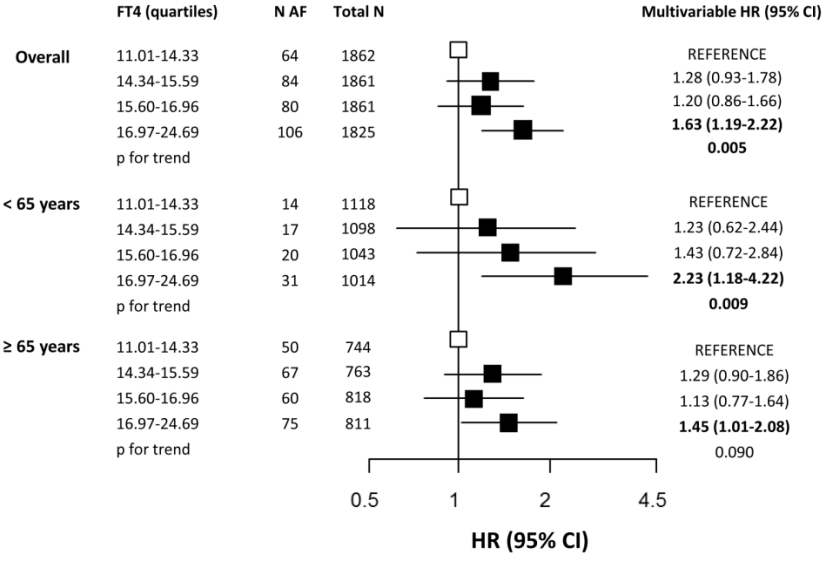
adding FT4 to the Simple Model. There was no significant improvement in NRI for the full or normal range of FT4. Excluding participants with thyroid function altering medication and excluding the first two or four years of follow-up, did not alter risk estimates (Table S4).

Table 4 Discriminative ability adding FT4 to CHARGE-AF Simple Model for 10 year risk prediction of incident atrial fibrillation*

Participants	C-Statistic Simple Model	C-Statistic Simple Model including FT4	p-value
All participants	0.722	0.729	0.039
Normal Range[a]	0.722	0.730	0.071
Age <65 years	0.694	0.712	0.132
Age ≥65years	0.677	0.683	0.299

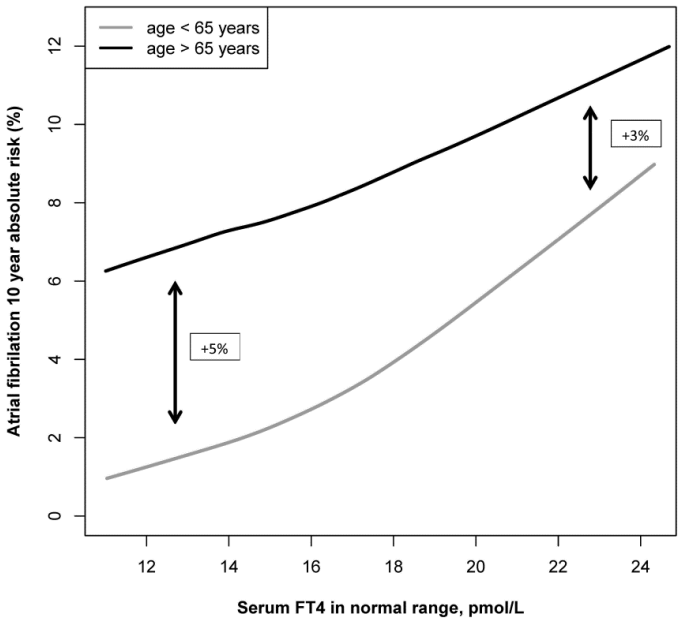
* CHARGE-AF Simple Model included age, race, height, weight, blood pressure, smoking, anti-hypertensive medication, diabetes, history of heart failure and history of myocardial infarction. For these analyses we excluded all non-white participants. Age-stratified analyses included age as a predictor. [a] normal range: TSH 0.4-4.0 mIU/L, FT4 11-25 pmol/L (Conversion 1 pmol/L=0.0777 ng/dL), excluding thyroid hormone users.AF, atrial fibrillation; FT4, free thyroxine; TSH, thyroid-stimulating hormone.

Figure 1 Stratified Analysis for the Association between FT4 Levels in Normal Range and Incident AF*



The normal range of TSH was defined as 0.4-4.0 mIU/L and of FT4 as 11-25 pmol/L (Conversion 1 pmol/L = 0.0777 ng/dL), thyroid hormone medication users were excluded.
The multivariable analyses were adjusted for age, gender, body mass index, smoking, hypertension, diabetes and cholesterol.Abbreviations AF = atrial fibrillation, TSH = thyroid-stimulating hormone, FT4 = free thyroxine, HR = hazard ratio, CI = confidence interval

Figure 2 Atrial fibrillation absolute 10-year risk for euthyroid subjects below and above 65 years of age plotted against FT4 within the normal range.*



* The normal range of FT4 was defined as 11-25 pmol/L (Conversion 1 pmol/L=0.0777 ng/dL)
Absolute risk analyses were conducted taking competing risk of death into account using a Fine and Gray model and adjusted for age and gender within strata.
FT4, free thyroxine.

DISCUSSION

Higher FT4 levels –within the normal range – are associated with an increased risk of AF, irrespective of age, gender and other potential confounders, with consistent results for both prevalent and incident AF. This effect was stronger among subjects younger than 65 years of age. We found higher 10 year absolute risks with increasing FT4 levels in participants older than 65 years of age with an increase in risk from 6% to 12%. In younger participants this was more marked, with an increase of risk from 1% to 9% with increasing FT4 levels within the normal range. Adding FT4 to the Simple Model for AF risk prediction improved discrimination of the model slightly.

Our overall results concerning relative risks are consistent with a previous study by Selmer et al. that reported a higher risk of AF towards hyperthyroidism [7]. Our results are also in line with two previous studies that focused on normal thyroid function and AF [10,11]. The population-based study by Cappola et al. (2843participants >65 years) found an increased risk with higher FT4 levels, within the normal range of thyroid function. This study however had a mean age of almost 75 years old and did not include younger participants. Furthermore, it did not provide information on potential absolute risks, differential risks or on the predictive ability of FT4 for AF.

A previous report from the RS including only 1426 participants, reported an increased risk with high-normal thyroid function 10 with an association between TSH levels and an increased risk of AF and a graded non-significant association with FT4. Our study shows that the associated risks are mainly with increased FT4 levels and not with TSH. Potential explanations for differences between previous and current report from the RS include population size (1426 vs 8740 participants), lack of subgroup analyses in the previous report, follow-up period and different characteristics of the study population. In the previous study, the included participants were slightly older at baseline (68.4v s 65.0 years), less AF events had occurred (105 vs 334) and follow-up started over 10 years earlier. A sensitivity analysis including only participants of this earlier study, with updated number of events and follow-up time showed results comparable to the findings of the current study, i.e. without an association with TSH but with an increased risk of AF with increased FT4 levels.

Thyroid hormones are known to have numerous direct effects on the cardiovascular system, including altering gene-expression of several cardiac genes[22], a decrease systemic vascular resistance[23] and alter systolic and diastolic cardiac function [24,25]. Thyroid hormone bioactivity is determined by the binding of the metabolically active form of thyroid hormone, triiodothyronine (T3), to its nuclear receptors, that function as transcription factors modulating gene expression. However, FT4 is the predominant marker of thyroid hormone in serum and important for the negative feedback mechanism. The level of serum FT4 is tightly regulated by the hypothalamic-pituitary-thyroid axis, with a different set point for each individual [26,27], which is under strong genetic influence[28]. This could

explain why even within the normal range of thyroid function, defined mostly by TSH, there is an increased risk of AF with higher FT4 levels. We find a higher risk of AF in both younger as well as older participants. However, in the younger participant group, this risk seems stronger. One explanation could be a changed set point among the elderly compared to younger participants. The individual set point can be modulated by several pathophysiological as well as physiological processes such as aging [29].Therefore, the same values of thyroid function serum makers, may hypothetically have different effects in younger versus older participants, especially over time. However, pathways possibly leading to the differences between different age groups still need to be determined and further explored.

The results of this study could have several implications. Currently, the normal range of thyroid function is defined biochemically, which is solely based on the measurement itself rather than on adverse clinical outcomes related to these measurement. It has therefore been debated whether the currently applied reference ranges should be redefined[30,31]. Our study provides evidence that variations within the reference range are associated with atrial fibrillation. Future studies should confirm these results and investigate whether variations within the reference range currently applied are associated with other cardiovascular outcomes.

The prevalence of AF and its burden on health care is increasing despite efforts to control risk factors [2,4]. Acknowledging non-classical risk factors might aid in the recognition and decision making concerning AF. With this study we show that even “high-normal thyroid function” has implications on relative risks, absolute risks and prediction of developing AF and perhaps should be taken into account in further research concerning risk prediction or screening of AF.

In recent years, the proportion of people treated for subclinical hypothyroidism has increased [32], mostly in an attempt to prevent possible adverse effects on the cardiovascular system. However, our results could suggest that treatment of subclinical hypothyroidism is not without danger and even when thyroid function is biochemically well-controlled, there can still be an increased risk of AF. Including only thyroid hormone users within the normal range of thyroid function, shows a similarly increased risk in higher FT4 level, even though not significant due to low

power in this specific subgroup. Population-based studies have shown that among patients treated with thyroid medication more than one fifth have TSH levels suppressed below normal. Future studies should determine whether the increased risk of AF is also applicable for those with high-normal thyroid hormone values due to replacement therapy.

A major strength of our study is the inclusion of a large number of participants in a population-based cohort and with longitudinal ascertainment of a large number of AF events. This allowed investigation of possible differential risks in several subgroups and calculation of absolute risks. Other strengths are the extensive evaluation and case-finding of participants with AF, both at baseline as well as during follow-up and ability to adjust for a wide variety of confounders. A limitation of our study is that despite this amount of variables included in the analyses, residual confounding cannot be excluded. Measurements of total T3 and Free T3 are not available in the Rotterdam Study and therefore the association between these thyroid function markers and AF could not be assessed. Also, we measured thyroid function only at baseline and therefore could not take changes in thyroid function into account, which is a limitation for most previous studies in this field [33,34]. Furthermore, this study is conducted in a mainly white population of 45 years and older and may not be generalizable to other populations.

Conclusions

There is an increased risk of AF with higher FT4 levels in the normal range of thyroid function. There is a trend of higher absolute risk increases with increasing FT4 levels from 1 to 9% in those younger than 65 years of age and from 6% to 12% in older participants. This suggests caution in decision making regarding intensity of treatment of subclinical hypothyroidism. Assessing thyroid function, even in the normal range, for risk prediction of AF development should be further investigated.

Online supplemental material

https://academic.oup.com/jcem/article-lookup/doi/10.1210/jc.2015-2480

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CHAPTER 2.3

THYROID FUNCTION AND QT VARIABILITY

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Submitted

**Denotes equal contribution*

ABSTRACT

BACKGROUND Short-term variability of QT intervals (STVqt) is a promising electrocardiographic marker for ventricular arrhythmias and sudden cardiac death (SCD). Both hypothyroidism and hyperthyroidism have been linked to ventricular arrhythmias; while higher thyroid function is associated with SCD. Our objective was to study the association between thyroid function and STVqt.

METHODS The association of thyroid function and STVqt was analyzed in a cross-sectional study using linear regression models in the Rotterdam Study, a cohort of community-dwelling participants. Thyroid function was expressed by thyroid-stimulating hormone (TSH) and free thyroxine (FT4), and by thyroid status (i.e. hypothyroidism, subclinical hypothyroidism, euthyroidism, subclinical hyperthyroidism, and hyperthyroidism). Nonlinearity was tested by adding polynomial terms of FT4. Analyses were stratified on age and sex. In sensitivity analyses we excluded users of thyroid medication, participants with anti-TPO antibodies above 35kU/mL and non-euthyroid participants.

RESULTS The study population comprised 8,708 participants, 56.9% were women. The mean age was 64.5 years. FT4 had a significant quadratic association with STVqt; both low and high levels of FT4 associated with a higher STVqt. The association was significantly stronger in men than in women (p interaction FT4 0.014; FT42 0.006). After excluding users of thyroid medication, TSH was significantly associated with a higher STVqt. STVqt was not significantly different across thyroid categories (p-trend 0.41).

CONCLUSIONS Both high and low levels of FT4 are associated with a higher STVqt, which is stronger in men. Further studies can elucidate if the association of FT4 and SCD is mediated via STVqt.

INTRODUCTION

The short-term variability of QT intervals (STVqt) is a marker of QT variability.[1] QT variability reflects the beat-to-beat changes in QT interval duration on the electrocardiogram (ECG).[1-3] It has been hypothesized that a high QT variability is indicative of repolarization instability. For example, QT variability has been investigated in studies that focused on cardiomyopathies or drug use as the substrate leading to repolarization instability.[2,4] Moreover, an increase in QT variability has been associated with an increased risk of ventricular arrhythmias[5] and sudden cardiac death (SCD).[6]

Thyroid hormones (triiodothyronine (T_3) and its precursor, thyroxine (T_4)), could also affect repolarization stability. Thyroid hormones can influence repolarization indirectly via the heart rate[7], or directly via potassium, sodium and calcium channels[8], or by changing the expression of Ca2+ ATPase and its inhibitor Phospholamban in the sarcoplasmic reticulum.[9] The possible effect of thyroid hormones on repolarization instability is reflected by the fact that higher thyroid hormone levels are associated with an increased risk of atrial fibrillation, even in euthyroid subjects[10], and that high free thyroxine (FT_4), even within the normal range, is associated with SCD.[3] On the other side of the thyroid spectrum, lack of thyroid hormone (i.e. hypothyroidism) is related to bradycardia and prolongation of the QT interval.[11] Case reports have suggested that both hyperthyroidism[12-14] and hypothyroidism [11,15-17] are associated with ventricular arrhythmias, albeit probably through different mechanisms.

Although both high thyroid function[3] and high QT variability[6] have been associated with SCD, the association of thyroid function with QT variability, and the marker STVqt, has not yet been studied. Analysis of this association would elucidate the relation of thyroid function with repolarization instability, and could provide pathophysiological insights into why higher FT_4 levels are associated with an increased risk of SCD. We therefore assessed the association of thyroid function with STVqt as measured on standard ECGs in a community dwelling middle-aged and elderly cohort.

METHODS

Research setting

The study is embedded in the prospective population-based Rotterdam Study. Design and rationale of the Rotterdam Study have been described in more detail elsewhere. [18,19] In short, 10,215 inhabitants aged 55 years and older from the well-defined Ommoord district in the city of Rotterdam were invited from 1990 to 1993 to participate in the initial cohort. Of the invitees, 7,983 persons agreed to participate (response rate 78%). In 2000, the cohort was extended by inviting 4,472 inhabitants of the same district who had turned 55 or who had moved into the district after the start of the initial cohort. In total, 3,011 individuals agreed to participate (response rate 67%). A second extension of the cohort was created in 2006 by inviting 6,057 persons living in the Ommoord district aged 45 years or over, of whom 3,932 subjects were included in the study (response rate 65%). Thus, by the end of 2008, 14,926 persons were included in the Rotterdam Study. Follow-up examinations were conducted every four to five years. This study used thyroid measurements from the third examination of the first cohort and the first examination of the second and third cohorts, 11,740 participants in total. Thyroid measurements taken in the first visit of the first cohort were not used because these were measured with a different assay. No thyroid measurements were taken during the second visit of the first cohort.

The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus MC and by the Ministry of Health, Welfare and Sport of the Netherlands, implementing the “Wet Bevolkingsonderzoek: ERGO (Population Studies Act: Rotterdam Study)”. We only included participants who provided written informed consent to participate in the study, to give access to information from their attending physicians and for follow-up monitoring. Figure 1 shows the flowchart of the study population.

Assessment of thyroid function

Thyroid function assessment included measurement of thyroid-stimulating hormone (TSH), FT_4, and thyroid peroxidase antibodies (TPOAb) in serum samples stored at -80°C (electrochemiluminescence immunoassay for thyroxine, thyrotropine and thyroid peroxidase antibodies, “ECLIA”, Roche, Basel, Switzerland). The cut-off

values of the normal range of TSH were set to 0.4-4.0 mIU/L, while the reference range for FT_4 was 11-25 pmol/L, in accordance with previous studies and in line with Dutch national guidelines. [3,10,20] TPOAb levels greater than 35 kU/mL were regarded as positive, as recommended by the assay manufacturer. Euthyroidism was defined as a TSH value within the reference range. Hypothyroidism was defined by TSH > 4.0 and FT_4 <11, whilst subclinical hypothyroidism was defined as TSH > 4.0 and FT_4 within the reference range. Hyperthyroidism was defined by TSH < 0.4 and FT_4 > 25, while subclinical hyperthyroidism was defined as TSH < 0.4 and FT_4 within the reference range.

Electrocardiography

ECGs were recorded during the same center visit as the thyroid function measurements. Standard 12-lead 10-second ECGs were recorded during rest with an ACTA Gnosis electrocardiograph (Esaote Biomedica, Florence, Italy). We used a sampling frequency of 500 Hz and the ECGs were stored digitally. ECGs were processed by the Modular ECG Analysis System (MEANS), which has been described previously and has been validated and applied extensively. [21,22] Exclusion criteria for ECGs were atrial fibrillation, atrial flutter, or pacemaker rhythm. We used short-term variability of QT intervals (STVqt)[1] as marker for QT variability. STVqt is defined as

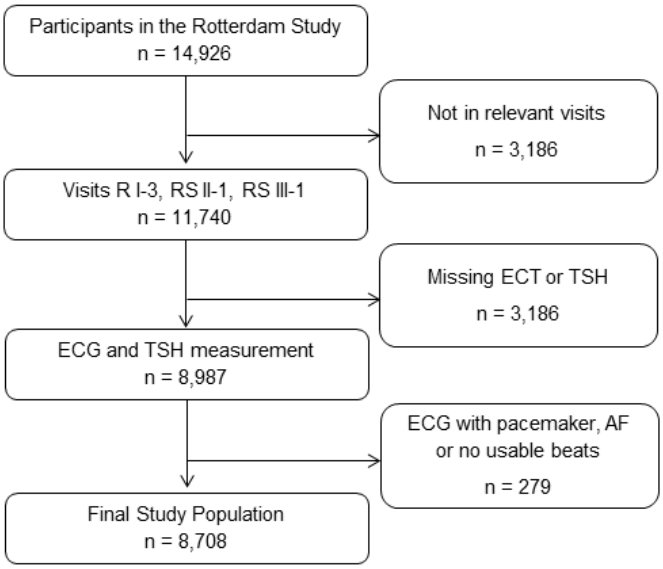
$$\mathrm{STVqt} = \sum_{i=1}^{n} \frac{|QT_{i+1} - QT_i|}{n\sqrt{2}}$$

where QT_i is the QT interval of beat i, and n is the total number of beats in the ECG recording.

QT intervals were measured automatically using fiducial segment averaging (FSA). [23] First, MEANS determines the locations of the individual QRS complexes on the ECG and initial fiducial points (onset of QRS complex and end of T wave) and a detection function consisting of the root-mean-square ECG signal, [24] is computed. Second, the fiducial point in each individual beat is shifted in an iterative procedure until maximum correlation is achieved between a small signal segment of the detection function around this fiducial point and the average of the segments around the fiducial points of the remaining complexes. The amount of shifting is

retained and constitutes the individual beat variation in the fiducial point estimate. Finally, the QT interval for each beat is calculated taking into account the shifts of the initial QRS onset and T end. MEANS automatically detects ectopic beats, and excludes them from further processing. To safeguard against signal segments with excessive noise or baseline wander, an additional test is applied after each FSA iteration. If the averaged absolute amplitudes of the difference between the ST-T wave of an individual beat and the averaged ST-T wave of the remaining beats is larger than a preset value, the beat is discarded and the iteration process is repeated for the remaining beats.

Figure 1 Flowchart of the study population



Abbreviations: RS, Rotterdam Study; RS I-3, Third visit of the first cohort; RS II-1, First visit of the second cohort; RS III-1, First visit of the third cohort; ECG, electrocardiogram; TSH, Thyroid-stimulating hormone; AF, Atrial fibrillation

Covariables

The following covariables were derived from the ECG: heart rate, heart-rate variability, (average) QT interval, and Sokolow-Lyon index. Heart-rate variability was expressed as the standard deviation of normal-to-normal RR intervals (SDNN,

in milliseconds) [25] and log-transformed in the analyses to approximate a normal distribution. The Sokolow-Lyon index was defined as the sum of the voltages (in millivolt) of the S wave in lead V1 and the R wave in lead V5 or V6, whichever was larger. The following clinical covariables were used: age, sex, smoking, BMI, hypertension, coronary heart disease (CHD), and diabetes mellitus. Smoking was based on a home interview. Participants were classified into never, former, and current smokers. BMI was defined as weight/height^2, with weight in kg and height in meters. Blood pressure was measured twice in the sitting position on the right upper arm. The average of two measurements was used. Hypertension was defined as a systolic blood pressure ≥140 mmHg, or a diastolic blood pressure ≥90 mmHg, or the use of blood-pressure-lowering medication with the indication hypertension. CHD was defined as a history of a myocardial infarction (MI) or a surgical or percutaneous coronary revascularization procedure. [26] Diabetes mellitus was defined as a fasting serum glucose level ≥7.0 mmol/L or a non-fasting serum glucose level ≥11.1 mmol/L (if fasting serum glucose was not present), or use of glucose-lowering medication, or a previous diagnosis of diabetes mellitus.

Statistical analyses

STVqt and TSH were log-transformed to normalize their distributions for all continuous analyses. Zero values for STVqt (n = 14) were set to one fourth of the lowest nonzero value to allow log-transformation. We implemented linear regression models to study the association of FT_4 with STVqt, using the Wald-test for confidence intervals (CIs) and p-values. The possibility of a nonlinear association between log-transformed TSH or FT_4 and STVqt was tested by successively adding polynomial terms of FT_4: FT_4^2, FT_4^3 and FT_4^4. The value of adding these terms was assessed with the likelihood ratio test.

All participants with a TSH measurement and an STVqt measurement were included in the main analysis (six missing values for FT_4 were imputed). Three sensitivity analyses were performed. First, to assess the possible influence of drug use, we excluded all users of thyroid function altering preparations (Anatomical Therapeutic Chemical (ATC) code H03A), anti-thyroid preparations (ATC H03B), iodine therapy (ATC H03C), corticosteroids for systemic use (ATC H02A and H02B), or amiodarone. Second, we limited the analyses to euthyroid participants (as defined above). Third, to rule out a potential effect of auto-immunity on STVqt,

we excluded participants with TPOAb >35 kU/l. Furthermore, we performed pre-specified analyses stratified by sex and stratified by age (older or younger than 65 years). For all analyses, we created a basic model adjusted for age and sex and a multivariable model with adjustment for age, sex, cohort, averaged QT interval, heart rate, and log-transformed heart-rate variability, BMI, hypertension, smoking status, serum total cholesterol, prevalent diabetes mellitus, and prevalent CHD. If the association of STVqt with FT_4 or with TSH was significant, we plotted the estimated regression line with 95% confidence intervals of FT_4 or TSH versus values of STVqt. The association of thyroid function with STVqt was additionally assessed using thyroid status, a categorical variable with the categories hypothyroid, subclinical hypothyroid, euthyroid, subclinical hyperthyroid and hyperthyroid. This categorical variable was analyzed in a linear regression model, using the same covariables and sensitivity analyses as in the analyses with TSH and FT_4.

After selecting those participants with both a TSH measurement and an ECG, remaining missing values in the other covariables were imputed with five-times imputation. There were no variables with more than 5% missing values. For all analyses we used SPSS (IBM SPSS Statistics for Windows, version 21.0. Armonk, NY). The regression line and 95% confidence intervals were calculated in R.

RESULTS

Population characteristic

There were 8,987 participants with a TSH measurement and an ECG (Figure 1). We excluded 279 participants because their ECG showed a pacemaker signal (n = 8), atrial fibrillation (n = 138), atrial flutter (n = 13), or because their ECG had no usable consecutive QT intervals (n = 120). Of the remaining 8,708 participants, 4,955 were women (56.9%) and the average age was 64.5 years, with a standard deviation of 9.7 years. Further characteristics of the study population are shown in Table 1.

Table 1 Baseline characteristics of included participants

Characteristic	Result
Participants, N	8,708
Women, N (%)	4,955 (56.9%)
Age (years), mean (SD)	64.5 (9.7)
Diabetes mellitus	1,604 (18.4%)
Smoking, N (%)	
never	2,704 (31.1%)
former	4,247 (48.8)
current	1,711 (19.6%)
Hypertension, N (%)	5,306 (60.9%)
Prevalent CHD, N (%)	575 (6.6%)
BMI (kg/m2), mean (SD)	27.3 (4.2)
Total cholesterol (mmol/L), mean (SD)	5.7 (1.0)
TSH (mU/l), median (IQR)	1.9 (1.3; 2.8)
FT4 (pmol/l), mean (SD)	15.7 (2.3)
TPOAb > 35 (kU/mL), N (%)	1,147 (13.2%)
Hypothyroidism, N (%)	67 (0.8%)
Subclinical hypothyroidism, N (%)	815 (9.4%)
Euthyroidism, N (%)	7,591 (87.2%)
Subclinical hyperthyroidism, N (%)	215 (2.5%)
Hyperthyroidism, N (%)	20 (0.2%)
Heart rate (bpm), mean (SD)	68.9 (10.9)
QT interval (ms) / QTc bazett, mean (SD)	405 (30) / 431 (24)
SDNN (ms), median (IQR)	16.4 (10.4; 27.0)
STVqt (ms), median (IQR)	1.8 (1.2; 3.0)

Abbreviations: BMI, body-mass index; CHD, coronary heart disease; FT4, thyroxine; IQR, interquartile range; QTc, heart-rate corrected QT interval; SD, standard deviation; SDNN, standard deviation of normal-to-normal RR intervals; STVqt, short-term variability of the QT interval; TPOAb, thyroid peroxidase antibodies; TSH, thyroid-stimulating hormone

The association of TSH and FT4 with STVqt

Table 2 shows the association of TSH and FT_4 with STVqt in the multivariable models. Both FT_4 and FT_4^2 were significantly associated with STVqt, indicating a U-shaped relationship of FT_4 with STVqt. TSH was not significantly associated with STVqt, except in the sensitivity analysis excluding users of thyroid-affecting medication. Otherwise, the sensitivity analyses did not reveal relevant changes in the association between thyroid function and STVqt. Results of the basic model were very similar to the multivariate model, e.g. main analysis FT_4 -0.086 (95%CI-0.124; -0.047), FT_4^2 0.003 (95%CI 0.001; 0.004) and thus not shown for all analyses.

Table 2 Association of thyroid function with log-transformed STVqt

Population	Determinant	Beta (95% CI)
All participants	log-transformed TSH	0.016 (-0.004; 0.036)
n = 8,708	FT_4	-0.079 (-0.117; -0.041)
	FT_4^2	0.002 (0.001; 0.003)
No thyroid medication	log-transformed TSH	0.023 (0.001; 0.045)
n = 8,181	FT_4	-0.088 (-0.138; -0.039)
	FT_4^2	0.003 (0.001; 0.004)
Euthyroid status	log-transformed TSH	0.013 (-0.023; 0.048)
n = 7,591	FT_4	-0.168 (-0.244; -0.092)
	FT_4^2	0.005 (0.003; 0.007)
TPOAb ≤ 35	log-transformed TSH	0.009 (-0.014; 0.033)
n = 7,552	FT_4	-0.076 (-0.122; -0.031)
	FT_4^2	0.002 (0.001; 0.004)

Notes: TSH and FT_4 were assessed in separate linear regression models. Estimates were adjusted for cohort, age, sex, QT interval, heart rate and heart-rate variability, BMI, hypertension, serum total cholesterol, prevalent diabetes, prevalent coronary heart disease and smoking status. *No thyroid medication* excludes users of thyroid function altering preparations (Anatomical Therapeutic Chemical (ATC) code H03A), anti-thyroid preparations (ATC H03B), iodine therapy (ATC H03C), corticosteroids for systemic use, (ATC H02A and H02B) and Amiodarone. Bold indicates P-value below 0.05.
Abbreviations: FT_4, Thyroxine; STVqt, short-term variability of the QT interval; TPOAb, thyroid peroxidase antibodies; TSH, thyroid-stimulating hormone.

Table 3 shows the analyses stratified on age and sex. There was a significant interaction of sex with FT_4 and FT_4^2 (p-values 0.014 and 0.006, respectively). The association of FT_4 with STVqt was significantly stronger in men than in women FT_4: -0.143 (95% CI -0.202; -0.085) in men versus -0.048 (95% CI -0.096; 0.001) in women, FT_4^2 : 0.004 (95% CI 0.003; 0.006) in men versus 0.001, 95% CI 0; 0.001 in women. In the stratification on age, there was no significant interaction with FT_4 or FT_4^2 when comparing those younger than 65 with those older than 65.

Figure 2 shows the regression lines of FT_4 and back-transformed STVqt, based on the model including all men in the left panel. The figure shows that STVqt is higher with an increased or a decreased FT_4. Figure 2 showing the same plot for all women in the right panel, reveals a weaker U-shaped association between FT_4 and STVqt. Supplementary Figure 1 and 2 show the same regression lines for men and women with euthyroid status, showing a similar pattern.

Table 3 Association of thyroid function with log-transformed STVqt stratified on age and sex

	Men; n = 3,753 **Beta (95% CI)**	**Women; n = 4,955** **Beta (95% CI)**
log-transformed TSH	-0.001 (-0.036; 0.034)	0.021 (-0.004; 0.046)
(log-transformed TSH)^2	0.022 (0.007; 0.038)	
FT_4	-0.136 (-0.195; -0.078)	-0.044 (-0.094; 0.006)
FT_4^2	0.004 (0.002; 0.006)	0.001 (0.000; 0.003)
	Age ≤ 65; n = 5,018 **Beta (95% CI)**	**Age > 65; n = 3,690** **Beta (95% CI)**
log-transformed TSH	0.004 (-0.023; 0.030)	0.028 (-0.003; 0.058)
FT4	-0.036 (-0.099; 0.026)	-0.102 (-1.53; -0.052)
FT_4^2	0.001 (-0.001; 0.003)	0.003 (0.001; 0.004)

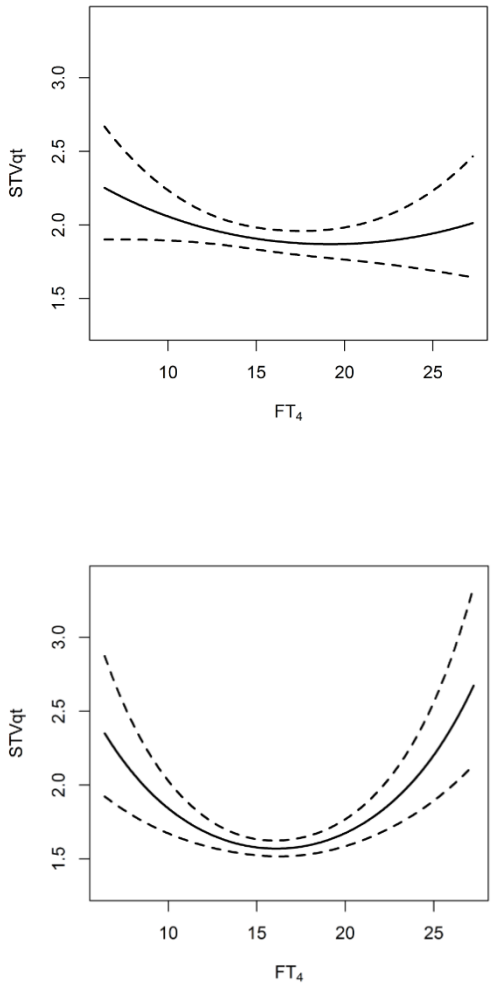
Notes: TSH and FT_4 were assessed in separate linear regression models. Estimates were adjusted for cohort, age, sex, QT interval, heart rate and heart-rate variability, BMI, hypertension, serum total cholesterol, prevalent diabetes, prevalent coronary heart disease and smoking status. Bold indicates P-value below 0.05

Abbreviations: FT_4, thyroxine; STVqt, short-term variability of the QT interval; TSH, thyroid-stimulating hormone.

Categorical analysis of the association between thyroid status and STVqt

The categorical analyses are shown in Supplementary Tables 1 and 2. Supplementary Table 1 first shows the main analysis, where subclinical hyperthyroid participants had the lowest beta for STVqt. However, differences with the other categories were not statistically significant, and the p-for trend was 0.41. In the sensitivity analysis excluding subjects that used thyroid function altering drugs, the hyperthyroid participants had the lowest beta for STVqt (Supplementary Table 1). When excluding participants with TPOAbs > 35, the hypothyroid participants had the lowest beta for STVqt. It should be noted that there were very low numbers (n = 8 and n = 10, respectively) in those categories. Supplementary Table 2 shows the categorical analysis stratified on sex. In the stratified analysis, no results were significant, except that hyperthyroid women had a significantly higher STVqt than euthyroid women.

Figure 2 Estimated regression line of STVqt by FT4 status with 95% confidence intervals in men (upper panel) and women (lower panel)



STVqt was used as a log-transformed variable in the analysis and back-transformed for presentation. The prediction for men was based on all included men (n = 3,753) and for women on all included women (n = 4,955). The analysis was adjusted for cohort, age, QT interval, heart rate, heart-rate variability, BMI, hypertension, serum total cholesterol, prevalent diabetes, prevalent coronary heart disease and smoking status. The continuous line indicates the estimate, the dotted lines indicate the limits of the 95% confidence interval.

DISCUSSION

Our study shows that there is a U-shaped association between FT_4 and STVqt. This association is significantly stronger in men than in women. TSH was only significantly associated with STVqt when excluding users of thyroid-affecting drugs. The analysis of overt thyroid dysfunction with STVqt showed no significant results, probably due to small numbers in several categories.

This is the first study that analyzes the association between thyroid function and QT variability.[4] Studies of heart-rate variability (HRV) found that HRV was decreased in both subclinical hyperthyroidism [27] and overt hypothyroidism, [28] suggesting a U-shaped association of thyroid function with heart-rate variability. Our study demonstrates a similar relationship of FT_4 with STVqt. We did not find a significant association of TSH with STVqt in the main analysis. Similarly, in previous studies in the elderly, FT_4, but not TSH, was associated with SCD [3] or atrial fibrillation.[10] This might be due to changes in the set point of the hypothalamic-pituitary-thyroid axis, with an altered TSH secretion in reaction to negative feedback of thyroid hormone due to aging. [20] The fact that the association of TSH with STVqt is significant when excluding thyroid-affecting drugs could indicate a different association between TSH and STVqt in users of thyroid-affecting drugs, which interferes with the association in the main analysis.

QT variability is thought to be increased when channels of the inward rectifying current (I_{kr}) in cardiac myocytes are blocked. Blockade of I_{kr} delays repolarization and increases the QT interval. [1] I_{kr} potassium channels can be blocked by the use of certain drugs, [4] or in congenital long-QT syndromes, [29] which are also associated with an increase in QT variability. It has been shown in animal models that an increase in FT_3 leads to a quick decrease in the I_{kr} current, prolonging repolarization. [30] it is possible that the I_{kr} potassium channels are the pathway by which higher levels of thyroid hormone lead to an increase in STVqt. The association of thyroid function and STVqt may also be indirect: case reports suggest that both hyperthyroidism [31] and hypothyroidism [32] can lead to dilated cardiomyopathy, and it is known that STVqt and other QT variability markers are increased in dilated cardiomyopathy. [2,33] However, we could not address dilated cardiomyopathy in our study, as we did not have data of this diagnosis, and future

studies should address this issue. Other possible pathways, e.g. common genetic factors, have also not yet been investigated.

Previous studies have reported that an increased QT variability is associated with ventricular arrhythmias [5,34] and sudden cardiac death. [35-38] Our study indicates that both lower and higher levels of FT4 lead to an increase in STVqt, which suggests that both hyperthyroidism and hypothyroidism could lead to ventricular arrhythmias. This is supported by a number of case reports of ventricular arrhythmias and sudden cardiac death in both hyperthyroidism [12-14] and hypothyroidism. [11,15-17] In a previous study we reported an association of higher FT_4 with an increased risk of SCD. [3] Also, there seemed to be a stronger positive association of FT_4 with SCD in men than in women, but this was not statistically significant. This suggests that STVqt could be involved in underlying mechanisms of the association between FT_4 and SCD. In our previous study of thyroid function and SCD, [3] there was no association between a low FT_4 and SCD, and it is possible that when a low FT_4 leads to a higher STVqt, other morbidity and mortality than SCD occurs. Also, the previously mentioned association of FT_3 and the I_{kr} potassium channels [30] cannot explain why hypothyroidism is associated with an increased STVqt. Thus, further studies are needed to elucidate the role of STVqt in mortality associated with thyroid disease.

We found that the (U-shaped) association of FT_4 with STVqt was stronger in men than in women, with significant interaction terms between sex and FT_4, FT_4^2. A previous study in the Rotterdam Study population reported that an increased FT_4 was associated with a higher risk of (average) QTc prolongation, but only in men. [39] Moreover, animal studies in dogs have shown that testosterone increases expression of potassium channels in cardiac myocytes and the QT interval on the ECG. [40] These studies suggest that repolarization of the heart affected both by testosterone and by thyroid hormone, perhaps leading to interaction between the effects of the two hormones.

Our study has a number of strengths and limitations. Strengths of our study include the use of a large population-based cohort of community-dwelling middle-aged and elderly participants. Furthermore, ECGs and thyroid measurements were performed prospectively without knowledge of the study question, and the ECGs were processed automatically, which reduced observer bias.

A limitation of our study is that most previous studies used longer ECG recordings (usually 3 to 5 minutes) than the standard 10-second ECGs used in this study, [4] and the relationship between STVqt measured on short ECGs and STVqt measured on longer ECGs has not yet been studied. However, despite this limitation, we were able to find a significant association between FT_4 and STVqt. Additionally, the results that are obtained in this middle-aged and elderly population might not be representative of younger populations. We could not evaluate temporal relationships between FT_4 and STVqt due to the cross-sectional design of our study.

In conclusion, we found that FT_4 has a U-shaped association with STVqt, as measured on 10-second ECGs. Since STVqt is a marker of repolarization instability, our study suggests that both high FT_4 and low FT_4 levels lead to repolarization instability. Further studies should elucidate the role of STVqt in mortality associated with thyroid disease, and possible sex differences in the relationship between thyroid function, STVqt and sudden cardiac death.

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Supplementary Table 1 Effect of thyroid status on log-transformed STVqt

	All participants		No thyroid medication		TPOAb ≤35	
Thyroid status	**n**	**β (95% CI)**	**n**	**β (95% CI)**	**n**	**β (95% CI)**
Hypothyroid	67	0.08 (-0.09; 0.27)	59	0.13 (-0.06 - 0.33)	10	-0.02 (-0.48; 0.45)
Sub. Hypothyroid	815	0.05 (0.00; 0.10)	712	0.06 (0.00; 0.12)	502	0.08 (0.01; 0.15)
Euthyroid	7,591	Reference	7,238	Reference	6,844	Reference
Sub. Hyperthyroid	215	-0.02 (-0.13; 0.08)	164	0.00; (-0.11; 0.12)	179	0.00 (-0.11; 0.11)
Hyperthyroid	20	0.06 (-0.27; 0.39)	8	-0.05 (-0.56; 0.47)	17	0.11 (-0.25; 0.47)

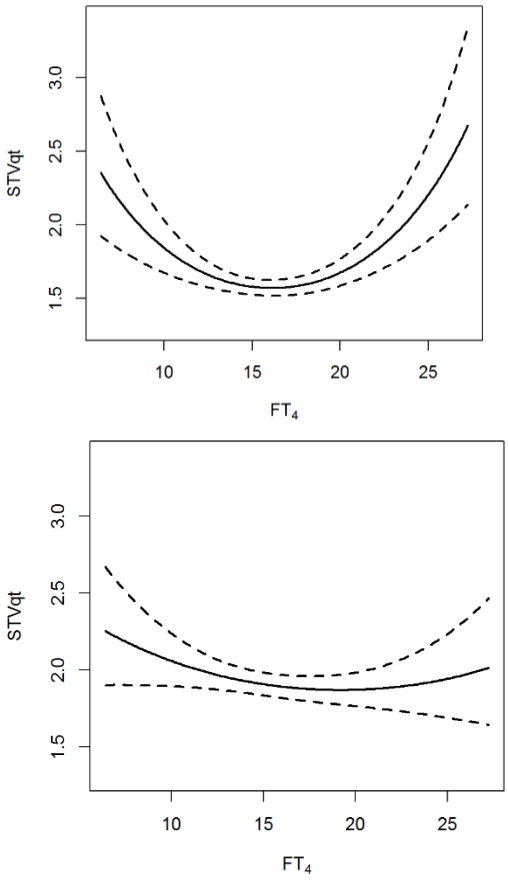
Notes: β Estimates were adjusted for cohort, age, sex, QT interval, heart rate and heart-rate variability, BMI, hypertension, serum total cholesterol, prevalent diabetes, prevalent coronary heart disease and smoking status. *No thyroid medication* excludes users of thyroid preparations (Anatomical Therapeutic Chemical (ATC) code H03A), anti-thyroid preparations (ATC H03B), iodine therapy (ATC H03C), corticosteroids for systemic use, (ATC H02A and H02B) and Amiodarone.
Abbreviations: STVqt, short-term variability of the QT interval; Sub, Subclinical; BMI, Body-mass index

Supplementary Table 2 Effect of thyroid status on log-transformed STVqt stratified on sex

	Men		Women	
Thyroid status	**n**	**β (95% CI)**	**n**	**β (95% CI)**
Hypothyroid	17	0.21 (-0.13; 0.55)	50	0.04 (0.01; 0.13)
Sub. Hypothyroid	240	0.03 (-0.06; 0.13)	575	0.05 (-0.17; 0.27)
Euthyroid	3,427	Reference	4,164	Reference
Sub. Hyperthyroid	66	0.10 (-0.08; 0.27)	149	-0.08 (-0.20; 0.05)
Hyperthyroid	3	0.41 (-0.40; 1.21)	17	0.00 (-0.37; 0.38)

Notes: β Estimates were adjusted for cohort, age, sex, QT interval, heart rate and heart-rate variability, BMI, hypertension, serum total cholesterol, prevalent diabetes, prevalent coronary heart disease and smoking status. *No thyroid medication* excludes users of thyroid preparations (Anatomical Therapeutic Chemical (ATC) code H03A), anti-thyroid preparations (ATC H03B), iodine therapy (ATC H03C), corticosteroids for systemic use, (ATC H02A and H02B) and Amiodarone.
Abbreviations: STVqt, short-term variability of the QT interval; Sub, Subclinical

Supplementary Figure 1 Estimated regression line with 95% confidence intervals of STVqt by FT_4 status in euthyroid men (upper panel) and euthyroid women (lower panel)



Notes: STVqt was used as a log-transformed variable in the analysis and back-transformed for presentation. The prediction was based on men with euthyroid status (n = 3,427) and women with euthyroid status (n = 4,164). The analysis was adjusted for cohort, age,QT interval, heart rate, heart-rate variability, BMI, hypertension, serum total cholesterol, prevalent diabetes, prevalent coronary heart disease and smoking status. The continuous line indicates the estimate, the dotted lines indicate the limits of the 95% confidence interval.
Abbreviations**:** STVqt, short-term variability of the QT interval; BMI, Body-mass index.

CHAPTER 2.4

THYROID FUNCTION AND THE RISK OF ATHEROSCLEROTIC CARDIOVASCULAR MORBIDITY AND MORTALITY

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Submitted

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 investigated the association of thyroid function with subclinical atherosclerosis, incident atherosclerotic cardiovascular (ASCV) events and ASCV mortality.

METHODS In a prospective population-based study, 9231 community-dwelling participants (mean age 64.7) were included. Outcomes under study were: 1.Presence of subclinical atherosclerosis, assessed by coronary artery calcification (CAC) score >100 AU; 2.ASCV events, defined as fatal and nonfatal myocardial infarction, other coronary heart disease (CHD) mortality or stroke; 3.ASCV mortality, defined as death due to CHD, cerebrovascular or other atherosclerotic diseases. Associations of thyroid-stimulating hormone (TSH) and free thyroxine (FT4) with the outcomes were assessed through logistic regression and Cox proportional-hazard models, adjusted for potential confounders including cardiovascular risk factors.

RESULTS During a median follow-up of 8.9 years (interquartile range: 4.5-11.8 years), 935 incident ASCV events and 580 ASCV deaths occurred. FT4 levels were positively associated with high CAC score (odds ratio [OR]; 95% confidence interval [CI]: 2.28; 1.30-4.03) and incident ASCV events (hazard ratio [HR]; CI: 1.87; 1.34-2.59). The risk of ASCV mortality increased with higher FT4 levels (HR; CI: 2.35; 1.61-3.41 per 1 ng/dl) and lower TSH levels (HR; CI: 0.92; 0.84-1.00 per 1 logTSH), with stronger estimates among participants with prevalent ASCV disease (HR; CI: 5.76; 2.79-11.89 for FT4 and 0.81; 0.69-0.95 for TSH) (p for interaction of FT4 and TSH with prevalent ASCV disease 0.002 and 0.04, respectively). Results remained similar or became stronger among euthyroid participants.

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

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 (TH) concentrations have commonly been linked with systolic hypertension[3,4] and hypercoagulation[5]; whereas lower circulating TH levels can instigate hyperlipidemia and inflammation.[6] Although atherosclerosis is a continuous process, prospective epidemiological studies so far have mainly focused on the relation between specific ranges of thyroid function and distinct atherosclerotic events, such as coronary heart disease (CHD) or stroke.[7-12] Results have been quite inconsistent. Some studies have reported that TSH levels ≥10 mIU/L or high-normal TSH levels may constitute an increased risk of CHD. [7,10] In contrast, other studies have reported a positive association of thyroid function with CHD or stroke risk.[9,11]Others have found no association.[8,12] 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 prospective population-based cohort study of middle-aged and elderly individuals, we examined the association of thyroid function with coronary artery calcification (CAC) (as a well-documented marker of subclinical atherosclerosis) 13, 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.[14] The Rotterdam Study was initiated in 1989, including 7983 participants aged 55 years or older (RS I) residing in Ommoord district of Rotterdam, the Netherlands. In 2000, the study was extended with a second cohort of 3011 subjects (RSII). In 2006, a third cohort of 3932 subjects aged 45 years or older was added (RSIII). As of now, the Rotterdam Study comprises a total of 14926 subjects, who undergo extensive follow-up medical examinations every 3 to 5 years.

The baseline for the current study included participants from the third visit of the first cohort (RS-I.3) and the first visit of the second (RS-II.1) and third cohorts (RS-III.1). Complete data on thyroid function and incident events were available in 9231 participants from these visits Supplemental Figure 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. All participants have given written informed consent.

Assessment of thyroid function

Thyroid function was assessed at baseline in three study cohorts using the same method and assay. Measurements of thyroid-stimulating hormone (TSH), free thyroxine (FT4), and thyroid peroxidase antibodies (TPOAb) were performed in baseline serum samples stored at -80°C using the electrochemiluminescence immunoassay “ECLIA” Roche. The reference ranges of serum TSH (0.40–4.0 mIU/L) and serum FT4 (0.86–1.94 ng/dL; equivalent to 11–25 pmol/L) were determined based on national guidelines and our previous studies.[15] Levels of TPOAb >35 kU/ml were regarded as positive, as recommended by the assay manufacturer.[15]

Assessment of CAC, ASCV events and ASCV mortality

Outcome measures under study were 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 2002 participants, during the third visit of the first cohort and the first visit of the second cohort. CAC was measured by electron beam computed tomography scans (C-150 Imatron GE) of the coronary arteries.[14] 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.[16] CAC score of 100 Agatson units (AU) or greater suggests clinically significant atherosclerotic plaque [2] and has been used in past consensus statements.[17] Therefore, we grouped participants into CAC score lower than 100 AU and CAC score of 100 AU or greater. ASCV events were defined as fatal and nonfatal MI, other CHD mortality, or stroke, as previously described.[18] Prevalent ASCV disease was defined as history of MI, stroke, coronary or other arterial revascularization.[18] Prevalent ASCV disease at baseline was assessed through interview and verified in medical records. ASCV mortality was defined as death due to CHD, cerebrovascular disease or other atherosclerotic diseases, and methods of ascertainment have been previously described.[18,19] Non-ASCV mortality was defined as death due to causes other than atherosclerotic disease. 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.

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). 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 medication.[20] 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. Heart failure (HF) was defined as the presence of typical symptoms and signs (i.e. breathlessness at rest or during exertion, ankle edema, and pulmonary crepitations), confirmed by the objective evidence of cardiac dysfunction (i.e. chest X-ray, echocardiography) or a positive response to the initiated treatment. The adjudication of HF was performed in accordance with the guidelines of the European Society of Cardiology.[21]

Statistical analysis

We used logistic regression models to investigate the cross-sectional association of thyroid function parameters with the risk of having CAC score of 100 AU or greater, among participants that were free of atherosclerotic cardiovascular disease. The association of thyroid function parameters 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 parameters 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. In the analysis of incident ASCV events, the end date of follow-up was considered the date of incident ASCV event, the date of death or January 1, 2012, whichever came first. In the analysis of ASCV mortality, the end date of follow-up was considered the date of death or January 1, 2012, whichever came first.

Additionally, 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) We excluded participants using thyroid function-altering medications, such as thyroid medications, analgesics (including non-steroidal anti-inflammatory drugs, paracetamol and muscle relaxants), corticosteroids or amiodarone; 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) To detect a potential influence of follow-up duration on our results, analyses of ASCV mortality were performed for lengths of follow-up 8, 10 and 12 years.

All analyses were adjusted for potential confounders, that were selected based on biological plausibility and previous literature. Model 1 was adjusted for age, gender and cohort. Model 2 was additionally adjusted for smoking status, alcohol intake, BMI, total cholesterol, triglycerides, systolic blood pressure, prevalent diabetes and use of antihypertensive and lipid-lowering medications. The analyses of ASCV mortality were additionally adjusted for presence of prevalent ASCV disease. Detailed descriptions of the research methods are provided in the Appendix. TSH was naturally log transformed, due to its skewed distribution. The proportional hazards assumption was assessed by Schoenfeld test and plots. No violation of the proportional hazards assumption was observed. Potential departure from linearity was explored by adding quadratic and cubic terms of covariates in the multivariable model, but none of these terms were significant. We checked for effect modification by separately adding product interaction terms of the exposure (TSH or FT4 or TPOAb) with each of the covariates of the most adjusted model.

Multiple imputations were performed for missing data (< 5% missings for all covariates). Schoenfeld test and plots were performed using R (survival package R-project, Institute for Statistics and Mathematics, R Core Team, version 3.2.3). All other statistical analyses were performed using IBM SPSS version 21 (IBM Corp).

RESULTS

We included 9231 participants with a maximum follow-up time of 14.7 years and a median of 8.9 years (interquartile range, 4.5-11.8 years). Baseline characteristics are presented in Table 1. The mean age of participants was 64.7 (± 9.7) years and 57.1% were women (Table 1). A total of 935 incident ASCV events (incidence rate, 12.8 per 1000 person-years) and 580 ASCV deaths (incidence rate, 7.6 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 completeness of follow-up for ASCV mortality was 99.0%.

Table 1 Baseline characteristics of 9231 participants*

Variable	Mean (SD)*
Age, years	64.7 (9.7)
Female, n (%)	5268 (57.1)
Smoking, n (%)	
current	1941 (21.0)
former	4375 (47.4)
never	2915 (31.6)
TSH, mIU/L, median, IQR	1.9 (1.2-2.8)
FT4, ng/dl	1.2 (0.2)
TPOAb positive, n (%)	1221 (13.2)
Use of thyroid medication, n (%)	294 (3.2)
Thyroid surgery, n (%)	165 (1.8)
History of thyroid disease, n (%)	750 (8.1)
BMI, 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 (%)	1432 (15.5)
Systolic blood pressure, mm Hg	139.3 (21.0)
Use of antihypertensive medications, n (%)	2095 (22.7)
History of diabetes, n (%)	1099 (11.5)
History of atherosclerotic cardiovascular disease, n (%)	714 (7.7)
Follow-up time for atherosclerotic events, median years, IQR	7.1 (4.3-11.6)
Follow-up time for atherosclerotic mortality, median years IQR	8.9 (4.5-11.8)

*unless specified otherwise
Abbreviations: BMI, body-mass index; FT4, free thyroxine; IQR, interquartile range; TPOAb, thyroid peroxidase antibodies (cutoff 35 kU/ml);.TSH, thyroid-stimulating hormone

Thyroid function and CAC score

Increasing FT4 levels were associated with higher odds of having CAC score of 100 AU or greater (OR, 2.28; CI, 1.30-4.03 per 1ng/dl; p-value 0.004) (Table 2). However, TSH levels were not associated with having a CAC score of 100 AU or greater (OR, 0.94; CI, 0.84-1.05 per 1 logTSH; p-value 0.29) (Table 2). The association remained similar or became stronger after restricting the analyses to participants with thyroid function within the reference ranges (OR, 2.42; CI, 1.14-5.13 per 1ng/dl FT4; p-value 0.02; OR, 0.91; CI, 0.72-1.16 per 1 logTSH; p-value 0.47) (Table 2). Furthermore, we found no association of TPOAb with the odds of having CAC score of 100 AU or greater (OR, 0.85; CI, 0.63-1.15; p-value 0.28) (Supplemental Table 1). In the analyses of CAC score, the interaction terms between the exposure and each covariate in the most adjusted model were not significant.

Thyroid function and incident ASCV events

There was a positive association of FT4 levels (HR, 1.87; CI, 1.34-2.59 per 1ng/dl; p-value 0.0002), but no association of TSH levels (HR, 0.97; CI, 0.90-1.04 per 1 logTSH; p-value 0.42) with the risk of incident ASCV events (Table 3). The associations became stronger after restricting the analyses to participants with thyroid function within the reference ranges (HR, 2.49; CI, 1.58-3.94 per 1ng/dl; p-value <0.0001; HR, 0.96; CI, 0.82-1.11 per 1 logTSH; p-value 0.59) (Table 3). This corresponds to a 2.69–times higher risk of incident ASCV events, for a participant with a FT4 in the higher limit of the reference range (1.94 ng/dl), compared with a participant with a FT4 in the lower limit of the reference range (0.86 ng/dl). The associations became stronger after excluding participants using thyroid function-altering medications or after censoring the analyses at the time of incident HF; and 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). Furthermore, we found no association of TPOAb with the risk of incident ASCV events (HR, 0.95; CI, 0.78-1.16; p-value 0.61) (Supplemental Table 1). In the analyses of incident ASCV events, the interaction terms between the exposure and each covariate in the most adjusted model were not significant.

Table 2 Cross-sectional association of thyroid function with high coronary artercy calcification (CAC) score*

Thyroid function	High CAC score/ TN (%)	OR (95% CI), Model 1	p-value	OR (95% CI), Model 2	p-value
Full range of measurement					
TSH mIU/L	817/1765 (46.3%)	0.98 (0.88; 1.10)	0.79	0.94 (0.84; 1.05)	0.29
FT4 ng/dL	818/1765 (46.3%)	2.15 (1.22; 3.77)	0.008	2.28 (1.30; 4.03)	0.004
Within the reference ranges (TSH 0.4-4.0 mIU/L FT4 0.85-1.95 ng/dl, excluding thyroid medication and thyroid disease history)					
TSH mIU/L	626/1337 (46.8%)	1.00 (0.78; 1.27)	0.99	0.91 (0.72; 1.16)	0.47
FT4 ng/dL	626/1337 (46.8%)	2.49 (1.17; 5.27)	0.01	2.42 (1.14; 5.13)	0.02

Model 1: age, gender and cohort. Model 2: Model 1, smoking, alcohol intake, body mass index, total cholesterol, triglycerides, systolic blood pressure, prevalent diabetes, use of antihypertensive and lipid-lowering medications. *High CAC score: CAC ≥100 AU. †Reference ranges:. ORs of TSH are per one unit increase of natural log transformed TSH. ORs of FT4 are per one ng/dl increase.Abbreviations: TN, total number; OR, odds ratio; CI, confidence interval; TSH, thyroid-stimulating hormone; FT4, free thyroxine.

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

Thyroid function	Events/ TN (%)	HR (95% CI), Model 1	p-value	HR (95% CI), Model 2	p-value
Full range of measurement					
TSH mIU/L	935/8513 (11.0%)	0.96 (0.90; 1.04)	0.38	0.97 (0.90; 1.04)	0.42
FT4 ng/dL	935/8511 (11.0%)	1.89 (1.37; 2.61)	<0.0001	1.87 (1.34; 2.59)	0.0002
Within the reference ranges (TSH 0.4-4.0 mIU/L FT4 0.85-1.95 ng/dl, excluding thyroid medication and thyroid disease history)					
TSH mIU/L	736/6834 (10.8%)	0.95 (0.81; 1.10)	0.49	0.96 (0.82; 1.11)	0.59
FT4 ng/dL	736/6834 (10.8%)	2.67 (1.69; 4.20)	<0.0001	2.49 (1.58; 3.94)	<0.001

Model 1: age, gender and cohort.Model 2: Model 1, smoking, alcohol intake, body mass index, total cholesterol, triglycerides, systolic blood pressure, prevalent diabetes, use of antihypertensive and lipid-lowering medications.HRs of TSH are per one unit increase of natural log transformed TSH. HRs of FT4 are per one ng/dl increase. Abbreviations: TN, total number; HR, hazard ratio; CI, confidence interval; TSH, thyroid-stimulating hormone; FT4, free thyroxine.

Thyroid function and ASCV mortality

Higher FT4 levels were associated with a higher risk of ASCV mortality (HR, 2.35; CI, 1.61-3.41 per 1ng/dl; p-value <0.0001) (Table 4). In line, higher TSH levels were associated with a lower risk of ASCV mortality (HR, 0.92; CI, 0.84-1.00 per 1 logTSH), though the association was borderline significant (p-value 0.06) (Table 4).

Table 4 Association of thyroid function with atherosclerotic cardiovascular mortality

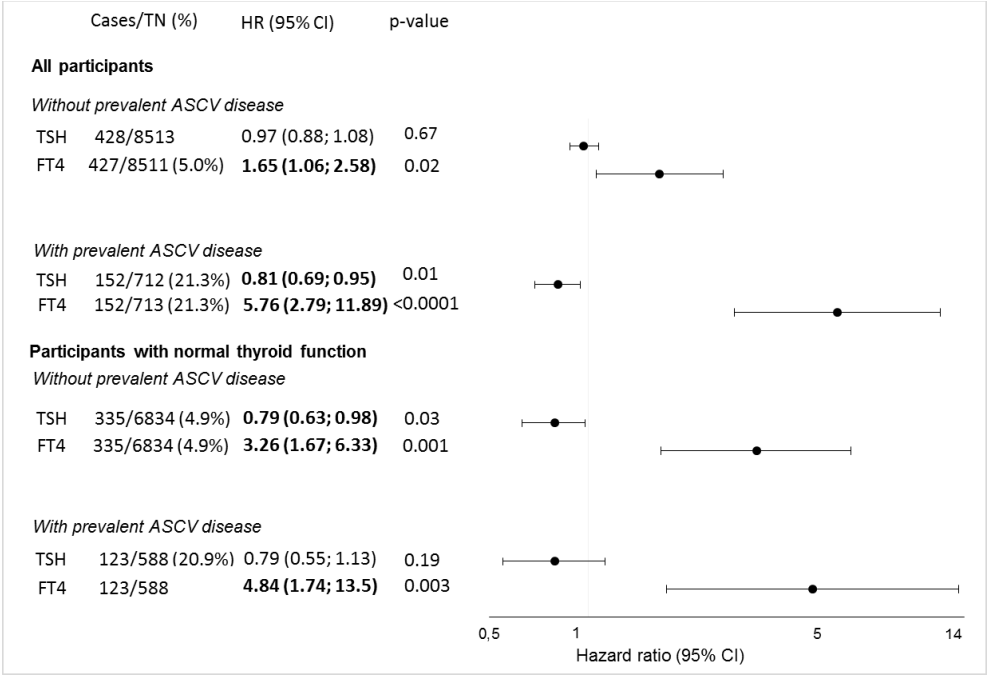
Thyroid function	Events/ TN (%)	HR (95% CI), Model 1	p-value	HR (95% CI), Model 2	p-value
Full range					
TSH, mIU/L	580/9226 (6.3%)	0.93 (0.85; 1.01)	0.12	0.92 (0.84; 1.00)	0.06
FT4, ng/dl	579/9225 (6.3%)	2.17 (1.53; 3.08)	<0.001	2.35 (1.61; 3.41)	<0.001
Thyroid function within the reference ranges*					
TSH, mIU/L	458/7423 (6.2%)	0.80 (0.67; 0.97)	0.02	0.79 (0.65; 0.95)	0.01
FT4, ng/dl	458/7423 (6.2%)	3.76 (2.17; 6.54)	<0.001	3.64 (2.08; 6.37)	<0.001

Model 1: age, gender and cohort. Model 2: Model 1, smoking, alcohol intake, body mass index, total cholesterol, triglycerides, systolic blood pressure, prevalent diabetes, use of antihypertensive and lipid-lowering medications. Both models are adjusted for presence of prevalent atherosclerotic cardiovascular disease at baseline. *Normal reference ranges were defined as serum TSH of 0.4-4.0 mIU/L and FT4 levels of 0.85-1.95 ng/dl, after excluding thyroid medication users and participants with history of thyroid disease. HRs of TSH are denoted per one unit increase of natural log transformed TSH (mIU/L). HRs of FT4 are per one unit increase in FT4 (ng/dl). Abbreviations: TN, total number; HR, hazard ratio; CI, confidence interval; TSH, thyroid-stimulating hormone; FT4, free thyroxine.

In the analysis of ASCV mortality, we found significant interaction terms of TSH and FT4 levels with prevalent ASCV disease at baseline (p for interaction 0.04 and 0.002, respectively), with age (p for interaction 0.04 and 0.01, respectively) and with gender (p for interaction 0.04 and 0.008, respectively). However, these interaction terms were not statistically significant among euthyroid participants (p for interactions > 0.05). Next, we stratified our population based on prevalent ASCV events, age and gender (Figure 1, Supplemental Table 4). Within the strata of age and gender, the interaction terms of TSH or FT4 concentrations with prevalent ASCV disease remained significant (p for interaction <0.05), whereas within the strata of prevalent ASCV disease, the interaction terms of TSH or FT4 concentrations with age or gender lost statistical significance (p for interaction >0.05). Therefore, the association between thyroid function and the risk of ASCV

mortality was assessed separately among subjects with and without ASCV disease (Figure 1). Among participants without prevalent ASCV disease, there was a positive association of FT4 levels (HR, 1.65; CI, 1.06-2.58 per 1ng/dl; p-value 0.02), but no association of TSH levels (HR, 0.97; CI, 0.88-1.08 per 1 logTSH; p-value 0.67) with the risk of ASCV mortality (Figure 1). Among participants with prevalent ASCV disease, higher FT4 (HR, 5.76; CI, 2.79-11.89 per 1ng/dl; p-value <0.0001) and lower TSH (HR, 0.81; CI, 0.69-0.95 per 1 logTSH; p-value 0.81) levels were associated with an increased risk of ASCV mortality (Figure 1).

Figure 1 Hazard ratios of atherosclerotic cardiovascular mortality, stratified by presence of ASCV disease at baseline.



Analyses were adjusting for age, gender, cohort, smoking, alcohol intake, body mass index, total cholesterol, triglycerides, systolic blood pressure, prevalent diabetes, use of antihypertensive and lipid-lowering medications. p for interaction of TSH and FT4 with prevalent ASCV disease were 0.04 and 0.002, respectively. Normal thyroid function was defined as serum TSH of 0.4-4.0 mIU/L and FT4 levels of 0.85-1.95 ng/dl, excluding thyroid medication use and thyroid disease history. Error bars represent the 95% CI of hazard ratios (black dots). Within brackets: Number of atherosclerotic cardiovascular deaths/ Total number. Abbreviations: TN, Total number; ASCV, atherosclerotic cardiovascular; TSH, thyroid-stimulating hormone; FT4, free thyroxine; CI, confidence interval.

The association of thyroid function with ASCV mortality became stronger after restricting the analyses to participants with thyroid function within the reference ranges (HR, 3.64; CI, 2.08-6.37 per 1ng/dl; p-value <0.0001; HR, 0.79; CI, 0.65-0.95 per 1 logTSH; p-value 0.01) (Table 4, Figure 1). This corresponds to a 3.93–times higher risk of ASCV mortality, for a participant with a FT4 in the higher limit of the reference range (1.94 ng/dl), compared with a participant with a FT4 in the lower limit of the reference range (0.86 ng/dl). The associations became stronger after excluding participants using thyroid function-altering medications or after censoring the analyses at the time of incident HF; and 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). Results of the main analysis were consistent with those of the 8, 10 and 12-year follow-up (Supplemental Table 5). The magnitude of association for ASCV mortality was larger than for non-ASCV mortality (Supplemental Table 6). Furthermore, we found no association of TPOAb with the risk of ASCV mortality (HR, 1.01; CI, 0.78-1.31; p-value 0.93) (Supplemental Table 1).

DISCUSSION

In this large prospective population-based cohort study, higher FT4 levels were consistently associated with an increased risk of atherosclerosis, independently of cardiovascular risk factors. The association was consistent throughout the spectrum of atherosclerosis; from subclinical atherosclerosis to overt atherosclerosis to atherosclerotic mortality. The association of thyroid function with atherosclerotic mortality was most pronounced in the presence of preexisting atherosclerotic disease.

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.[15] Our study suggests that the association of thyroid function with atherosclerosis is independent of these cardiovascular risk factors, as our results remained statistically significant after

accounting for serum lipid levels, BMI and AF. After accounting for AF, our risk estimates slightly attenuated, suggesting that AF could be involved in the pathways linking thyroid function to atherosclerosis but does not fully explain the association. 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.[22,23] 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 alternative pathways.

Plausible mechanisms that can link high thyroid function to atherosclerosis include endothelial damage, hemostasis, thrombosis and hemodynamic changes. First, excess TH concentrations can increase the production of reactive oxygen species that further induce the expression of adhesion molecules on endothelial cells[24] Hence, hyperthyroidism has been commonly associated with early atherosclerosis and markers of endothelial dysfunction such as E-selectin, intracellular adhesion molecule-1 and vascular cell-adhesion molecule.[25] Second, TH regulates the synthesis of procoagulant proteins.[26] Excess FT4 levels have been linked with increased concentrations of various procoagulant proteins, namely von Willebrand factor, fibrinogen, factors VIII and IX, that can accelerate plaque vulnerability and rupture [5]. Third, high levels of TH 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 vs 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.[27-30] In an attempt to identify potential subgroups at risk, prior research has suggested that the effect of thyroid function on mortality might be age- [27-30] or gender- dependent.[28,29] Generally, studies performed in older participants have reported an increased risk of mortality with higher FT4 levels[28,29], whereas studies including younger participants have failed to show an association.[31,32] Additional studies have reported an association of thyroid function with mortality risk exclusively in men [33] or women.[7] Our data on atherosclerotic mortality primarily revealed an effect modification by age and gender, with generally stronger risk estimates in males and older subjects. However, the effect disappeared after stratifying by preexisting atherosclerotic disease. This might indicate that the presence of atherosclerotic disease *per se* rather than aging or gender can potentiate the link between thyroid function and atherosclerotic mortality. Prevalent atherosclerotic disease can therefore be an effect modifier of the association between thyroid function and atherosclerotic mortality. Alternatively, one might argue that TH is not a contributor, but rather a marker of subclinical atherosclerosis or a marker of increased mortality in the setting of chronic atherosclerosis. In particular, it could be hypothesized that health problems underlying atherosclerotic disease can affect thyroid parameters. This condition, known as non-thyroidal illness, is typically characterized by normal serum TSH levels combined with low serum triiodothyronine and FT4 levels.[34] In contrast, we found an association of higher rather than lower FT4 levels with an increased risk of atherosclerotic manifestations. Additionally, non-thyroidal illness occurs mainly in critically ill patients, whereas our population consists of relatively healthy community-dwelling adults. Furthermore, our study showed that higher FT4 levels among participants without preexisting atherosclerotic disease were associated with higher risk of atherosclerotic events and atherosclerotic mortality. We took reverse causation into account by excluding events that occurred during the first 2 years of follow-up; and results remained similar. Overall, these data suggest that it is more likely that thyroid function affects atherosclerotic manifestations than vice-versa.

Variations of thyroid function within the reference range markedly affected the risk of atherosclerotic morbidity and mortality in our participants. In line with these

results, a recent individual participant data analysis reported a positive association between thyroid function within the reference range and the risk of stroke.[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], though this could be due to the relatively low proportion of CHD deaths (3.3%). Notably, we observed larger risk estimates after restricting the analyses to participants with thyroid function within the reference range, without history of thyroid disease and not using thyroid function-altering medications. It could therefore be argued that participants with thyroid dysfunction could have become more health conscious and prone to treatment, which could have reduced their risk for atherosclerotic morbidity and mortality over time. Finally, these data provide supporting evidence for a re-evaluation of TSH and FT4 reference ranges, which are currently based on arbitrary statistical approaches (2.5th and 97.5th 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] Thus, the challenge for future research will be to integrate the associated risk of relevant adverse outcomes, in order to eventually define the clinically relevant normal range of thyroid function.

In our study population, there was a positive association between FT4 levels and atherosclerotic outcomes. Although the association between TSH levels and atherosclerotic outcomes was in the expected opposite direction of FT4, it sometimes did not reach statistical significance. Similar observations have been also reported by studies investigating the relationship of thyroid function with various clinical endpoints.[9,28] Serum FT4 levels are tightly regulated by the hypothalamic-pituitary-thyroid axis, with a different set point for each individual. This might explain why FT4 levels are associated with various clinical endpoints, especially within the euthyroid range which is generally defined by TSH. Alternatively, these results may reflect a slight shift in the TSH-FT4 set point, which may be due to aging.[35] To our knowledge, this is the first population-based cohort study to investigate the relationship of thyroid function with atherosclerosis throughout its spectrum; from subclinical atherosclerosis to overt atherosclerosis to atherosclerotic mortality. Another major strength is our prospective design with 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. Several limitations should also be considered. Thyroid function was measured only at baseline and we had no information regarding its fluctuations over time. Nevertheless, due to the intra-individual variability of TSH and FT4 levels, the lack of repeated measurements would tend to underestimate the association between thyroid function and atherosclerotic outcomes rather than generate spurious findings[36] In addition, our results were consistent within the normal range of thyroid function, which is considered to be stable with very small intra-individual variability.[37] We lacked information on serum triiodothyronine levels. However, TSH and FT4 represent the most relevant measurements of thyroid function in clinical practice. Though thyroid function and CAC score were measured in a subpopulation, the general characteristics of that subpopulation were not substantially different from those of the larger population. Given that our study comprised mainly white middle-aged and older adults, the generalizability of our findings to nonwhite and younger populations remains to be investigated. Lastly, the possibility of residual confounding in an observational study design cannot be entirely ruled out.

Conclusions

Higher FT4 levels in middle-aged and elderly subjects were associated with an increased risk of atherosclerotic morbidity and mortality, independently of cardiovascular risk factors. The association of thyroid function with atherosclerotic mortality was most pronounced in the presence of atherosclerotic disease. These findings suggest that FT4 measurement can be a predictive marker of atherosclerotic mortality, especially among subjects with atherosclerotic disease. Furthermore, our findings underscore the importance of identifying the modifiable mediators of the association between thyroid function and atherogenesis. Preventive strategies targeting thyroid function or certain mediators could further lead to a reduction in atherosclerotic events. Lastly, our findings provide supporting evidence for a re-evaluation of the current reference ranges of TSH and FT4 tests, which are based on arbitrary statistical approaches rather than on clinical outcomes such as atherosclerotic morbidity and mortality.

Supplemental material can be found online.

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CHAPTER 2.5

DEFINING OPTIMAL HEALTH RANGE FOR THYROID FUNCTION BASED ON THE RISK OF CARDIOVASCULAR DISEASE

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ABSTRACT

BACKGROUND Reference ranges of thyroid stimulating hormone (TSH) and free thyroxine (FT4) are defined by their distribution in apparently healthy populations, (2.5th and 97.5th percentiles) irrespective of disease risk and used as cut-offs for defining and clinically managing thyroid dysfunction. Our objective is to provide a proof of concept in defining thyroid function optimal health ranges based on cardiovascular disease (CVD) mortality risk.

METHODS A total of 9,233 participants from the Rotterdam Study (mean age 65.0 years) were followed up (median 8.8 years) from baseline to date of death or end of follow-up (2012), which ever came first (689 cases of CVD mortality). We calculated 10-year absolute risks of CVD mortality (defined according to SCORE project) using a Fine and Grey competing risk model per percentile of TSH and FT4, modelled non-linearly and sex- and age-adjusted.

RESULTS Overall, FT4 > 90th percentile was associated with a predicted 10-year CVD mortality risk >7.5% (p =0.005). In men, FT4 > 97th percentile was associated with a risk of 10.8% (p<0.001). In participants ≥ 65 years, absolute risk estimates were <10.0% below the 30th percentile (~14.5 pmol/L or 1.10 ng/dL) and ≥15.0% above the 97th percentile of FT4 (~22 pmol/L or 1.70 ng/dL).

CONCLUSIONS We describe absolute 10-year CVD mortality risks according to thyroid function (TSH and FT4) and suggest optimal health ranges for thyroid function can be defined according to disease risk and are possibly sex and age-dependent. These results need to be replicated with sufficient samples and representative populations.

INTRODUCTION

Reference ranges of blood and other clinical tests are predominantly statistically defined using the 2.5th and 97.5th percentile interval of the population distribution in an apparently healthy population. These reference ranges are typically established under the assumption of a normal distribution or a log-normal distribution and are therefore also referred to as “normal ranges”. This definition of a reference range does not account for whether individuals are symptomatic or at risk of potential adverse events or disease. Nevertheless, these biochemically defined reference values are frequently used to define sickness and health in clinical practice ignoring the inherent risk of the population.

The reference ranges of thyroid function tests, defined by thyroid stimulating hormone (TSH) and free thyroxine (FT4), are examples of reference ranges defined by their distribution. TSH and FT4 reference ranges are currently used as cut-offs to define subclinical and overt thyroid disease, and guide treatment decisions. However, accumulating evidence suggests that subclinical thyroid dysfunction, defined by TSH outside of the reference range but FT4 within the reference range, is also associated with various clinical adverse outcomes, including coronary heart disease (CHD) and cardiovascular mortality, at the extremes.[1,2] Moreover, even differences in thyroid function within the defined reference range are associated with differing risk of cardiovascular events including atrial fibrillation, stroke, sudden cardiac death and cardiovascular mortality.[3-7] Based on the increased risk of CHD in subclinical hypothyroidism, current guidelines advocate treatment with levothyroxine above a TSH of 10 mIU/L, independent of FT4.[8] Extending this concept, the re-evaluation of thyroid function ranges could take clinical adverse events into account and thus move from reference ranges towards “optimal health ranges” for thyroid function. This approach has been successfully applied to management of myocardial infarction, stroke and diabetes using cholesterol, blood pressure or glucose measurements.[9] For example, the defined range for total cholesterol does not rely on the distribution of total cholesterol in a specific population, but rather on the associated 10-year risk of cardiovascular mortality.[9] Pursuing the same strategy for thyroid function might not be as straightforward as for other biomarkers. The risk of

adverse events is relevant for both high and low thyroid function, suggesting a non-linear association, in contrast to cholesterol for example, where the focus is on the high end of the measurement. Furthermore, thyroid dysfunction is not solely associated with cardiovascular disease (CVD), but has important implications for bone health and possibly also cognitive health. [10-13]

We therefore aimed to calculate the 10-year absolute risk of cardiovascular mortality in a large population-based cohort study by the two most commonly used parameters of thyroid function, TSH and FT4. We further aimed to define optimal health ranges based on provided absolute risk estimates in the whole cohort as well as by sex and age groups.

METHODS

The Rotterdam Study

The Rotterdam Study is a prospective population-based cohort study that investigates determinants and occurrence of age-related diseases in a middle-aged and elderly population in Rotterdam, the Netherlands. The aims and design of the Rotterdam Study have been described in detail elsewhere.[14] The Rotterdam Study consists of three independent cohorts: RS Cohort 1 (RSI), including 7,983 participants aged ≥55 (baseline 1990-1993), RS Cohort II (RSII), including 3,011 participants aged ≥55 (baseline 2000-2001) and RS Cohort 3 (RSIII), including 3,932 participants aged ≥45 (baseline 2006-2008). The Rotterdam Study has been approved by the medical ethics committee according to the Population Screening Act: Rotterdam Study, executed by the Ministry of Health, Welfare and Sports of the Netherlands.

Study population

We selected data from participants from the third visit of the first cohort (1997-1999, n=4797) and the first visits of the second (2000-2001, n=3011) and third cohort (2006-2008, n=3932), if TSH or FT4 measurements were performed and participants were not using thyroid function altering medication, including levothyroxine, anti-thyroid drugs, amiodarone or corticosteroids. We did not use the first visit of the first cohort as thyroid function was measured with a different assay.

All participants in the present analysis provided written informed consent to participate and to obtain information from their treating physician. All study participants were followed up from the day of baseline laboratory testing to date of death or end of follow-up January 1, 2012 which ever came first.

Assessment of thyroid function and other baseline measurements

TSH and FT4 measurements were performed using the same methods and assay in blood samples collected between 1997 and 2008, depending on the cohort and stored at -80°C (electrochemiluminescence immunoassay for free thyroxine and thyrotropin, "ECLIA", Roche). Body mass index was calculated as body mass (kg) divided by the square of the body height (m). Serum cholesterol was measured using standard laboratory techniques. Systolic blood pressure was calculated as the average of two consecutive measurements. Over 95% of participants were in fasting state when blood was drawn (morning) at the Rotterdam Study center visit. Information on tobacco smoking was derived from baseline questionnaires. Information on medication use was obtained from questionnaires in combination with pharmacy records.

Outcome definition

As primary outcome of interest we selected CVD since it is a leading burden of disease, morbidity and mortality.[15] Additionally, the association of subclinical and overt thyroid dysfunction with CVD mortality are well-established.[1] Secondary outcomes of interest were CHD and stroke (fatal and non-fatal). Methods for collection of data and outcome definitions have been previously described.[14,16,17] Information on the vital status of all participants was obtained on a weekly basis from the central registry of the municipality in Rotterdam and through digital linkage with records from GPs working in the study area. The cause of death was established by abstracting information from the medical records of the general practitioners or nursing home physicians and hospital discharge letters. Cardiovascular mortality was defined as according to the SCORE project definition of fatal CVD including the ICD-10 codes I10-25, I44-51, I61-73, and R96.[9,18] To test the robustness of our findings we repeated the absolute risk estimate calculations using the CVD mortality defined according to previously published definition of the Rotterdam Study, which also included non-atherosclerotic cardiovascular mortality.[16] CHD was defined as myocardial infarction, cardiac revascularization

procedure or CHD mortality. Stroke was defined according to World Health Organization (WHO) criteria as a syndrome of rapidly developing clinical signs of focal (or global) disturbance of cerebral function, with symptoms lasting 24 hours or longer or leading to death, with no apparent cause other than of vascular origin, including ischemic or hemorrhagic strokes. Outcomes were adjudicated by a committee who were blinded to lab results.

Statistical analysis

Absolute values of TSH and especially FT4 are assay dependent, but the different immunoassays of TSH or FT4 correlate well in non-pregnant adult populations[19,20], as previously also shown in the Rotterdam Study.[21] Therefore, to enhance generalizability of our results, we analyzed the association of TSH or FT4 in percentiles with the outcomes defined below. Absolute 10-year risk estimates of CVD mortality used the percentiles of TSH and FT4 and were calculated according to the Fine and Gray model, taking the competing risk of non-CVD deaths into account and were adjusted for age and sex.[22] The competing risk for the CHD and stroke analyses were non-CHD and non-stroke deaths respectively. In addition, we performed predefined analyses stratifying for age categories and gender. We performed sensitivity analyses using a Rotterdam Study based definition for CVD mortality[16], additionally adjusting the TSH analyses for FT4 and vice versa as well as additionally adjusting the analyses for cardiovascular risk factors used in the SCORE project charts (i.e. smoking, systolic blood pressure, and cholesterol).[9] We used the following cut-offs for the risk estimates and color denomination of risk categories, which were slightly adjusted from the SCORE project due to the higher average age in our population: low risk (< 2.0%, blue), low-intermediate risk (2.0-5.0%, green), intermediate risk (5.0-7.5%, yellow), high-intermediate risk (7.5-10.0%, orange) and high risk (≥ 10.0%, red).

For the CHD analyses we excluded all those with prevalent or missing information on CHD at baseline (n=685). For the stroke analyses we excluded all participants with missing information at baseline or a history of stroke (n=319). We performed a goodness-of-fit test for the Fine and Gray model for the absolute risk estimations, using the Zou Laird Fine test, and this revealed no linear, quadratic or log time varying effects of TSH or FT4 (p-value > 0.1 for all analyses). Linearity of absolute risk estimates was tested with restricted cubic splines with 3 knots at the 10th, 50th

and 90th percentile. Analyses were performed in R (survival, rms, crrSC and cmprsk packages R-project, Institute for Statistics and Mathematics, R Core Team (2013), Vienna, Austria, version 3.0.2).

RESULTS

We included a total of 9,233 participants with a mean age of 65.0 (standard deviation 9.8) years of which 55.9% were female (Table 1). During an average follow-up of 8.8 years, with a total of 75,981 person-years, 2166 deaths occurred of which 689 were CVD deaths according to the SCORE criteria and 692 according to the Rotterdam Study criteria. There were 642 CHD events and 553 stroke events during follow-up. Completeness of follow-up was 99.6%.[23]

Table 1 Baseline characteristics of included participants in the study

Variable	Mean (SD)[a]
Number of participants	9233
Age, years	65.0 (9.8)
Female, N (%)	5157 (55.9)
History of diabetes, N (%)	1097 (11.9)
BMI kg/m2	27.2 (4.2)
Cholesterol mmol/L	5.7 (1.0)
Smoking, N (%)	
current	1975 (21.4)
past	4380 (47.4)
never	2878 (31.2)
Systolic BP, mmHg	139.5 (21.0)
TSH ImU/L median (IQR)	1.90 (1.29-2.74)
FT4 pmol/L	15.6 (2.2)
FT4 ng/dL	1.21 (0.2)

[a] Values are means and SD unless otherwise specified Abbreviations: BMI = body-mass index; BP = blood pressure; FT4 = free thyroxine; IQR = inter-quartile range; N= number; SD = standard deviation; TSH = thyroid-stimulating hormone

Absolute risk estimates cardiovascular mortality

Ten-year absolute risk estimates for CVD mortality across the range of TSH and FT4 are plotted in Figure 1. CVD mortality increased with higher FT4 levels (p-value 0.005) and lower TSH levels, although not statistically significantly for the latter. The best fit for both TSH and FT4 analyses was non-linear (p for non-linearity < 0.001, Figure 1). Table 2 shows the different percentile cut-offs of TSH and FT4 values with the predicted absolute 10-year risk estimates, based on the

non-linear association. Overall, FT4 values above the 97th percentile (absolute level of approximately 22 pmol/L or 1.7 ng/dL) were associated with a predicted 10-year risk of 9.6% (p-value = 0.005). FT4 levels above the 90th percentile corresponded to an increased risk of 7.5% and higher for CVD mortality (absolute level of approximately 19 pmol/L or 1.5 ng/dL). Sensitivity analyses did not change the definition of the cut-offs meaningfully (Supplemental Table 1). TSH levels were inversely associated with CVD mortality but not statistically significant (Table 1). For men, a risk of ≥10.0% occurred at the 97th percentile of FT4 (p-value < 0.001) and a risk of ≥7.5% already occurred at the 60th percentile (Table 3). In women, there was no association of the thyroid function markers and risk of CVD mortality (Table 3). In participants younger than 65 years of age, the risk of CVD mortality increased with decreasing TSH levels (p-value = 0.009) with a risk of ≥ 2.0 % from the 30th percentile and lower (~1.40 mIU/L), while FT4 levels were not association with CVD mortality (Table 4). In participants older than 65 years of age (Table 4), the absolute risk estimates were <10.0% below the 30th percentile and ≥ 15.0% higher than the 97th percentile of FT4.

Absolute risk estimates CHD and stroke

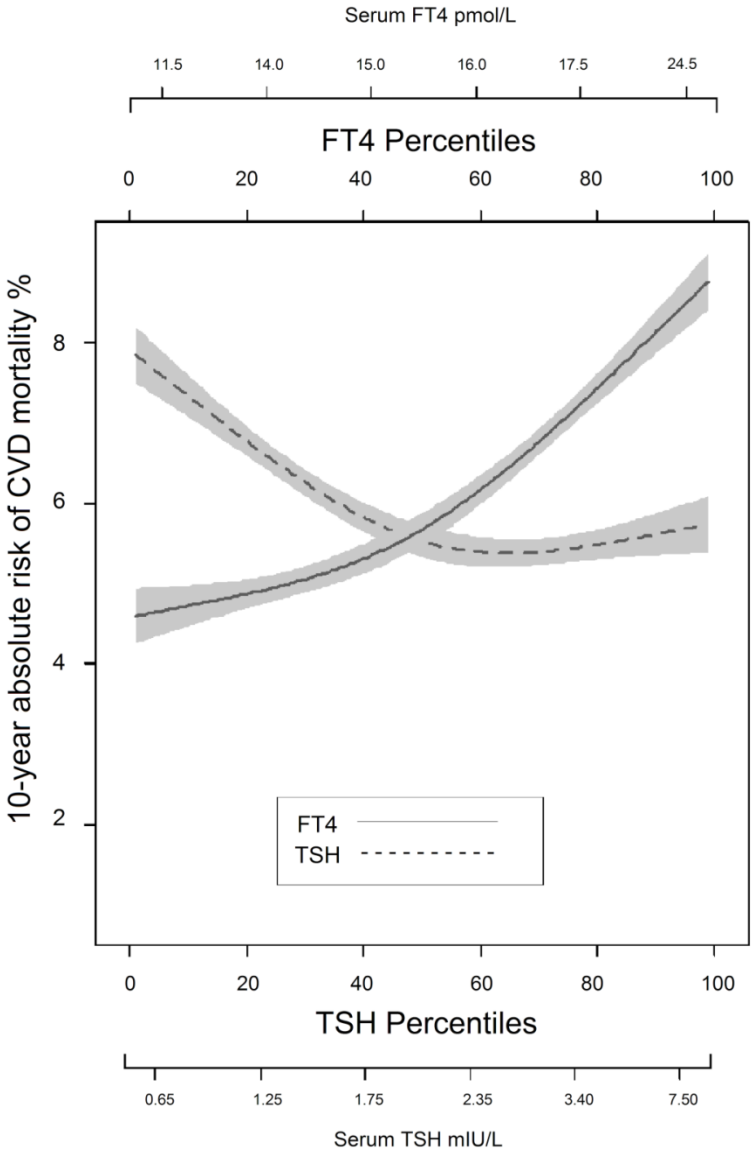
Supplemental Figure1 plots the absolute risk estimates of CHD and stroke against the continuous FT4 and TSH levels. In the Fine and Grey models, the association of TSH or FT4 with CHD events was not statistically significant (p-value > 0.5). Higher FT4 levels were associated with an increased risk of stroke (p-value = 0.009). TSH levels were inversely associated with the risk of stroke, but this did not reach statistical significance. The best fit for the CHD analyses was linear, while the best fit for the stroke analyses was non-linear (p for non-linearity <0.001, Supplemental Figure 1).

Table 2 Absolute 10-year risk estimates for CVD mortality according to percentiles of TSH and FT4 (n= 9227)*

Predicted 10-year absolute risk of event (n= 689 cases)															
TSH, percentile	**<2**	**2-5**	**5-10**	**10-20**	**20-30**	**30-40**	**40-50**	**50-60**	**60-70**	**70-80**	**80-90**	**90-95**	**95-97**	**>97**	**P-trend**
Absolute risk	8.3%	8.3%	7.4%	6.9%	6.5%	5.9%	6.0%	5.5%	5.5%	5.4%	5.3%	6.0%	5.5%	6.0%	0.59
N	*149*	*164*	*471*	*944*	*959*	*952*	*930*	*958*	*944*	*953*	*933*	*444*	*257*	*169*	
Mean TSH	*0.03*	*0.19*	*0.53*	*0.97*	*1.26*	*1.52*	*1.76*	*2.04*	*2.36*	*2.77*	*3.45*	*4.54*	*5.74*	*13.53*	
FT4 percentiles	**<2**	**2-5**	**5-10**	**10-20**	**20-30**	**30-40**	**40-50**	**50-60**	**60-70**	**70-80**	**80-90**	**90-95**	**95-97**	**>97**	**P-trend**
Absolute risk	4.5%	4.4%	5.1%	4.7%	4.7%	5.2%	5.8%	6.0%	6.2%	6.9%	7.5%	8.4%	8.9%	9.6%	0.005
N	*185*	*190*	*476*	*941*	*952*	*961*	*940*	*953*	*939*	*947*	*911*	*463*	*238*	*131*	
Mean FT4 pmol/L	*8.93*	*11.57*	*12.57*	*13.46*	*14.16*	*14.73*	*15.27*	*15.80*	*16.36*	*17.01*	*17.83*	*18.85*	*19.82*	*22.01*	
Mean FT4 ng/dL	*0.69*	*0.90*	*1.00*	*1.05*	*1.10*	*1.14*	*1.19*	*1.23*	*1.27*	*1.32*	*1.39*	*1.46*	*1.54*	*1.71*	

Models are adjusted for age and sex and computed using a competing risk model. Risk legend: low risk (< 2.0% , blue), low-intermediate risk (2.0-5.0%, green), intermediate (5.0-7.5%, yellow), high-intermediate risk (7.5-10.0%, orange), high risk (≥ 10.0%, red)
Abbreviations: CVD = cardiovascular disease; FT4 = free thyroxine; N = number; TSH = thyroid stimulating hormone
* 6 people excluded due to missing cause of death

Figure 1 Absolute 10-year risk of CVD mortality by TSH and FT4



Absolute 10-years risks of CVD mortality were calculated taking competing risk of death by other causes into account, and are plotted against TSH and FT4 percentiles and absolute values, with 95% confidence intervals. P for non-linearity < 0.001 for both TSH and FT4 analyses. Abbreviations: CVD cardiovascular disease FT4 free thyroxine, TSH thyroid-stimulating hormone.

Table 3 Absolute 10-year risk estimates for CVD mortality according to percentiles of TSH and FT4 (n= 9227)*

Men, N= 4072 cases = 357															
TSH, percentile	**<2**	**2-5**	**5-10**	**10-20**	**20-30**	**30-40**	**40-50**	**50-60**	**60-70**	**70-80**	**80-90**	**90-95**	**95-97**	**>97**	**P-trend**
Absolute risk	11.4%	8.6%	8.8%	8.0%	7.1%	7.0%	7.3%	6.4%	6.6%	6.4%	6.4%	7.8%	7.2%	7.1%	0.46
N	*44*	*78*	*216*	*461*	*461*	*472*	*452*	*450*	*408*	*418*	*354*	*159*	*60*	*39*	
FT4 percentiles	**<2**	**2-5**	**5-10**	**10-20**	**20-30**	**30-40**	**40-50**	**50-60**	**60-70**	**70-80**	**80-90**	**90-95**	**95-97**	**>97**	**P-trend**
Absolute risk	4.4%	5.3%	6.1%	5.4%	5.5%	6.1%	6.8%	7.5%	7.6%	8.3%	8.4%	9.0%	9.0%	10.8%	<0.001
N	*62*	*51*	*199*	*377*	*352*	*412*	*393*	*450*	*425*	*461*	*458*	*244*	*128*	*60*	
Women, N = 5155, cases = 332															
TSH, percentile	**<2**	**2-5**	**5-10**	**10-20**	**20-30**	**30-40**	**40-50**	**50-60**	**60-70**	**70-80**	**80-90**	**90-95**	**95-97**	**>97**	**P-trend**
Absolute risk	7.0%	8.1%	6.3%	5.9%	5.9%	4.7%	4.6%	4.7%	4.6%	4.5%	4.6%	5.0%	5.1%	5.9%	0.99
N	*105*	*86*	*255*	*483*	*498*	*480*	*478*	*508*	*536*	*535*	*579*	*285*	*197*	*130*	
FT4 percentiles	**<2**	**2-5**	**5-10**	**10-20**	**20-30**	**30-40**	**40-50**	**50-60**	**60-70**	**70-80**	**80-90**	**90-95**	**95-97**	**>97**	**P-trend**
Absolute risk	4.8%	4.3%	4.2%	4.2%	4.3%	4.5%	5.0%	4.7%	5.1%	5.6%	6.7%	7.8%	8.8%	8.6%	0.27
N	*123*	*139*	*277*	*564*	*600*	*549*	*547*	*503*	*514*	*486*	*453*	*219*	*110*	*71*	

Models are adjusted for age and sex and computed using a competing risk model. Risk legend: low risk (< 2.0% , blue), low-intermediate risk (2.0-5.0%, green), intermediate (5.0-7.5%, yellow), high-intermediate risk (7.5-10.0%, orange), high risk (≥ 10.0%, red)
Abbreviations: CVD = cardiovascular disease; FT4 = free thyroxine; N = number; TSH = thyroid stimulating hormone
* 6 people excluded due to missing cause of death

Table 4 Absolute 10-year risk estimates for CVD mortality according to percentiles of TSH and FT4 (n= 9227)*

Age < 65 years, N= 5172 cases = 82															
TSH, percentile	**<2**	**2-5**	**5-10**	**10-20**	**20-30**	**30-40**	**40-50**	**50-60**	**60-70**	**70-80**	**80-90**	**90-95**	**95-97**	**>97**	**P-trend**
Absolute risk	2.6%	3.0%	2.6%	2.4%	2.2%	1.9%	1.7%	1.4%	1.3%	1.2%	1.0%	0.9%	0.8%	0.9%	0.009
N	*56*	*59*	*234*	*490*	*523*	*557*	*532*	*573*	*564*	*580*	*554*	*233*	*134*	*83*	
FT4 percentiles	**<2**	**2-5**	**5-10**	**10-20**	**20-30**	**30-40**	**40-50**	**50-60**	**60-70**	**70-80**	**80-90**	**90-95**	**95-97**	**>97**	**P-trend**
Absolute risk	1.2%	1.1%	1.3%	1.3%	1.3%	1.5%	1.5%	1.8%	1.8%	1.9%	2.1%	2.2%	2.4%	2.4%	0.20
N	*96*	*97*	*285*	*565*	*556*	*561*	*516*	*526*	*508*	*535*	*512*	*239*	*115*	*61*	
Age ≥ 65 years, N = 4055, cases = 607															
TSH, percentile	**<2**	**2-5**	**5-10**	**10-20**	**20-30**	**30-40**	**40-50**	**50-60**	**60-70**	**70-80**	**80-90**	**90-95**	**95-97**	**>97**	**P-trend**
Absolute risk	11.8%	11.5%	12.2%	11.9%	11.5%	11.3%	11.2%	11.1%	11.1%	11.2%	10.9%	11.4%	10.5%	10.8%	0.76
N	*93*	*105*	*237*	*454*	*436*	*395*	*398*	*385*	*380*	*373*	*379*	*211*	*123*	*86*	
FT4 percentiles	**<2**	**2-5**	**5-10**	**10-20**	**20-30**	**30-40**	**40-50**	**50-60**	**60-70**	**70-80**	**80-90**	**90-95**	**95-97**	**>97**	**P-trend**
Absolute risk	8.1%	7.9%	10.2%	9.3%	9.2%	10.2%	10.7%	11.1%	11.4%	13.1%	14.1%	14.7%	14.9%	15.7%	0.005
N	*89*	*93*	*191*	*376*	*396*	*400*	*424*	*427*	*431*	*412*	*399*	*224*	*123*	*70*	

Models are adjusted for age and sex and computed using a competing risk model. Risk legend: low risk (< 2.0% , blue), low-intermediate risk (2.0-5.0%, green), intermediate (5.0-7.5%, yellow), high-intermediate risk (7.5-10.0%, orange), high risk (≥ 10.0%, red)
Abbreviations: CVD = cardiovascular disease; FT4 = free thyroxine; N = number; TSH = thyroid stimulating hormone
* 6 people excluded due to missing cause of death

DISCUSSION

This is the first study to propose reference ranges of TSH and FT4 to be based upon the disease risk (i.e. absolute risk estimates of CVD) as a proof of concept. Based on our findings, the proposed upper limit for FT4 could be the 90th percentile, independent of TSH levels. The optimal health ranges for thyroid function based on cardiovascular disease seem to differ between men and women and the associations were not statistically significant in women. In participants older than 65 years of age, the absolute risk estimates of CVD were <10.0% below the 30th percentile (~14.5 pmol/L or 1.1 ng/dL) and ≥ 15.0% higher than the 97th percentile of FT4 (~22 pmol/L or 1.7 ng/dL). The associations of TSH and FT4 with CVD mortality were non-linear. The association of thyroid function with stroke followed a similar pattern, but the association with CHD showed a linear association.

Reference ranges for the thyroid function biomarkers TSH and FT4 have been derived mainly statistically from the 2.5th and 97.5th percentile, similar to reference ranges of other laboratory results and clinical tests.[24-26] Subclinical and overt thyroid disease are subsequently defined by these biochemical and statistical reference ranges which, in general, do not take future health and disease risks into account. However, some guidelines do uphold additional cutoffs for treatment based on studies showing an increased risk of cardiovascular disease at certain levels.[8,27] For example, the European Thyroid Association guidelines on subclinical hypothyroidism [8], make a distinct separation between TSH levels below and above 10 mIU/L for consideration of levothyroxine treatment. These recommendations are based on a study by the Thyroid Studies Collaboration that provided evidence for a higher relative risk of CHD with TSH levels higher than 10 mIU/L.[1] However, to our knowledge, there are no studies specifically addressing the optimal health ranges based on absolute risk estimates of adverse health outcomes.

Overall, our study shows an absolute 10-year risk of 7.5% or higher with FT4 levels above the 90th percentile, corresponding to a cut-off level of FT4 approximately 19 pmol/L (~1.5 ng/dL). This is however, as expected, different in participants younger than 65 years of age compared to those older than 65 years. Also, there seems to be a differential association of thyroid function with absolute risk of CVD when

comparing men to women. This can, at least partially, be attributed to the difference in background absolute risk between the two sexes, where, also in our study, women have an inherent lower risk of CVD. However, aside from background risk of CVD, there also seems to be a thyroid dependent differential risk when comparing men to women, which could be explained by e.g. a difference in set point between the sexes. [28] Our findings need to be confirmed and validated across different populations, but could suggest a sex-specific reference range ought to be considered. These findings need to be confirmed and validated across different populations, but could suggest a sex-specific reference range is needed.

In our study, higher FT4 levels are associated with an increased risk of CVD mortality whereas TSH levels showed an expected opposite relation with CVD mortality which did not reach statistical significance. The current study is not the first to report an association of FT4 with clinical events, while the association is lower or absent with TSH.[3,6,21] Based on the log-linear relationship between TSH and FT4, TSH is perceived as the most sensitive marker in subjects with thyroid disease. The lack of association with TSH is therefore remarkable. One explanation could be that in euthyroid subjects, TSH predominantly reflects the pituitary-thyroid axis set point rather than disease risk,[29] while, independent of TSH, circulating FT4 (and subsequently FT3 acting intracellular) represents the bioavailable thyroid hormone that can be taken up by cells, thereby leading to clinical consequences of thyroid hormones peripherally.

Cholesterol is a modifiable risk factor for CVD mortality and diagnosis and treatment targets for cholesterol are included within optimal primary and secondary prevention of CVD mortality. In our study, we show that FT4 is also a potentially modifiable risk factor for CVD and CVD mortality, especially in men and the elderly. For cholesterol, the average risk difference, as derived from the SCORE risk chart for low risk countries [9], when comparing 65 year old men with a cholesterol level of 7 mmol/L to 65 year-old men with a cholesterol level of 4 mmol/L is approximately 4.0%. This is similar to the risk difference when comparing men with an average age of 65 years in the highest 10th percentile of FT4 (cut-off ~ 1.5 ng/dL) to those in lowest 10th percentile (cut-off ~ 1.0 ng/dL), namely 4.3%. Whether modifying higher FT4 levels with anti-thyroid drugs will indeed result in this cardiovascular mortality risk reduction still needs to be determined.

There are several strengths to our study including the population-based design, the large size of the study population, the completeness of follow-up and the fact that outcomes were defined independently from baseline thyroid function. Nevertheless, the currently proposed optimal health ranges should be interpreted with caution. First of all, even though CVD is one of the most important clinical outcomes, the presented absolute risk estimates are solely based on cardiovascular mortality and our findings as such should be considered as a proof of concept. Furthermore, The Netherlands is classified as a low cardiovascular mortality risk country by the European Society of Cardiology and therefore estimates are not generalizable to countries with higher CVD mortality risk.[30] The Rotterdam Study consists of participants of 45 years and older and mainly Caucasians with, on average, a sufficient iodine status.[31,32] Also, only one baseline measurement of thyroid function was available, which holds true for most population-based cohort studies. The intra-individual set-point is much tighter than the inter-individual set-point, meaning that within an individual the changes in time are much smaller than between individuals.[33] Nevertheless, we could not investigate how changes in thyroid function could affect CVD risk and whether repeated measurements of thyroid function could better differentiate risk among cohort participants. The absolute levels of TSH and especially FT4 depend on the assay used and are therefore variable. Immunoassays for FT4 are affected by changes in serum binding proteins that occur in disease and pregnancy.[34] We therefore used the percentiles of the measurements to study the associations and define the optimal health ranges, because of the strong correlation between the different assays of TSH or FT4 in community-dwelling non-pregnant populations. These results are therefore potentially more generalizable to other populations. This is also the reason to advice that the calculation of these percentiles is country, iodine status, region and if possible even laboratory specific.

The mentioned limitations of our study also highlight the need for further research. Therefore our approach to define thyroid function adequacy focused on cardiovascular mortality need to be confirmed in similar populations but also replicated in complementary populations such as younger participants, other ethnicities and in regions with different current and historical iodine status.[35] Cardiovascular disease is an established and well-studied outcome in relation to

thyroid function. However, recently, there is increasing interest in the association of thyroid function with other outcomes as well, such as cognition. Therefore, importantly, consensus is needed on which clinical outcomes are or could be relevant in defining the optimal health ranges for thyroid function, beyond cardiovascular disease. Lastly, and beyond the discussion on thyroid function optimal health ranges, consensus is also needed on which cardiovascular risk is considered too high and whether this is similar for all populations. For example, a 10-year absolute risk of 2.5% for CVD mortality for a person of 45 years of age might not be deemed equally acceptable compared to the same risk in a person of 75 years.

This is a population-based study, and therefore risks and benefits of treatment decisions were not explored. While randomized controlled trials are the best evidence for defining treatment cut-offs, they are costly and not always able to address the timeliest issues. In the absence of results from such trials in the near future, defining the optimal health ranges by determining the absolute risk estimates of disease, in various observational studies from representative populations, is perhaps the most feasible.

In summary, we propose an approach to define thyroid function based not only on population's distribution but taking into account health and disease risk. We describe the absolute 10-year risk of cardiovascular mortality associated with TSH and FT4 and provide an example of defining optimal health ranges based on cardiovascular mortality risk using data from a large population-based study. Further research is needed to investigate optimal health ranges based on thyroid-relevant clinical outcomes in sufficiently powered studies with representative samples from multiple populations.

Online supplemental material

https://academic.oup.com/jcem/article-abstract/doi/10.1210/jc.2017-00410/3828544/Defining-optimal-health-range-for-thyroid-function?redirectedFrom=fulltext

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CHAPTER 3

THE BRAIN

CHAPTER 3.1

SUBCLINICAL THYROID DISORDERS AND THE RISK OF STROKE: SYSTEMIC REVIEW AND META-ANALYSIS

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ABSTRACT

BACKGROUND Subclinical thyroid dysfunction has been associated with coronary heart disease, but the risk of stroke is unclear. Our aim is to combine the evidence on the association between subclinical thyroid dysfunction and the risk of stroke in prospective cohort studies.

METHODS We searched Medline (OvidSP), Embase, Web-of-Science, Pubmed Publisher, Cochrane and Google Scholar from inception to November 2013 using a cohort filter, but without language restriction or other limitations. Reference lists of articles were searched. Two independent reviewers screened articles according to pre-specified criteria and selected prospective cohort studies with baseline thyroid function measurements and assessment of stroke outcomes. Data were derived using a standardized data extraction form. Quality was assessed according to previously defined quality indicators by two independent reviewers. We pooled the outcomes using a random-effects model.

RESULTS Of 2274 articles screened, six cohort studies, including 11,309 participants with 665 stroke events, met the criteria. Four of six studies provided information on subclinical hyperthyroidism including a total of 6029 participants and five on subclinical hypothyroidism (n=10,118). The pooled hazard ratio (HR) was 1.08 (95% CI: 0.87-1.34) for subclinical hypothyroidism (I^2 of 0%) and 1.17 (95% CI: 0.54-2.56) for subclinical hyperthyroidism (I^2 of 67%) compared to euthyroidism. Subgroup analysis yielded similar results.

CONCLUSIONS Our systematic review provides no evidence supporting an increased risk for stroke associated with subclinical thyroid dysfunction. However, the available literature is insufficient and larger datasets are needed to perform extended analyses. Also, there were insufficient events to exclude clinically significant risk from subclinical hyperthyroidism, and more data are required for subgroup analyses.

INTRODUCTION

Thyroid disease has been associated with several metabolic disorders as well cardiovascular disease [1,2] and cardiovascular mortality.[3,4] The cardiovascular system is one of the main targets of thyroid hormones, which decrease systemic vascular resistance [5], alter systolic and diastolic cardiac function [4] and directly increase cardiac contractility and heart rate.[6] They also have effects on several cardiovascular risk factors including changed lipid profile [7] and increased risk of atrial fibrillation and other supraventricular arrhythmias [8]. Many of these effects are also seen in subclinical thyroid dysfunction.[9]

Subclinical thyroid dysfunction is defined by serum thyroid stimulating hormone (TSH) values outside the reference range, but with normal concentrations of free thyroxine (T4), as well as free triiodothyronine (T3) in the case of subclinical hyperthyroidism.[10,11] The reference ranges depend on several factors including the thyroid function assay used[10] and iodine status of the population.[12] Thyroid dysfunction in the subclinical range is very common, with a prevalence of subclinical hypothyroidism varying between 4-14% in adults [13-15] which increases with age.[14] Subclinical hyperthyroidism is less common in the general population with a prevalence ranging from 0.7% [16] up to 10% in women.[17]

Two meta-analyses of individual participant data of prospective cohorts showed an increased risk of coronary heart disease in subclinical hypothyroidism[18], as well as subclinical hyperthyroidism.[19] Stroke, which is worldwide the second most common cause of death and one of the leading causes of disability [20], shares many of the same risk factors as other cardiovascular disease, including high blood pressure, high cholesterol, obesity and atrial fibrillation. The link between overt hyperthyroidism and atrial fibrillation [2] and ischemic stroke has been established, even in young adults.[21] The association between overt hypothyroidism, atrial fibrillation and cardioembolic stroke has been suggested, but is not established.[2,22]

While subclinical thyroid dysfunction influences several of the mentioned cardiovascular risk factors, there remains debate to what extent this actually affects stroke risk.[9] Prior studies assessing this association are few, with conflicting outcomes and have never been systematically analyzed. With this meta-analysis,

we aim to determine whether subclinical thyroid dysfunction is associated with an increased risk of stroke in prospective cohort studies.

METHODS

Eligibility criteria

We searched for published studies of prospective cohorts that satisfy the following criteria: (i) measurement of thyroid function at baseline in subjects above the age 18, (ii) assessment of stroke and/or transient ischemic attack (TIA) outcomes (iii) inclusion of subclinical thyroid dysfunction group and a comparison group with euthyroidism and (iv) evaluation of the association of altered thyroid function on stroke providing a measure of this association with either a risk ratio, odds ratio or hazard ratio. We excluded studies including participants with only overt thyroid disease or only participants taking thyroid-function altering medication, or with only stroke and/or TIA patients. We did include studies with a proportion of participants taking thyroid function altering medication. In prospective cohort studies this will probably not exceed 10% of the studied population. We did however conduct a sensitivity analysis excluding those studies. Our outcome of interest was fatal and non-fatal stroke.

Study search and identification

We conducted a systematic literature search for studies on the association between subclinical thyroid dysfunction and stroke published between earliest inception and November 2013 in several databases (Supplemental Material). The databases searched were: Medline (OvidSP), Embase, Web-of-Science, Pubmed Publisher, Cochrane and Google Scholar. We used a cohort filter designed by BMJ Evidence Centre information specialists [23] to select prospective studies for both the Medline as well as the Embase database, but not for the other databases. Filters for observational studies have shown to perform well in Medline and Embase with a sensitivity of >99% and reduce the amount of retrieved articles up to 30%.[24] We did not use any other filters or restrictions including language restrictions. In addition, we searched in other sources including bibliographies of key articles in the field and those included in this review.

Study selection

Two reviewers (LC, CB) screened the abstracts and titles of the search result independently and in duplicate. Articles of prospective cohorts studying the association between subclinical thyroid dysfunction and stroke and/or TIA were included. When potentially eligible studies were retrieved, the full text publications were evaluated according to the eligibility criteria. The inter-reviewer agreement was calculated according to the kappa-statistic (κ), which was fair to good (κ= 0.61) for abstract and title, and excellent (κ= 1.0) for full-text screening. Disagreements were resolved by either consensus or discussion with a third independent reviewer (RP). Subclinical hypothyroidism was defined as an elevated TSH and normal free T4 (FT4), subclinical hyperthyroidism as a decreased TSH and normal (F)T4/T3. We used TSH and (F) T4/T3 -cutoffs as reported by each cohort separately.

Data Collection and Quality Assessment process

Standardized data collection forms were used to extract data from the individual cohorts of the included studies concerning participant characteristics, used reference ranges for thyroid function measurements, including the minimally and maximally adjusted HR's for the outcome events of interest, types of analysis and covariates adjusted for (Table 1). The correctness of the abstracted information was confirmed by a second reviewer (RP) and corrected and/or completed where needed. Two independent reviewers assessed study quality using previous criteria for the assessment of key indicators of cohort study quality.[25] The components assessed were: (i) whether the study was population based, (ii) whether the study had a formal adjudication procedures for stroke defined as having clear criteria for the outcome that were reviewed by experts for each potential case, (iii) which methods were used for stroke ascertainment, (iv) which if any adjudication was performed without knowledge of thyroid status, (v) what was the loss of follow-up and (vi) what were the adjustments for the multivariate analysis, if any. We also assessed study quality using the Newcastle Ottawa Scale (NOS) for cohort studies.[26] Two reviewers (LC, CB) rated all studies for quality and any disagreement was resolved by a third reviewer (OF).

Statistical analysis

We used the most adjusted HRs and 95% confidence intervals (CI) available provided by the included studies as the primary analysis. We used the random-

effects method by DerSimonian and Laird [27] to assess the pooled estimates and 95% CIs of the risk of subclinical hypothyroidism and hyperthyroidism on stroke. In addition we also conducted a fixed-effect analysis for comparison. We used the Cochrane Q test and I^2 index with a conservative p-value of 0.10 to evaluate heterogeneity across individual studies.[28] I^2 values of <25% indicate low, 25 and 50% moderate and > 50% high heterogeneity. We also evaluated publication bias visually through funnel plots and statistically with an Egger test.[29]

For the analyses we used Review Manager (RevMan) Version 5.2. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2012. To explore sources of heterogeneity, we planned sensitivity analyses to exclude non-population based studies, exclude studies including a proportion of levothyroxine users, including only studies with a adjudication procedure, including studies with (F)T4/T3 measured in all participants and a sensitivity analysis using only the minimally adjusted HRs. We also planned subgroup analyses stratifying for different age groups, gender, TSH levels.

RESULTS

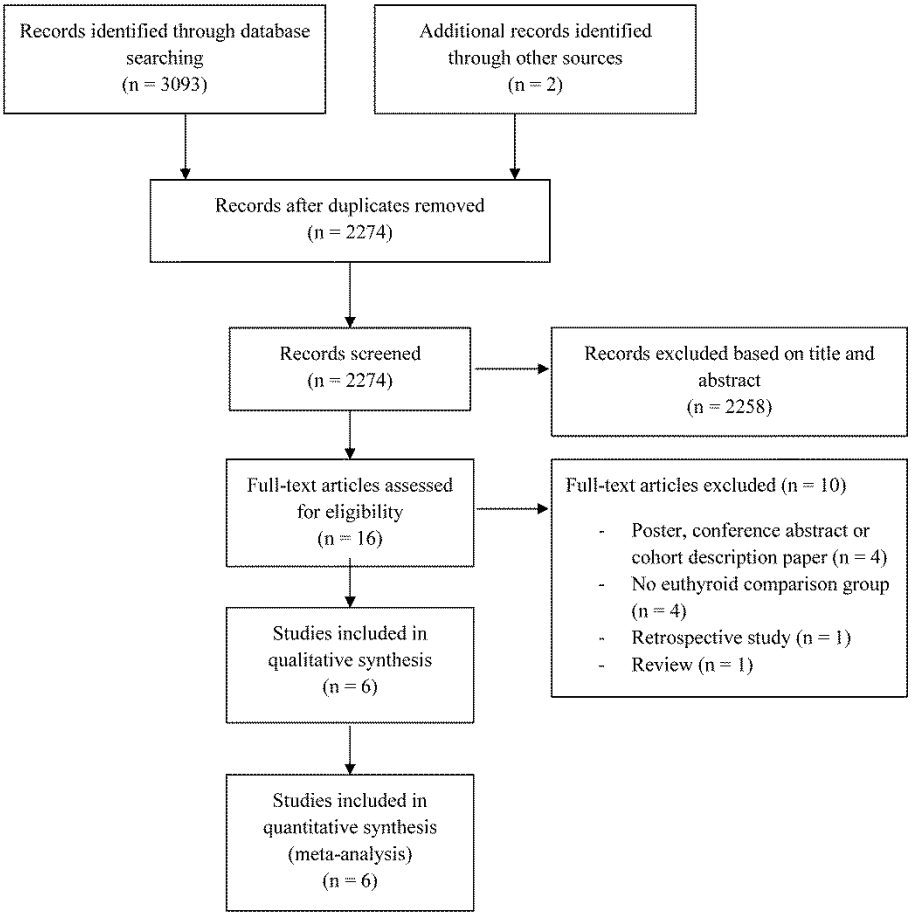
Study selection

We identified 3095 reports, of which 2274 remained after removing duplicates (Figure 1). We excluded 2258 based on the title and abstract as they were unrelated to the association between subclinical thyroid dysfunction and stroke. Of the remaining 16 articles, we excluded 10 articles because they did not meet inclusion criteria after full-text screening, leaving six reports that met the eligibility criteria and were included in further qualitative and quantitative analyses.

Study Characteristics

The 6 studies selected for the analysis enrolled 11,309 participants in total (Table 1) and included 665 stroke events. The included studies were from Europe, Japan and the USA. Out of the six studies, five studies provided information on subclinical hypothyroidism analyzing a total of 10118 participants with 620 stroke events, and four studies provided data on subclinical hyperthyroidism analyzing a total of 6029 participants with 301 stroke events.

Figure 1 Study Selection



The mean age ranged from 58.5 up to 74.7 years at baseline, while the overall mean age was 69 years. Out of the 6 cohorts, 3 excluded participants with thyroid-altering medication and three studies measured T4 (and/or T3) only in abnormal TSH values. None of the studies evaluated stroke as a primary outcome and none specified the type of stroke. The percentage of subclinical thyroid dysfunction varied across studies ranging from 1.5% to 5.9% for subclinical hyperthyroidism and from 5.1% to 15.3% in subclinical hypothyroidism. The TSH cut-offs for subclinical hyperthyroidism ranged between 0.1 mIU/L and 0.6 mIU/L and for

subclinical hypothyroidism between 4.0 mIU/L and 5.0 mIU/L. The follow-up duration varied from 4 years up to 12.5 years. All studies except for one [30] used a second or third generation TSH-assay. The first-generation TSH-assay used by this study is insufficiently sensitive to detect a TSH in the range of subclinical hyperthyroidism. However, the Nagasaki Study did not provide information on subclinical hyperthyroidism and was only included in the subclinical hypothyroidism analysis.

Quality assessment

Five out of the 6 studies were population based and one study was a randomized clinical trial (Table 1). Three studies had formal adjudication procedure for stroke of which 2 studies also reported adjudication without knowledge of thyroid status. The studies with no formal adjudication procedure used the International Classification of Diseases (ICD) to ascertain stroke outcomes. None except for one cohort [31] reported loss to follow-up and all but one [31] reported HR adjusted for covariates (Supplemental Material Table S1). The studies showed similar scores on the NOS assessment scale overall, but scored differently on the separate quality item (Supplemental Material Table S2). As an overall quality check, and in order to ensure transparent reporting of this systematic review and meta-analysis, the PRISMA guidelines were followed and the PRISMA checklist is provided (Supplemental Material Table S3).

Subclinical hyperthyroidism and stroke

Of the 6 included studies, 4 provided data on subclinical hyperthyroidism and the risk of stroke. Two studies included in this analysis [31,32] showed an increased risk, with one study providing an adjusted HR reaching statistical significance with a HR 3.39 (95% CI 1.15 – 10.00) [32]. The two other studies showed a statistically non-significant decreased risk for stroke associated with subclinical hyperthyroidism [33,34] (Table 2). The overall pooled estimated HR using a random effects model showed no association of subclinical hyperthyroidism with stroke with a HR of 1.17 (95% CI: 0.54 to 2.56) and substantial heterogeneity (Q-statistic p- value < 0.05 and I^2 of 67%, Figure 2A). Sensitivity analyses excluding studies without formal stroke adjudication procedures, not population based or including participants on levothyroxine yielded similar results (Table 2).

Table 1 Description, characteristics and results of included studies on the effect of subclinical thyroid dysfunction and stroke risk

First author, name of cohort, year of start	Description of population	Journal, year publication	Mean age	Total no.	No. euthyroid subjects (%)	No. Subclinical hyperthyroid subjects (%)	No. Subclinical hypothyroid subjects (%)	F (%)	Exclusion of Thyroid altering medication?
Schultz, Frederiksberg, 1998	General population in Copenhagen, > 50yrs, normal LVF. Denmark	Horm Metab Res, 2011	67.9	605	549 (90.7)	25 (4.1)	31 (5.1)	352 (58.2)	no
Rodondi, Health ABC, 1997	Community-dwelling adults aged 70-79 years from Medicare. USA	Arch Intern Med, 2005	74.7	2730	2392 (87.6)	NA	338 (12.4)	1392 (51.0)	no*
Imaizumi, Nagasaki, 1984	Atomic bomb survivors in Nagasaki. Japan	JCEM, 2004	58.5	2550	2293 (89.9)	NA	257 (10.1)	1551 (60.8)	yes
Parle, Birmingham, 1988	Community-dwelling patients ≥ 60 years old in Birmingham, England	Lancet, 2001	70.4	1191	1026 (86.1)	70 (5.9)	76 (6.4)	681 (57.2)	yes
Cappola, CHS, 1989	Community-dwelling adults from Medicare aged ≥ 65 years. USA	JAMA, 2006	72.7	3233	2639 (81.6)	47 (1.5)	496 (15.3)	1926 (59.6)	yes
Drechsler, 4D study, 1998	RCT atorvastatin in DM II, hemodialysis patients,18-80 years, Germany	Am J Kidney Dis., 2013	65.6 ‡	1000	781 (78.1)	137 (13.7)	16 (1.6)	431 (43.1)	no

Abbreviations: LVF = left ventricular function RCT = Randomized controlled trial, DM II = type 2 diabetes* excluded only 2 taking anti-thyroid drugs, ‡ calculated from provided mean ages per thyroid subgroup.

Table 1 (continued) Description, characteristics and results of included studies on the effect of subclinical thyroid dysfunction and stroke risk

Name of Cohort	Stroke events (no.)	Stroke mortality (no.)	Total mortality (no.)	Follow-up duration (years)	Reference range TSH mIU/L	Reference range (F)T4	Reference range (F)T3	T4/T3 measured in all?
Frederiksberg	28	NR	88	5 (median)	0.4 - 4.0	T4: 4.7-12.3 ng/dL**	T3:65-196 pg/dL***	yes
Health ABC	153	NR	324	4 ‡	0.1 - 4.5	FT4: 0.8-1.8 ng/dL	NA	no¶
Nagasaki	44	12	152	6 (max)	0.6 - 5.0	FT4: 0.8-2.5 ng/dL	NA	yes
Birmingham	NA	45	509	8.3 (mean)	0.5 - 5.0	FT4: 0.7-1.9 ng/dL**	FT3:130-519 pg/dL***	no¶
CHS	320	NR	847	12.5 (mean)	0.45 - 4.5*	FT4: 0.7-1.7 ng/dL	NA	no¶
4 D study	75	NR	471	3.94 (mean)	0.30 - 4.0*	FT4: 0.9-1.9 ng/dL**	FT3:175-494 pg/dL***	yes

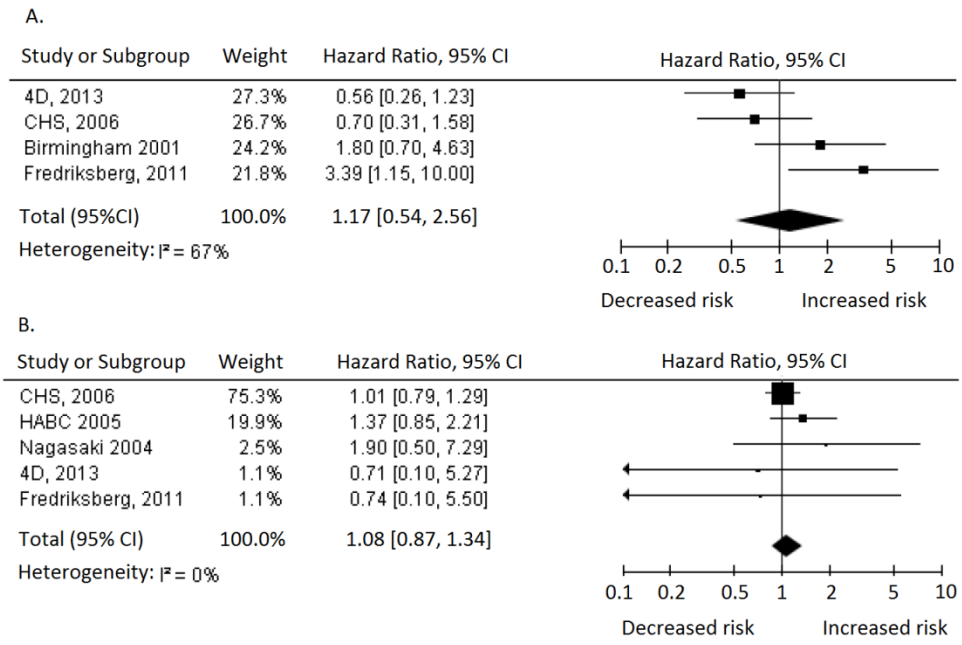
‡ Unclear descriptive, * Subclinical hypothyroidism groups defined as: CHS: TSH 4.5 – 20 mIU/L, 4D study: TSH 4.0 – 15 mIU/L, ** conversion 1 pmol/L = 0.0777 ng/dL, *** conversion 1 pmol/L = 65 pg/dL, ¶ T4 and or T3 only measured in higher and or lower levels of TSH.

Table 1 (continued) Description, characteristics and results of included studies on the effect of subclinical thyroid dysfunction and stroke risk

Name of Cohort	TSH ImU/L, Mean (SD or IQR)					HR (95% CI)	
	Hypothyroid	Subclinical Hypothyroid	Euthyroid	Subclinical Hyperthyroid	Hyperthyroid	Subclinical Hyperthyroidism	Subclinical Hypothyroidism
Frederiksberg*	NR	5.84 (4.49-7.35)	1.36 (0.93-1.95)	0.26 (0.12-0.34)	NR	3.39 (1.15-10.00)	0.74 (0.10-5.50)
Health ABC	NR	NR	NR	NR	NR	NA	1.37 (0.85 – 2.20)
Nagasaki	NR	6.79 (3.45)**	2.82 (1.01)**	NA	NA	NA	1.9 (0.5 – 7.3) †
Birmingham	NR	NR	NR	NR	NR	1.8 (0.7 – 4.7)†	NA
CHS	28.1 (15.7)	6.67 (2.54)	2.20 (0.99)	0.5 (0.13)	NA	0.70 (0.31 – 1.57)	1.01 (0.79 – 1.29)
4 D study	NR	5.9 (2.9)	1.2 (0.8)	0.14 (0.1)	NR	0.56 (0.27 – 1.23)	0.71 (0.10 – 5.27)

Abbreviations: NR = Not reported, NA = Not applicable. Abbreviations: NR = Not reported, if information is part of the scope of the study, but not reported. NA = Not applicable, if information is not part of the scope of the study. † Only stroke mortality; * provided TSH and interquartile range, ** calculated by using provided data in the publication.

Figure 2 Forest Plots for subclinical thyroid disease and risk of stroke



2A. Subclinical Hyperthyroidism and stroke. 2B. Subclinical Hypothyroidism and stroke. Overall hazard ratios are displayed as diamonds. The forest plots are ordered by study weight. 4D = Die Deutsche Diabetes Dialyse Studie, CHS = Cardiovascular Health Study, HABC = Health, Aging and Body Composition study.

Subclinical hypothyroidism and stroke

In the five studies that included results on the effect of subclinical hypothyroidism on the risk of stroke the pooled HR was 1.08 (95% CI: 0.87 to 1.34) without evidence for heterogeneity (p- value of 0.70, I^2 of 0%) (Figure 2B). The results were mainly due to the large weight (75%) of one particular study. Sensitivity analyses did not show any relevant difference in the results, except stratifying by age with a higher risk in the population aged < 65 years (HR 1.90, CI 95% 0.50 – 7.29), but with only one study contributing with participants with a mean age below 65 years old.

Subgroup and sensitivity analyses

Pre-specified subgroup analyses were performed on different age categories, different cut-off points for TSH levels as well as the inclusion of levothyroxine users

in the study and showed no significant differences. Due to the lack of data, no subgroup analysis on gender could be performed (Table 2). Sensitivity analysis using only minimally adjusted HRs did not alter the risk estimates substantially (Table 2).

Evaluation of publication bias

Neither the visual assessment of the funnel plots (Supplemental Material Figure S1) nor the Egger test (p=0.76) showed signs for publication bias for the association between subclinical hypothyroidism and the risk of stroke. However the Egger test did show significant publication bias for the analysis on subclinical hyperthyroidism (p= 0.003) Furthermore, the funnel plots for subclinical hyperthyroidism and risk of stroke also showed publication bias with the Frederiksberg study being a possible outlier. Excluding this study in a sensitivity analysis yielded different risk estimates for subclinical hyperthyroidism, slightly reducing statistical heterogeneity (Table 2). The results for subclinical hypothyroidism did not change by this additional analysis.

DISCUSSION

In our systematic review and meta-analysis we did not find evidence supporting an increased risk of stroke in participants with subclinical thyroid dysfunction. This is in line with previous studies that found no association between subclinical hyperthyroidism and stroke [19,35] or subclinical hypothyroidism and stroke.[35,36] The number of studies retrieved was low and the study quality (assessed by scoring key indicator of quality and the NOS scale) was heterogeneous with few studies reporting adjudication of the outcome without prior knowledge of the thyroid status. Five studies adjusted for at least age and sex, but all studies included a different number of additional covariates for both minimal as maximal adjustment making them hard to compare. For example, only three out of the six studies corrected for smoking status.[30,33,37] while smoking is associated with both stroke as with thyroid disease.[38] Smoking is negatively associated with hypothyroidism and positively associated with hyperthyroidism with current smokers having lower levels of TSH.[38] It has even been suggested that smoking might mediate the associations found between thyroid function and BMI [39].

Stroke shares many of the same risk factors with other cardiovascular diseases. Subclinical hyperthyroidism significantly increases the risk of atrial fibrillation, as demonstrated in an individual participant level meta-analysis by Collet et al.[19] In subclinical hypothyroidism, relevant changes in low-density lipoprotein cholesterol (LDL-cholesterol) were seen, mainly with a TSH higher than 10 mIU/L.[40] Although the association between subclinical thyroid dysfunction and various risk factors for stroke have been established, the risk of stroke in subclinical thyroid dysfunction remains unclear.

This is the first systematic review and meta-analysis on the association between subclinical thyroid dysfunction and the risk of stroke. We conducted an extensive literature search in several electronic databases with as little limitations as possible in order to retrieve the maximum amount of literature available on the topic.

However, the number of retrieved papers was still small, revealing a scarcity of literature on this issue. Moreover almost all studies were conducted in populations of 65 years or older, limiting the generalizability to other populations. The only study with a younger population looking at subclinical hypothyroidism found an increased risk with a hazard ratio of 1.90, even though statistically not significant.[30] Looking at different age groups would be of special interest as previous studies evaluating the association between subclinical thyroid dysfunction, cardiovascular disease and mortality suggest an age dependent effect.[25,41]

Another important limitation is the lack of information on different TSH levels. Previous studies showed an association between different TSH levels and the risk of cardiovascular disease [18] and cardiovascular mortality.[19] Only one study [37] in our meta-analysis included different TSH-levels in the analysis, showing no clear dose-response relation. Only two [32,33] out of the four studies included in the subclinical hyperthyroidism analysis provided information on atrial fibrillation, which did not allow for stratification. We were not able to explore age-related nor TSH level associated risks of stroke in subclinical thyroid dysfunction in our meta-analysis, due to limited data. Also, we were not able to stratify for follow-up time, in order to take into account the time between exposure and outcome, due to the difference in definition of follow-up time (Table 1).

Table 2 Stratified and sensitivity analyses of the association between subclinical thyroid disease and the risk of stroke

	Subclinical Hyperthyroidism			Subclinical Hypothyroidism		
	Pooled HR (95% CI)	No. of studies	p for heterogeneity*	Pooled HR (95% CI)	No. of studies	p for heterogeneity*
Eligible Study Model						
Random Effects	1.17 (0.54-2.56)	4	0.03	1.08 (0.87-1.34)	5	0.70
Fixed Effects	1.05 (0.67-1.63)	4	0.03	1.08 (0.87-1.34)	5	0.70
Definition of Subclinical Hyperthyroidism						
TSH cutoff < 0.45mIU/l	1.33 (0.23-7.69)	2	0.008	NA	NA	NA
Exclusion of studies with thyroxine users ‡	1.09 (0.43-2.74)	2	0.14	1.03 (0.81-1.31)	2	0.36
Measurement of T4 in all†	1.33 (0.23-7.69)	2	0.008	1.20 (0.45-3.19)	3	0.63
Stratified by Mean Age at Inclusion in the Cohorts **						
<65 years	NA	NA	NA	1.90 (0.50-7.29)	1	NA
≥65 years	1.17 (0.54-2.56)	4	0.03	1.07 (0.86-1.32)	4	0.67
<70 years	1.33 (0.23-7.69)	2	0.008	1.20 (0.45-3.19)	3	0.63
≥70 years	1.09 (0.43-2.74)	2	0.14	1.10 (0.84-1.43)	2	0.27
Adjustments						
Minimally Adjusted Results §	1.26 (0.65-2.47)	4	0.07	1.06 (0.86-1.31)	5	0.68
Characteristics of Study Quality						
Formal Stroke Adjudication Procedures	0.63 (0.36-1.10)	2	0.71	1.09 (0.88-1.35)	4	0.56
Excluding studies						
One study that was not population based	1.54 (0.62, 3.83)	3	0.06	1.09 (0.88-1.35)	4	0.56
One study as an outlier in funnel plot	0.85 (0.44, 1.65)	3	0.16	1.09 (0.88-1.35)	4	0.56

Abbreviations: NA = not applicable, * p > 0.10, ratios are homogeneous † these studies measure T4 or T3 only in higher and/or lower TSH levels ‡ studies that excluded thyroid hormone recipients were included in this analysis ¶ lowest prevalence versus highest prevalence of subclinical thyroid disease § adjusted for the least amount of covariates per study. ** p for interaction: for age threshold 70 in subclinical hypothyroidism 0.25, in subclinical hyperthyroidism 0.88, age threshold 65 in subclinical hypothyroidism 0.23

Furthermore, we were not able to take possible treatment during follow-up into account, neither did we have information on the progression of the thyroid function or thyroperoxidase (TPO) autoantibody status of participants. Finally, (Free)T3 was not measured or taken into account in all studies allowing for possible misclassification of subclinical hyperthyroidism, which might have been the case in two studies.[31,33]

Reference ranges differed substantially between the individual studies, especially for subclinical hyperthyroidism, with lower limits of TSH ranging from 0.1 to 0.6 mIU/L. This might be one of the reasons for heterogeneity. All included studies evaluated stroke as a secondary outcome next to other cardiovascular diseases and deaths or total mortality. This is reflected by the lack of subgroup analyses per cohort and the inability of our study to stratify for different possible risk populations, such as gender specific analyses. For the subclinical hyperthyroidism analysis we found statistically significant heterogeneity, complicating the interpretation of the meta-analysis results. One of the reasons might be the underlying differences in populations ranging from healthy volunteers [32] to patients on hemodialysis [34] and from multiracial [33,37] to a solely Asian population [30], leading to possible selection bias. In order to examine the issue of heterogeneity in the subclinical hyperthyroidism analysis, we conducted sensitivity and subgroup analyses, which led to a reduction in heterogeneity by excluding studies that included levothyroxine users, including studies with formal stroke adjudication procedures only and stratifying by age (e.g. cut-off at 70 years of age). However, these analyses included no more than 2 studies and none reached statistical significance for the outcome measure. Furthermore, the visual assessment of publication bias, showed no evidence for publication bias for the analysis on subclinical hypothyroidism but some for subclinical hyperthyroidism. This remains a concern in meta-analyses, especially when only a small number of studies are retrieved.

While we could not provide evidence for an effect of subclinical thyroid dysfunction on the risk of stroke, these results should be interpreted with caution. Thyroid dysfunction, even in the subclinical range gives rise to several cardiovascular risk factors. In subclinical hypothyroidism some seem to be reversible if treated with levothyroxine.[42,43] Furthermore, restoring thyroid function with levothyroxine treatment in subclinical hypothyroid individuals showed a reduction of almost 10%

of the carotid artery mean intima media thickness (IMT).[44] As IMT has shown to be a risk factor of cardiovascular disease and stroke[45], this decline in IMT might also result in a decreased risk of stroke. Also, the association between subclinical hyperthyroidism and atrial fibrillation has been clearly demonstrated[19] and therefore an association between subclinical hyperthyroidism and stroke could be presumed. However, we were not able to demonstrate this expected risk increase in our meta-analysis for subclinical hyper- nor for hypothyroidism. This might be due to the limited number of populations evaluated and gathered (hence the lack of power) by the included studies, not representing specific populations at risk. Treatment of subclinical thyroid dysfunction in relation to cardiovascular disease is still controversial and highly debated. Consensus guidelines have advocated treatment of subclinical hyperthyroidism but only in the elderly or patients with cardiac risk factors, heart disease or osteoporosis.[46]. Levothyroxine treatment has shown to improve several subclinical cardiovascular disease markers, e.g. IMT and endothelial dysfunction [43,47,48], in subclinical hypothyroidism, but no large controlled trials have been performed to evaluate the effect in preventing cardiovascular events. Moreover, there are concerns about the risk of overtreatment, as population-based studies reported that among patients treated with thyroid medication, only 60% were within the normal biochemical range of TSH with more than one fifth having a TSH level that was suppressed below normal.[15] Randomized clinical trials, like the ongoing IEMO 80+ Thyroid Study and Thyroid Hormone Replacement for Subclinical Hypothyroidism (TRUST) trial (ClinicalTrials.gov Identifier: NCT01660126), are expected to give more insight into the benefit of treatment in the general population as well as specific subgroups.

In summary, we found no association between subclinical thyroid dysfunction and the risk of stroke. However, the available literature is insufficient and more research is needed. Future studies should focus on the association between subclinical thyroid dysfunction and the risk of stroke as a primary outcome and be adequately powered to conduct subgroup analyses including different age groups, TSH levels and gender differences.

Online supplemental material

https://link.springer.com/article/10.1007%2Fs10654-014-9946-8

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CHAPTER 3.2

SUBCLINICAL HYPOTHYROIDISM AND THE RISK OF STROKE AND MORTALITY: A META-ANALYSIS OF INDIVIDUAL PATIENT DATA

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ABSTRACT

BACKGROUND There is paucity in literature concerning the association of subclinical hypothyroidism with the subsequent risk of stroke.

METHODS Published prospective cohort studies were identified through a systematic search through November 2013 without restrictions in several databases. Unpublished studies were identified through the Thyroid Studies Collaboration. We collected individual participant data (IPD) on thyroid function and stroke outcome. Euthyroidism was defined as thyrotropin (TSH) levels 0.45-4.49 mIU/L, subclinical hypothyroidism as TSH levels 4.5-19.9 mIU/L with normal thyroxin levels.

RESULTS We collected IPD on 47,573 adults (3451 subclinical hypothyroidism) from 17 cohorts, followed-up 1972-2014 (489,192 person-years). Age- and sex-adjusted pooled hazard ratio (HR) for participants with subclinical hypothyroidism compared to euthyroidism was 1.05 (95% CI, 0.91-1.21) for stroke events (combined fatal and non-fatal stroke) and 1.07 (95% CI, 0.80-1.42) for fatal stroke. Stratified by age, the HR for stroke events was 3.32 (95% CI, 1.25-8.80) for individuals aged 18-49 years. There was an increased risk of fatal stroke in the age groups 18-49 and 50-64 years with a HR of 4.22 (95% CI, 1.08-16.55) and 2.86 (95% CI, 1.31-6.26), respectively (p trend 0.04). We found no increased risk for those 65-79 years (HR 1.00, 95% CI, 0.86-1.18) or ≥80 years (HR 1.31, 95% CI, 0.79-2.18). There was a pattern of increased risk of fatal stroke with higher TSH concentrations.

CONCLUSIONS Although no overall effect of subclinical hypothyroidism on stroke could be demonstrated, an increased risk in subjects younger than 65 years and those with higher TSH concentrations was observed.

INTRODUCTION

Subclinical hypothyroidism is defined as an elevated thyrotropin (TSH) level above the upper limit of the reference range with a free thyroxin (FT4) value that is normal [1-3]. It has a prevalence varying between 4-14% in adults [4-6] with a higher prevalence in iodine-sufficient populations [7] and older individuals [5]. Subclinical hypothyroidism has been associated with hypercholesterolemia [6,8,9], atherosclerosis [10], and an increased carotid intima-media thickness (IMT) [11]. Furthermore, the association between subclinical hypothyroidism and risk of clinical cardiovascular outcomes such as coronary heart disease [12] and heart failure [13], has been established in specific subgroups with higher TSH levels [12]. Also, higher risks of cardiovascular disease (CVD) in subclinically hypothyroid individuals have been found in younger populations but not in the oldest old [14,15].

Although CVD and stroke share risk factors, published data on the association between subclinical hypothyroidism and stroke are insufficient and conflicting [16]. Even the largest prospective cohort studies have limited power, with most studies suffering from lack of generalizability and inability to conduct subgroup analyses on specific age groups or different TSH levels [17-19]. A recent systematic review and meta-analysis of published data showed no association between subclinical hypothyroidism and the risk of stroke [16]. However, meta-analysis of aggregated published data does not always allow for examination of specific subgroups that may have differential risk. Hence, we aimed to evaluate the association between subclinical hypothyroidism and stroke by conducting an individual participant data (IPD) analysis, with pre-specified stratified analyses to examine the effects of age, sex and degree of TSH elevation on this association.

METHODS

Data sources and study selection

We conducted a systematic review and meta-analysis, contacted experts in the field and reviewed reference lists to identify eligible studies [16]. The systematic literature search was conducted in Medline (OvidSP), EMBASE, Web-of-science, PubMed publisher, Cochrane and Google Scholar from inception to the 18th of

November 2013 (Supporting Information). We included publications from longitudinal studies that measured at least TSH and (F)T4 at baseline in adults and assessed stroke events and/or fatal stroke prospectively. Further details of the systematic literature search and meta-analysis have been previously described in detail elsewhere [16]. We identified six studies [17-22] that met the inclusion criteria. We identified additional studies with unpublished data within the Thyroid Studies Collaboration (TSC), a consortium of cohort studies investigating the association between thyroid dysfunction and clinical outcomes. Through contact with experts in the field, we were able to identify one more unpublished study [23]. Investigators from eligible studies were invited to join the IPD analysis, of which one declined to participate [22]. This study included 549 euthyroid subjects with 23 stroke events and 31 subclinical hypothyroid subjects with 1 stroke event.

Data extraction

We requested individual participant characteristics related to prior cardiovascular risk factors and disease, including total cholesterol, systolic blood pressure (both as continuous variables), history of diabetes, smoking and previous cerebrovascular disease. We also collected available information on medication use (thyroid hormone replacement, anti-thyroid, lipid-lowering and anti-hypertensive therapy), demographic information (age, sex and ethnicity), anthropometric measurements (height and weight) and the outcome. Primary outcome measures were stroke events (fatal and non-fatal) and fatal stroke. Stroke was defined according to World Health Organization (WHO) criteria as a syndrome of rapidly developing clinical signs of focal (or global) disturbance of cerebral function, with symptoms lasting 24 hours or longer or leading to death, with no apparent cause other than of vascular origin, including ischemic or hemorrhagic strokes. Some studies [24,25] used variations of this definition (Supplemental Table 1).

Thyroid function testing

We used a common definition of subclinical hypothyroidism and euthyroidism in order to increase comparability between the different studies and in concordance with previous analyses [12,13,26], expert reviews [1,3] and several large cohorts [17,25,27]. We defined subclinical hypothyroidism as a serum TSH level of 4.5 mIU/L or greater to less than 20.0 mIU/L, with a normal (free) T4 concentration.

Euthyroidism was defined as TSH level between 0.45 and 4.49 mIU/L. Most studies used a third-generation TSH radioimmunoassay, but the Whickham Survey used a first-generation assay that reports higher measured TSH values than current assays,[28] for which we adjusted the range to 6.0 – 21.4 mIU/L to define subclinical hypothyroidism, as previously described [12,29]. In addition the Whickham Survey was the only study to perform total T4 assays [29]; the remainder performed free T4 (FT4) assays.

For (F)T4 values, we used site- and method-dependent cutoffs, as these measurements are more assay dependent. We excluded participants with TSH levels below 0.45 mIU/L or above 19.9 mIU/L and those with abnormal (F)T4 values (n=3967). When (F)T4 values were missing (n= 10,541), we considered participants with a TSH level between 4.5 and 20 mIU/L as having subclinical hypothyroidism, due to a low likelihood of overt hypothyroidism with this degree of TSH elevation [30].

Statistical analysis

We performed a Cox proportional hazard model in each cohort separately to assess the association between subclinical hypothyroidism and stroke events and fatal stroke (IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp). The Cox-proportional hazard assumption was met by each cohort, as assessed by Schoenfeld residual plots. We used a random-effects model according to DerSimonian and Laird[31] to pool estimates of the outcomes. Pooled estimates were summarized in forest plots using the metafor package for R (R-project, Institute for Statistics and Mathematics, R Core Team (2013), Vienna, Austria, version 3.0.2). Heterogeneity across studies was measured using the I^2 statistic and tested using the Q-statistic [32].

We adjusted for age and sex in the primary analysis. We also conducted a multivariable analysis additionally adjusting for systolic blood pressure, smoking, total cholesterol and diabetes. These covariates were available in all cohorts except for the Birmingham cohort [21]. We conducted multiple imputation in cohorts when there was ≥ 5% of missing data for the smoking, total cholesterol, systolic blood pressure and prevalent diabetes covariates. We considered the age and sex adjusted analysis the primary analysis because 1) covariates used in the multivariable analyses could also be considered as mediators 2) it includes all

studies in contrast to the multivariable analysis that does not include the Birmingham cohort.

In order to identify populations at risk and possible sources of heterogeneity, we conducted pre-defined subgroup and sensitivity analyses. We performed stratified analyses by age, sex and degree of TSH elevation. Based on expert reviews [1,3] and following our previous approach [12,13] we stratified subclinical hypothyroidism into the following TSH categories: 4.5-6.9 mIU/L, 7.0-9.9 mIU/L and 10.0-19.9 mIU/L. If a study did not have an event in the (stratified) study-specific analysis, we used Firth's penalized maximum likelihood bias reduction method for the Cox model [33,34] to estimate hazard ratios (HRs) and 95% confidence intervals (CIs).

We conducted the following sensitivity analyses: 1) excluding three [20,23,35] studies that did not have stroke events (non-fatal and fatal) in the subclinical hypothyroidism group 2) excluding participants who had thyroid-function altering medication at baseline and during follow-up 3) excluding studies that included transient ischemic attack (TIA) as a stroke event 4) excluding participants with a history of stroke 5) excluding participants with missing (F)T4 levels 6) using only unimputed data 7) cohorts with potential co-morbidities and 8) including the published risk estimates of the study that declined to participate in the meta-analysis [22]. We assessed age- and sex-adjusted funnel plots and conducted Egger tests [36] to evaluate potential publication bias.

RESULTS

We found 18 prospective cohort studies that met the criteria. From these we included 17 from the United States [17,19,35], Europe [15,20,21,23,24,27,29,37-40], Australia [25], Brazil [41] and Japan [18] that prospectively assessed stroke outcomes and agreed to share individual participant data (Table 1, Supplemental Figure 1).

Table 1 Baseline Characteristics of Individuals in the Included Studies (n = 47,573)

Study, Start year	Description of Study Sample	No.	Median Age (Range), years[a]	Women No. (%)	Subclinical Hypothyroidism No. (%)	Thyroid Medication No. (%) at baseline[b]	Thyroid Medication No. (%) follow up[c]	Median Duration (IQR), years
4D Study[20], 1998	Trial of atorvastatin in type 2 diabetes and hemodialysis patients, Germany	883	66 (30-83)	400 (45.3)	10 (1.1)	44 (5.0)	62 (7.0)	1.5 (0.2-3.6)
Brazilian Thyroid Study[41], 1999	Adults from Japanese descent living in São Paulo, Brazil	991	57 (30-92)	523 (52.8)	101 (10.2)	0	NA	7.3 (7.0-7.5)
Busselton Health Study[25], 1981	Adults in Busselton, Western Australia	2001	51 (18-90)	984 (49.2)	89 (4.4)	15 (0.7)	33 (1.6)	20 (19.5-20.0)
Birmingham Study[21], 1988	CDA's aged ≥ 60 y from primary care practice in Birmingham, England	1107	69 (60-94)	628 (56.7)	92 (8.3)	0	29 (2.6)	10.2 (5.7-10.6)
Cardiovascular Health Study[17], 1989	CDA's with Medicare eligibility in 4 US communities	3017	71 (64-100)	1812 (60.1)	492 (16.3)	0	153 (5.1)	13.9 (8.6-16.4)
EPIC-Norfolk Study[27], 1995	Adults living in Norfolk, England	12709	58 (40-78)	6874 (54.1)	723 (5.7)	0	NA	13.4 (12.6-14.3)
Health, Aging, and Body Composition Study[19], 1997	CDA's with Medicare eligibility in 2 US communities	2677	74 (69-81)	1346 (50.3)	335 (12.5)	232 (8.7)	338 (12.6)	11.9 (7.5-12.2)
InCHIANTI Study[37], 1998	Adults aged 20-102 years living in Chianti geographic area, Italy	1099	71 (21-102)	612 (55.7)	33 (3.0)	21 (1.9)	NA	9.07 (8.1-9.2)
Leiden 85-plus Study[15], 1997	Adults aged 85 years living in Leiden, The Netherlands	493	85 (NA)	322 (65.3)	35 (7.1)	14 (2.8)	20 (4.1)	5.2 (2.5-8.6)
MrOS Study[35], 2000	Community-dwelling U.S. men aged 65 years and older	1558	73 (65-99)	0	148 (9.5)	110 (7.1)	NA	12.0 (8.2-12.7)

Table 1 Baseline Characteristics of Individuals in the Included Studies (n = 47,573) (continued)

Study, Start year	Description of Study Sample	No.	Median Age (Range), years[a]	Women No. (%)	Subclinical Hypothyroidism No. (%)	Thyroid Medication No. (%) at baseline[b]	Thyroid Medication No. (%) follow up[c]	Median Duration (IQR), years
Nagasaki Adult Health Study[18], 1984	Atomic bomb survivors in Nagasaki, Japan	2766	57 (38-92)	1688 (61.0)	424 (15.3)	39 (1.4)	6 (0.2)	13.0 (12.3-13.6)
Pisa cohort[39], 2000	Patients admitted to cardiology department in Pisa, Italy[d]	2922	63 (19-92)	935 (32.0)	227 (7.8)	12 (0.4)	0	2.5 (1.6-3.7)
PREVEND Study[23], 1997	Adults living in Groningen, The Netherlands	2562	46 (28-75)	1306 (51)	51 (2.0)	27 (1.1)	34 (1.3)	10.9 (10.6-11.1)
PROSPER trial[40], 1997	Trial on the benefits of pravastatin vs. placebo in adults	5525	75 (69-83)	2801 (50.7)	446 (8.1)	211 (3.8)	264 (4.8)	3.3 (3.0-3.5)
Rotterdam Study[10, 38], 1989	Adults ≥55 years living in Rotterdam, The Netherlands	1697	68 (55-93)	1036 (61.0)	104 (6.1)	30 (1.8)	NA	16.8 (11.1-18.9)
SHIP Study[24], 1997	Adults in West Pomerania, North-East of Germany	3118	47 (20-81)	1587 (50.9)	13 (0.4)	159 (5.1)	214 (6.9	11.3 (10.6-11.8)
Whickham Survey[29], 1972	Adults living in & near Newcastle upon Tyne, England	2448	46 (18-92)	1308 (54.4)	128 (5.2)	99 (4.0)	71 (2.9)	19 (15.0-20.0)
Overall		**47,573**	**65 (18-102)**	**24,162 (50.8)**	**3451 (7.3)**	**1103 (2.3)**	**1224 (2.6)**	**11.6 (5.0-13.8)**

Abbreviations: CDA = community-dwelling adult; IQR = interquartile range (25th-75th percentile); NA = not available.

[a] Participants younger than 18 years of age were not included

[b] Participants with missing information on thyroid medication at baseline: CHS 1, HABC 7, Whickham 3, RS 482, MrOS 64

[c] Participants with missing information on thyroid medication at follow-up: Birmingham 1026, Whickham 1489

[d] Excluded patients with acute coronary syndrome or severe illness

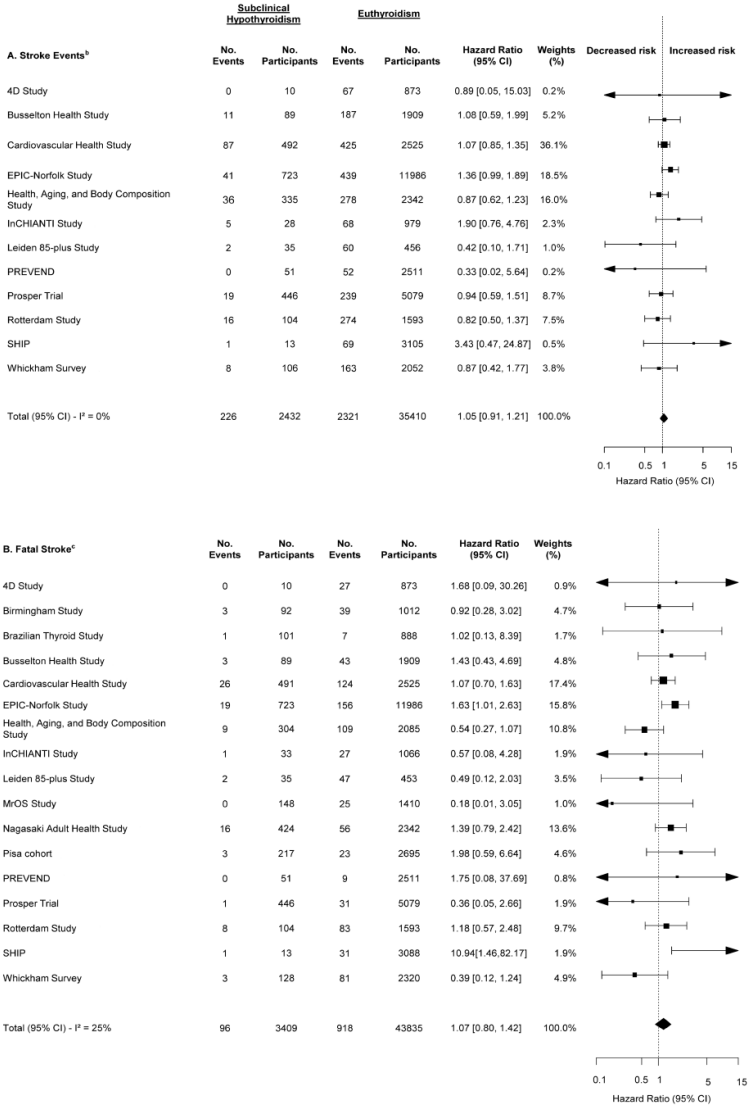
One study [42] was excluded from our analyses because no outcome events occurred. The included studies provided information on a total of 47,573 participants with a follow-up from 1972 to 2014, a median follow-up ranging between 1.5 and 20 years and a total follow-up of 489,192 person-years. All studies, except one [43,44], included both female (50.8%) and male participants. The prevalence of subclinical hypothyroidism ranged from 0.4 to 16.3%, with an overall average of 7.3% (n= 3451) of which 62% were female. All cohorts reported fatal stroke and 12 studies also reported stroke events, including fatal and non-fatal stroke, contributing to the stroke events analysis among 37,842 participants. During follow-up 2547 stroke events occurred and 1014 participants had a fatal stroke.

All studies provided information on the proportion of participants taking thyroid medication at baseline, which varied from 0 to 8.7%. All but five studies also provided follow-up information on thyroid function-altering medication use, with a range between 0 and 12.6%. One study[24] used questionnaires for the assessment of stroke events. Formal adjudication, defined as having clear criteria for the outcomes that were reviewed by experts for each potential case, was used for stroke events in six studies [10,15,17,19,22,40] and for fatal stroke in two additional studies [35,39]. Three studies [18,35,39] required multiple imputation due to more than 5% missing data for covariates.

The age- and sex-adjusted pooled HR for participants with subclinical hypothyroidism compared to euthyroidism was 1.05 (95% CI, 0.91-1.21) for stroke events and 1.07 (95% CI, 0.80-1.42) for fatal stroke (Figure 1). We found no heterogeneity for the stroke events analysis (I^2=0%) and little heterogeneity for fatal stroke (I^2=25%).

Subsequent subgroup analyses showed an increased risk of stroke events (HR 3.32, 95% CI, 1.25-8.80) and fatal stroke (HR 4.22, 95% CI, 1.08-16.55) in the 18-49 year age group with subclinical hypothyroidism compared to euthyroidism, but the number of events was small (Table 2, Figure 2). For the 50-64 year age group, we found an increased risk of fatal stroke with a HR of 2.86 (95% CI, 1.31-6.26), p for trend across age groups = 0.04.

Figure 1 The Risk of Stroke Events and Fatal Stroke in Subclinical Hypothyroidism vs Euthyroidism[a]



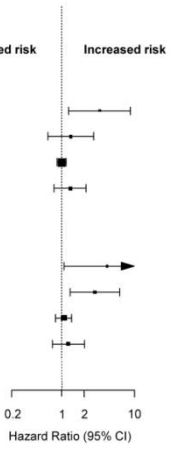
A. Stroke Events[b]	Subclinical Hypothyroidism No. Events	Subclinical Hypothyroidism No. Participants	Euthyroidism No. Events	Euthyroidism No. Participants	Hazard Ratio (95% CI)	Weights (%)
4D Study	0	10	67	873	0.89 [0.05, 15.03]	0.2%
Busselton Health Study	11	89	187	1909	1.08 [0.59, 1.99]	5.2%
Cardiovascular Health Study	87	492	425	2525	1.07 [0.85, 1.35]	36.1%
EPIC-Norfolk Study	41	723	439	11986	1.36 [0.99, 1.89]	18.5%
Health, Aging, and Body Composition Study	36	335	278	2342	0.87 [0.62, 1.23]	16.0%
InCHIANTI Study	5	28	68	979	1.90 [0.76, 4.76]	2.3%
Leiden 85-plus Study	2	35	60	456	0.42 [0.10, 1.71]	1.0%
PREVEND	0	51	52	2511	0.33 [0.02, 5.64]	0.2%
Prosper Trial	19	446	239	5079	0.94 [0.59, 1.51]	8.7%
Rotterdam Study	16	104	274	1593	0.82 [0.50, 1.37]	7.5%
SHIP	1	13	69	3105	3.43 [0.47, 24.87]	0.5%
Whickham Survey	8	106	163	2052	0.87 [0.42, 1.77]	3.8%
Total (95% CI) - I^2 = 0%	226	2432	2321	35410	1.05 [0.91, 1.21]	100.0%

B. Fatal Stroke[c]	No. Events	No. Participants	No. Events	No. Participants	Hazard Ratio (95% CI)	Weights (%)
4D Study	0	10	27	873	1.68 [0.09, 30.26]	0.9%
Birmingham Study	3	92	39	1012	0.92 [0.28, 3.02]	4.7%
Brazilian Thyroid Study	1	101	7	888	1.02 [0.13, 8.39]	1.7%
Busselton Health Study	3	89	43	1909	1.43 [0.43, 4.69]	4.8%
Cardiovascular Health Study	26	491	124	2525	1.07 [0.70, 1.63]	17.4%
EPIC-Norfolk Study	19	723	156	11986	1.63 [1.01, 2.63]	15.8%
Health, Aging, and Body Composition Study	9	304	109	2085	0.54 [0.27, 1.07]	10.8%
InCHIANTI Study	1	33	27	1066	0.57 [0.08, 4.28]	1.9%
Leiden 85-plus Study	2	35	47	453	0.49 [0.12, 2.03]	3.5%
MrOS Study	0	148	25	1410	0.18 [0.01, 3.05]	1.0%
Nagasaki Adult Health Study	16	424	56	2342	1.39 [0.79, 2.42]	13.6%
Pisa cohort	3	217	23	2695	1.98 [0.59, 6.64]	4.6%
PREVEND	0	51	9	2511	1.75 [0.08, 37.69]	0.8%
Prosper Trial	1	446	31	5079	0.36 [0.05, 2.66]	1.9%
Rotterdam Study	8	104	83	1593	1.18 [0.57, 2.48]	9.7%
SHIP	1	13	31	3088	10.94[1.46,82.17]	1.9%
Whickham Survey	3	128	81	2320	0.39 [0.12, 1.24]	4.9%
Total (95% CI) - I^2 = 25%	96	3409	918	43835	1.07 [0.80, 1.42]	100.0%

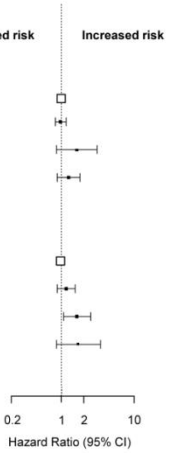
a Hazard ratios (HRs) and their 95% confidence intervals (CIs) are represented by squares. Sizes of data markers are proportional to the inverse of the variance of the hazard ratios.
b Data for stroke events were available in 12 studies. Three hundred eighty-seven participants were excluded from the analysis of stroke event due to missing follow-up data.
c Data for fatal stroke were available in 17 studies. Three hundred twenty-nine participants were excluded from the analysis of fatal stroke, due to missing cause of death.

Figure 2 Hazard Ratios (HRs) for Stroke Events and Fatal Stroke for Subclinical Hypothyroidism Stratified by Age vs Euthyroidism and According to Elevated Thyroid–Stimulating Hormone (TSH) Categories[a]

Panel A	Subclinical Hypothyroidism No. Events	Subclinical Hypothyroidism No. Particpants	Euthyroidism No. Events	Euthyroidism No. Particpants	HR Ratio (95% CI)[b]
Stroke events by age[b]					
18 – 49y	3	207	61	8348	3.32 [1.25, 8.80]
50 – 64y	20	498	361	9225	1.34 [0.65, 2.80]
65 -79y	172	1555	1631	16,056	1.00 [0.86, 1.18]
≥ 80y	31	172	268	1781	1.31 [0.79, 2.18]
					p for trend= 0.07
Fatal stroke by age[c]					
18 – 49y	2	311	12	9568	4.22 [1.08, 16.55]
50 - 64y	13	911	104	12,378	2.86 [1.31, 6.26]
65 – 79y	64	1918	634	19,542	1.07 [0.83, 1.39]
≥ 80y	17	268	168	2348	1.23 [0.74, 2.04]
					p for trend= 0.04



Panel B	No. Events	No. Particpants	HR Ratio (95% CI)
Stroke events by TSH level, mIU/L[b]			
0.45 - 4.49	2301	35,250	reference
4.5 - 6.9	161	1799	1.01 [0.86, 1.19]
7.0 - 9.9	53	507	1.62 [0.89, 2.94]
10.0 - 19.9	32	286	1.27 [0.90, 1.80]
			p for trend = 0.05
Fatal stroke by TSH level, mIU/L[c]			
0.45 - 4.49	910	43,648	reference
4.5 - 6.9	72	2544	1.18 [0.83, 1.69]
7.0 - 9.9	22	699	1.63 [1.09, 2.43]
10.0 - 19.9	10	353	1.69 [0.88, 3.27]
			p for trend = 0.07



[a] Hazard ratios (HRs) and their 95% confidence intervals (CIs) are represented by squares. Sizes of data markers are proportional to the inverse of the variance of the hazard ratios. Unfilled squares indicate the reference categories. For the analysis stratified by age, HRs for stroke events and fatal stroke were adjusted for sex and age as a continuous variable to avoid residual confounding within age strata.

[b] Data for stroke events were available in 12 studies. Three hundred eighty-seven participants were excluded from the analysis of stroke event due to missing stroke event data.

[c] Data for fatal stroke were available in 17 studies. Three hundred twenty-nine participants were excluded from the analysis of fatal stroke, due to missing cause of death.

Table 2 Stratified Analyses for the Associations between Subclinical Hypothyroidism and the Risk of Stroke and Fatal Stroke

		Stroke events[a]			Fatal Stroke[b]		
		No. events/ Total participants	**Age & sex adjusted HR (95% CI)**	**Multivariable[c] HR (95% CI)**	**No. events/ Total participants**	**Age & sex adjusted HR (95% CI)**	**Multivariable[c] HR (95% CI)**
Total Population		2547/37,842	1.05 (0.91, 1.21)	0.97 (0.77, 1.22)	1014/47,244	1.07 (0.80, 1.42)	1.11 (0.82, 1.50)
	Men[d]	1177/17,644	1.12 (0.88, 1.42)	1.07 (0.90, 1.27)	452/23,238	1.19 (0.83, 1.70)	1.19 (0.82, 1.72)
	Women[d]	1370/20,198	1.07 (0.90, 1.27)	1.17 (0.92, 1.49)	562/24,006	1.19 (0.86, 1.64)	1.24 (0.83, 1.84)
	p for interaction		*0.76*	*0.55*		*0.99*	*0.88*
Age[e]	18 – 49y	64/8555	3.32 (1.25, 8.80)	3.34 (1.18, 9.46)	14 / 9,879	4.22 (1.08, 16.55)	4.80 (1.03, 22.30)
	50 – 64y	381/9723	1.34 (0.65, 2.80)	1.34 (0.69, 2.62)	117/13,289	2.86 (1.31, 6.26)	1.99 (1.05, 3.74)
	65 -79y	1803/17,611	1.00 (0.86, 1.18)	1.02 (0.87, 1.20)	698/21,460	1.07 (0.83, 1.39)	1.09 (0.82, 1.45)
	≥80	299/1953	1.31 (0.79, 2.18)	1.43 (0.93, 2.18)	185/2,616	1.23 (0.74, 2.04)	1.34 (0.75, 2.40)
	p for trend		*0.07*	*0.11*		*0.04*	*0.08*
Age[e]	18-64y	445/18,278	1.37 (0.71, 2.63)	1.46 (0.78, 2.73)	131/23,168	2.51 (1.42, 4.44)	2.29 (1.41, 3.74)
	≥65y	2102/19,564	1.04 (0.90, 1.20)	1.03 (0.71, 1.49)	883/24,076	0.99 (0.81, 1.21)	1.04 (0.81, 1.32)
	p for interaction		*0.42*	*0.35*		*0.003*	*0.005*
TSH, mIU/L	0.45 - 4.49	2301 / 35,250	reference	reference	910 / 43,648	reference	reference
	4.5 - 6.9	161 / 1799	1.01 (0.86, 1.19)	1.01 (0.85, 1.19)	72 / 2544	1.18 (0.83, 1.69)	1.09 (0.71, 1.67)
	7.0 - 9.9	53 / 507	1.62 (0.89, 2.94)	1.68 (0.91, 3.09)	22 / 699	1.63 (1.09, 2.43)	1.65 (1.16, 2.33)
	10.0 - 19.9	32 / 286	1.27 (0.90, 1.80)	1.26 (0.89, 1.79)	10 / 353	1.69 (0.88, 3.27)	1.79 (0.88, 3.63)
	p for trend		*0.05*	*0.05*		*0.07*	*0.05*

Abbreviations: CI, confidence interval; HR, hazard ratio; TSH, thyroid-stimulating hormone.

[a] Data were available from 12 studies, 387 participants were excluded due to missing stroke event data.

[b] 329 participants were excluded due to missing data on cause of death.

[c] Adjusted for sex, age, systolic blood pressure, smoking and prevalent diabetes at baseline. The Birmingham Study was excluded in this analysis because of lack of data on cardiovascular risk factors.

[d] These analyses were not adjusted for sex.

[e] These HRs were adjusted for sex and age as continuous variable to avoid residual confounding within age strata.

When participants were pooled into two categories, younger and older than 65 years, in a post-hoc analysis, we found a significantly increased risk of fatal stroke with a HR of 2.51 (95% CI, 1.42-4.44) in the younger group, p for interaction = 0.003 (Table 2). When looking at incidence rate per 100,000 person-years for stroke events in the pooled dataset within each age group we find 58 for the18-49 year age group, 330 for the 50-64 year group, 1127 for the 65-79 group and 2991 for those 80 years and older. For fatal stroke this was 11, 74, 370 and 1183 per 100,000 person-years for the respective age groups.

There was a non-significant pattern of increased risk of fatal stroke with higher TSH concentrations. In the age- and sex-adjusted analyses, the HR for fatal stroke was 1.18 (95% CI, 0.83-1.69) in participants with TSH levels between 4.5 and 6.9 mIU/L, 1.63 (95% CI, 1.09-2.43) for those with TSH levels between 7.0 and 9.9 mIU/L, and 1.69 (95% CI, 0.88-3.27) for those with TSH levels between 10.0 and 19.9 mIU/L, compared to the euthyroid group (p for trend 0.07). There was no observed difference by sex (p for interaction > 0.5).

Multivariable analyses, adjusting for sex, age, smoking, total cholesterol, systolic blood pressure and history of diabetes yielded similar results, with the exception of fatal stroke analysis in the age group between 50 and 65 years of age, which was attenuated after adjustment (Table 2). This was likely due to eliminating heterogeneity in this subgroup, with an I^2 of 29% in the age- and sex-adjusted analysis and 0% in the multivariable analysis.

Sensitivity analyses excluding several studies, excluding thyroid medication users, using only non-imputed data, additional adjustments and other sensitivity analyses did not meaningfully affect the risk estimates (Supplemental Table 2). We found no evidence of publication bias, either with visual assessment of age- and sex-adjusted funnel plots or with the Egger test for stroke events (p = 0.67) or fatal stroke (p = 0.58).

DISCUSSION

In our IPD analysis of 47,573 participants from 17 prospective cohort studies, no overall effect of subclinical hypothyroidism was observed on the risk of stroke events or fatal stroke compared to euthyroidism in age- and sex-adjusted analyses.

However, younger participants had an increased risk of stroke events and fatal stroke in subclinical hypothyroidism compared to euthyroidism. There was an increase in fatal stroke in those younger than 65 years and in participants with a TSH level of 7.0 to 9.9 mIU/L, but a non-significant p for trend (0.07). This is the first IPD analysis to investigate the association between subclinical hypothyroidism and stroke. We are also the first to detect differences by age in associations between subclinical hypothyroidism and a clinical outcome in an IPD analysis.

The mechanisms by which subclinical hypothyroidism increases the risk of stroke, as found in specific subgroups, could be explained by the increased prevalence of cardiovascular risk factors in those with subclinical hypothyroidism. Thyroid hormones have direct effects on the cardiovascular system and are known to decrease systemic vascular resistance [45] and alter systolic and diastolic cardiac function [46]. Thyroid hormone deficiency increases the risk of several cardiovascular risk factors including hypertension [47], dyslipidemia [48] and atherosclerosis[49]. These changes have also been observed in subclinical thyroid dysfunction[10,50]. However, our multivariable IPD analyses yielded similar results to the age- and sex-adjusted analyses. Adjusting for smoking, total cholesterol, systolic blood pressure and diabetes only slightly changed the risk estimates in the age-stratified analysis of fatal stroke for participants between 50 and 65 years old. The fact that adjustment for traditional cardiovascular risk factors did not substantially alter risk estimates suggests an independent association of subclinical hypothyroidism on the risk of stroke and also indicates that total cholesterol, systolic blood pressure and diabetes are not relevant factors mediating the hypothetical pathway between subclinical hypothyroidism and stroke. Another explanation might be that this is due to some unmeasured confounders or mediators.

Various abnormalities in the hemostatic system have been reported in overt [51,52] and subclinical hypothyroidism[53-55]. Alterations in coagulability and the fibrinolytic system have been linked to a high risk of CVD [56]. This might also be one of the mechanisms that play a role in the increased risk of stroke in subclinical hypothyroidism. We were not able to discriminate between haemorragic and ischemic stroke in the current study as most cohorts did not collect these data. Another pathway linked with both thyroid function and risk of stroke is atrial fibrillation [26]. This however, seems unlikely as atrial fibrillation is linked to overt and

subclinical hyperthyroidism and not to hypothyroidism [26]. The exact mechanistic relationship between subclinical hypothyroidism and the risk of stroke still remains to be determined.

In our study, younger individuals with subclinical hypothyroidism had a higher risk of stroke events and fatal stroke compared with euthyroid subjects within the same age groups. Although a higher risk in those younger than 65 years of age has previously been reported in a meta-analysis of published data studying the association between subclinical thyroid disease and coronary heart disease[57], this was not confirmed by an IPD analysis investigating the same association[12]. Several population based studies and published data meta-analysis found an association between subclinical hypothyroidism and various clinical outcomes, including self-reported health [58], ischemic heart disease [14,18,29,57] and cognition [15] when including younger age groups but not in older populations. However, these differences in association by age have not been observed in IPD analyses prior to ours.

In our IPD-analysis, the relationship between subclinical hypothyroidism and the risk of stroke seen in younger individuals does not seem to hold in populations of 65 years and older. This seems counterintuitive as both the prevalence and incidence of subclinical hypothyroidism and stroke are higher in elderly than in younger populations. An explanation for the absence of the association in elderly subjects could be that adverse outcomes of subclinical hypothyroidism (e.g. hyperlipidemia) are leveled out in this particular group due to slowing of metabolic rate and energy expenditure [59], reduced sensitivity to adrenergic stimulation [60] or other counterbalancing protective factors. Also, differences in stroke etiology in younger versus older individuals could explain the difference in risk estimates by age group. For example, stroke in younger adults is more often hemorrhagic compared to older individuals [61]. Subclinical and overt hypothyroidism are linked to hypocoagulability [51] and could through this pathway have a stronger effect on younger adults rather than on the elderly. There might also be the possibility of competing risk of events in the elderly. However, this rarely leads to meaningful changes in relative risk estimates of the hazard ratio [62]. Another possible explanation for the different risks across age groups might be a changed hypothalamus-pituitary-thyroid set point in elderly, leading to higher TSH levels [63,64]. In this case subclinical hypothyroidism, defined with a TSH > 4.5 mIU/L may

not reflect thyroidal status as well as in younger individuals [65-67] and subclinical hypothyroidism and stroke would exist simultaneously rather than have a causal relation in those older than 65 years of age. It is debated whether age-specific reference ranges are needed to define the normal range and herewith also the altered state of thyroid function. Some studies have found relevant reclassification of thyroid status by applying age-specific reference ranges of TSH [68], while others have not [69]. The question remains whether the definition of the normal range should be based on age-specific biochemical cut-offs or rather based on risk of clinical adverse events associated with these cut-offs. The findings of our study suggest that for older subjects a TSH cut-off higher than 4.5 ImU/L could be applied, while this cut-off might be too high for younger individuals. However, further studies are needed to determine the risks and benefits of redefining the cut-offs of thyroid function.

We found a higher risk of fatal stroke in a subset of subclinically hypothyroid individuals with TSH levels between 7.0 and 9.9 mIU/L, when compared to individuals with values within the TSH reference range. We were not able to demonstrate an association for the subgroup with a TSH level between 10.0 and 19.9 mIU/L, which is probably due to lack of power, as the point estimate for fatal stroke was higher than for TSH levels between 7.0 and 9.9 mIU/ml, suggesting a dose-response relationship.

The strengths of our study is that we were able to perform an IPD analysis including over 47,000 participants from 17 cohort studies, based on published and unpublished data. We did an extensive literature search and included all available published data on the association between subclinical hypothyroidism and the risk of stroke and fatal stroke. Furthermore we were able to find additional cohorts with unpublished longitudinal data with information on thyroid function and stroke outcomes. One of the advantages of performing an IPD analysis is that it enables the standardization of the definition of exposures and covariates used for the time-to-event analyses, allowing a more uniform interpretation. Although we found similar overall results in this IPD analysis compared to the previous study-level meta-analysis [16], we did observe additional important findings in subgroups that were not detected by meta-analyzing the aggregate data. This highlights the

strength of an IPD analysis, as it provides a better opportunity for subgroup and sensitivity analyses.

Despite the large number of participants, we had limited power mainly for the stratified analyses. Power calculations showed that our study had a statistical power of 80% to detect a HR of 1.57 for stroke events and a HR of 1.61 for stroke mortality. The power was limited especially in the age subgroup analyses under 50 where the number of events was decreased, reflected in the wide confidence intervals. There were also limited numbers of events in those with TSH levels between 10.0 and 19.9 mIU/L. Information on thyroid medication use during follow-up, which could alter risk over time, was not available for some cohorts. We were unable to perform analyses stratified by type of stroke (ischemic vs. hemorrhagic) due to limited number of events in each stratum or by race due to having few non-white participants. Furthermore, thyroid function was determined only at baseline in most cohorts and therefore it was not possible to take the evolution of thyroid dysfunction over time into account. As a number of participants with mildly elevated TSH levels will normalize in the course of time, a second measurement of thyroid function would have enabled us to specifically investigate participants with persistent subclinical hypothyroidism, where perhaps the effects are more pronounced.

In summary we found no association between subclinical hypothyroidism and overall risk of stroke events or fatal stroke. In stratified analyses, younger participants, particularly those under the age of 50 years, had increased stroke risk, though the number of events was small. Those with TSH of 7.0-9.9 mIU/L also had an increased risk of fatal stroke compared to their euthyroid counterparts. Our data are reassuring for those over the age of 65 years and those with TSH levels between 4.5 and 6.9 mIU/L, who represent the majority of participants with subclinical hypothyroidism. Whether treatment of subclinical hypothyroidism will result in a decrease of risk of stroke in younger subjects or those with higher TSH levels needs to be answered by a sufficiently powered randomized clinical trial.

Online supplemental material

https://academic.oup.com/jcem/article-lookup/doi/10.1210/jc.2015-1438

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CHAPTER 3.3

VARIATIONS WITHIN THYROID REFERENCE RANGE AND THE RISK OF STROKE EVENTS AND FATAL STROKE: A META-ANALYSIS OF INDIVIDUAL PARTICIPANT DATA

Chaker L, Baumgartner C, den Elzen WP, Collet TH, Ikram MA, Blum MR, Dehghan A, Drechsler C, Luben RN, Portegies ML, Iervasi G, Medici M, Stott DJ, Dullaart RP, Ford I, Bremner A, Newman AB, Wanner C, Sgarbi JA, Dörr M, Longstreth WT Jr, Psaty BM, Ferrucci L, Maciel RM, Westendorp RG, Jukema JW, Ceresini G, Imaizumi M, Hofman A, Bakker SJ, Franklyn JA, Khaw KT, Bauer DC, Walsh JP, Razvi S, Gussekloo J, Völzke H, Franco OH, Cappola AR, Rodondi N, Peeters RP; for the Thyroid Studies Collaboration

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ABSTRACT

BACKGROUND The currently applied reference ranges for thyroid function are under debate. Despite evidence that thyroid function within the reference range is related with several cardiovascular disorders, its association with the risk of stroke has not been evaluated previously.

METHODS We identified studies through systematic literature search and the Thyroid Studies Collaboration, a collaboration of prospective cohort studies. Studies measuring baseline thyroid-stimulating hormone (TSH), free thyroxine (FT4) and stroke outcomes were included and we collected Individual Participant Data (IPD) from each study, including thyroid function measurements and incident all stroke (combined fatal and non-fatal) and fatal stroke. The applied reference range for TSH levels was between 0.45-4.49 mIU/L.

RESULTS We collected IPD on 43,598 adults with TSH within the reference range from 17 cohorts, with median follow-up of 11.6 years (interquartile range 5.1-13.9), including 449,908 person-years. Age- and sex-adjusted pooled HR for TSH was 0.78 (95% Confidence Interval [CI], 0.65-0.95, across the reference range of TSH) for all stroke and 0.83 (95% CI, 0.62-1.09) for fatal stroke. For the FT4 analyses, the HR was 1.08 (95% CI, 0.99-1.15, per SD increase) for all stroke and 1.10 (95% CI, 1.04-1.19) for fatal stroke. This was independent of cardiovascular risk factors including systolic blood pressure, total cholesterol, smoking and prevalent diabetes.

CONCLUSIONS Higher levels of TSH within the reference range may decrease risk of stroke, highlighting the need for further research focusing on the clinical consequences associated with differences within the reference range of thyroid function.

INTRODUCTION

Subclinical hypothyroidism is associated with hypertension, hyperlipidemia, atherosclerosis and an increased risk of coronary artery disease (CAD) whereas subclinical hyperthyroidism is associated with an increased risk of atrial fibrillation and CAD.[1-4] Subclinical thyroid dysfunction is defined by a thyroid-stimulating hormone (TSH) outside the reference range with a free thyroxine (FT4) within the reference range.

However, the currently applied reference ranges for thyroid function are under debate [5,6] as thyroid function within these reference ranges is also associated with several adverse health outcomes.[7-9] A previous systematic review found that lower TSH values and higher FT4 values within the reference range are associated with reduced bone mineral density, atrial fibrillation and an increased risk of fractures.[8] Furthermore, higher levels of TSH and lower levels of FT4 within the reference range are associated with cardiovascular events and an unfavorable metabolic profile.[8] On the other hand, a previous individual participant data (IPD) analysis provided no evidence for a higher risk of coronary heart disease within the reference range as currently defined.[10]

A considerable amount of data exist on the association of thyroid function within the reference range and cardiovascular risk factors such as atrial fibrillation, hypercholesterolemia and hypertension.[8] While these risk factors related to differences within the reference range are also associated with cardiovascular disease, few data are available on clinical outcomes and no data are available on the risk of stroke, the second major vascular cause of morbidity and mortality worldwide.[11] A previous study-level meta-analysis on the association of subclinical thyroid dysfunction and stroke risk included only a small number of studies and did not include any analyses on TSH within the reference range.[12] Assessing the consequences of differences within the reference range of thyroid function on clinical outcomes is important for understanding the definition of the reference range and to improve care and preventive measures. Furthermore, it can help identify clinical outcomes that need to be addressed in future randomized controlled trials assessing the benefits and risks of thyroid treatment in subclinical thyroid dysfunction.[13]

Therefore we aimed to investigate the association between TSH and FT4 differences within the reference range and the risk of stroke (fatal and non-fatal) in an IPD analysis. An IPD analysis provides the opportunity to standardize definitions of thyroid function and statistical analyses, include unpublished data and pool results from several cohorts. Also, an IPD can provide the opportunity to conduct subgroup analyses due to the large number of events included.

METHODS

Data sources and study selection

Studies were identified through the Thyroid Studies Collaboration (TSC). The TSC is a consortium of cohorts with thyroid function measurements at baseline and prospective follow-up of cardiovascular outcomes.[1,4,10,14-16] Its primary purpose is to examine the association of subclinical thyroid dysfunction and cardiovascular disease. Eligible cohorts were originally identified through systematic literature reviews[1] and this has been described in detail previously.[12] From the 19 cohorts identified by these two literature searches, 17 cohorts had information available on baseline thyroid function and follow-up stroke incidence, agreed to participate and were therefore eligible for the current study. No additional inclusion criteria were applied. None of the cohorts has previously published on the risk of stroke within the reference range of thyroid function, and 5 cohorts [17-21] previously published on the association of subclinical thyroid dysfunction and the risk of stroke (Table 1). Investigators from the 17 eligible studies were invited to join the IPD analysis. The local Medical Ethics Committees of each included study approved the distinct original study protocols, and informed consent was obtained from all study participants by the original cohort studies.

Data extraction

We requested individual participant characteristics related to prior cardiovascular risk factors and disease, including systolic blood pressure, serum total cholesterol, history of diabetes, smoking, previous cardiovascular disease and previous stroke. We also collected available information on demographic information (age, sex, race), anthropometric measurements (height and weight), medication use (thyroid hormone replacement, lipid-lowering and anti-hypertensive therapy) and the

outcome. Individual participant information from all cohorts were collected and analyzed in one center (Rotterdam, The Netherlands). The primary outcome measures were all stroke (combined fatal and non-fatal) and fatal stroke. Stroke was defined according to World Health Organization (WHO) criteria as a syndrome of rapidly developing clinical signs of focal (or global) disturbance of cerebral function, with symptoms lasting 24 hours or longer or leading to death, with no apparent cause other than of vascular origin, including ischemic or hemorrhagic strokes.

Thyroid function testing definition

We used a common definition of the reference range of thyroid function (i.e. euthyroidism) in order to increase comparability among the different studies and in concordance with previous analyses, [1,4,16] expert reviews [22,23] and several large cohorts.[17,24,25] Euthyroidism was defined as TSH level between 0.45 and 4.49 mIU/L.[1] Most studies used a third-generation TSH radioimmunoassay, but the Whickham Survey used a first-generation assay that reports higher measured TSH values than current assays, [26] for which we adjusted the range to 0.5-6.0 mIU/L to define euthyroidism, as previously described.[1,15,27] In addition, the Whickham Survey was the only study to perform total T4 assays [27]; the remainder of the cohorts performed FT4 assays.

For FT4 values, we excluded studies that only measured FT4 in TSH values outside of the reference range for these analyses.[17,20,21,28] In studies that measured FT4 independent of TSH values, we used all FT4 levels with individuals with TSH in the reference range, not limited by the FT4 reference range.

Data synthesis and statistical analysis

We performed a Cox proportional hazards model in each cohort separately to assess the association of TSH or FT4 continuously with all stroke and fatal stroke (IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp). We investigated the linearity assumption using cubic restricted splines (rms package, R-project, Institute for Statistics and Mathematics, R Core Team (2013), Vienna, Austria, version 3.0.2). Due to departure from linearity for the TSH analysis in the 4D cohort (p for non-linearity = 0.03), TSH was log transformed for all continuous analyses (natural logarithm). We found no departure from non-linearity in the transformed TSH or any of the FT4 analyses and no threshold effect was therefore

detected. The analyses are presented as Hazard Ratios (HR) across the reference range of TSH (0.45-4.49 mIU/L). This corresponds to the HR when comparing participants with a TSH in the upper limit of the reference range (4.49 mIU/L) to participants with a TSH in the lower limit of the reference range (0.45 mIU/L). The FT4 analyses were performed in a standardized manner (per SD) as well as per 1 ng/dL increase, for which the Whickham study [27] was excluded. We assessed the proportional hazard assumption in each cohort for each outcome, by Schoenfeld residual plots and the Schoenfeld test. All studies met the proportional hazard assumption except for the Birmingham study and PROSPER trial for the analyses with TSH, for which we performed a sensitivity analysis excluding these two cohorts. There was no interaction between FT4 and TSH levels for the all stroke events or stroke mortality analyses (p=0.099 and p = 0.28 respectively), as assessed by introducing an interaction term between FT4 (ng/dL) and TSH values.

We used a random-effects model according to DerSimonian and Laird [29] to pool outcomes estimates (two-step approach). Pooled estimates were summarized in forest plots using the metafor package for R (R-project, Institute for Statistics and Mathematics, R Core Team (2013), Vienna, Austria, version 3.0.2). Heterogeneity across studies was measured using the I^2 statistic and 95% confidence interval (95% CI).[30]

The primary analyses were adjusted for age and sex. We also conducted multivariable analyses additionally adjusting for systolic blood pressure, smoking, total cholesterol and diabetes. These covariates were available in all cohorts except for the Birmingham cohort, where none was available [20]. We conducted multiple imputation of covariates in cohorts when there was ≥ 5% of missing data for the smoking, total cholesterol, systolic blood pressure or prevalent diabetes covariates, which was the case for one study.[19] We considered the age and sex adjusted analysis the primary analysis because 1) covariates used in the multivariable analyses could also be considered as mediators 2) it includes all studies, whereas the multivariable analysis does not include the Birmingham cohort.

In order to evaluate the robustness of our findings and identify possible sources of heterogeneity and populations at risk, we conducted pre-defined subgroup and sensitivity analyses. We performed stratified analyses by age, sex, history of

stroke, subtype of stroke (including only classified strokes) and race, in concordance with previous reports.[1,4] If the parameter estimates were infinite due to a small number of events in a stratified study-specific analysis, we used Firth's penalized maximum likelihood bias reduction method for the Cox proportional hazards model [31,32] to estimate hazard ratios (HRs) and 95% CIs.

For the continuous TSH analyses, we conducted the following sensitivity analyses: 1) excluding participants who had thyroid-hormone replacement at baseline and during follow-up 2) excluding studies that included transient ischemic attack as a stroke event 3) excluding studies with self-reported stroke data 4) excluding studies that did not meet the proportional hazard assumption 5) excluding cohorts with potential co-morbidities (e.g. diabetes patients) and 6) excluding studies without formal adjudication procedures. We also conducted additional multivariable analyses including prevalent atrial fibrillation, prevalent cardiovascular disease, body mass index (BMI) or lipid-lowering, and anti-hypertensive therapy at baseline to the previous multivariable model. Furthermore, we performed the following methodological sensitivity analyses: 1) perform the meta-analysis in a two-step approach using the restricted maximum-likelihood estimator also using the metafor package and 2) calculate the risk estimates using a one-step frailty Cox proportional hazards model (coxme package, R-project, Institute for Statistics and Mathematics, R Core Team (2013), Vienna, Austria, version 3.0.2.) We assessed age- and sex-adjusted funnel plots and conducted Egger tests [33] to evaluate potential publication bias statistically. There was no specific funding for this study.

RESULTS

We identified 17 cohorts from the United States [17,21,34], Europe [18,20,24,27,28,35-40], Australia [25], Brazil [41] and Japan [19] that assessed stroke outcomes prospectively and agreed to share IPD (Table 1). The included studies provided information on a total of 43,598 participants with thyroid function within the reference range and a follow-up from 1972 to 2014, a median follow-up ranging between 1.5 and 20 years and a total follow-up of 450,684 person-years. All studies, except one [34], included both female (49.6%) and male participants. All cohorts reported fatal stroke and 12 studies reported both fatal and non-fatal stroke, contributing to the all stroke

analyses among 34,853 participants. During follow-up, 2271 participants had a stroke, with an incidence rate of 6.3 per 1000 person-years and 907 a fatal stroke with 2.0 per 1000 person-years. The FT4 analyses included 24,888 participants for all stroke and 32,580 for fatal stroke. Two studies [25,39] used variations of the WHO criteria to define all stroke and fatal stroke (Supplemental Table 1) and four studies included information on type of stroke (hemorrhagic versus ischemic).[17,21,28,40] One study[39] used questionnaires for the assessment of non-fatal stroke. Formal adjudication, defined as having clear criteria for the outcomes that were reviewed by experts for each potential case, was used for all stroke in six studies [17,21,28,36,42,43] and for fatal stroke in two additional studies.[34,38]

All but three cohorts had information on participants' race.[18,24,25] For the additional multivariate analyses, information on AF at baseline was available for eight studies.[17,18,21,25,35,36,39,40,42] Data on lipid-lowering and hypertensive medications were not available in all but two studies.[19,24] Data on history of cardiovascular disease were not available for two studies.[34,35]

All studies provided information on the proportion of participants taking thyroid hormone medication at baseline. In all but four cohorts, none of the participants used thyroid medication at baseline. In the cohorts where thyroid medication was used, the proportion varied from 1 to 6%. All but six studies also provided follow-up information on thyroid hormone replacement use, with a range between 0 and 3%.

Table 1 Baseline Characteristics of Individuals in the Included Studies (n = 43,598)

Study, Year (Reference)	Description of Study Sample	No.	Median Age (Range), years*	Women No. (%)	Thyroid Med No. (%) at baseline†	Thyroid Med No. (%) follow up‡	TSH, Median (IQR)	FT4 Mean (SD) §	Follow-up median (IQR)	Person years
4D Study, 1998, (18)	Trial of atorvastatin in DMII and hemodialysis patients, Germany	841	66 (30-83)	368 (43.8)	0	11 (1.3)	1.10 (0.77-1.60)	13.90 pmol/L (2.92)	1.5 (0.2-3.6)	1666
Birmingham Study, 1988, (20)	CDA's aged ≥ 60 y from primary care practice in Birmingham, England	1015	69 (60-94)	550 (54.2)	0	NA	1.60 (1.10-1.20)	NA	10.2 (5.7-10.6)	8301
Brazilian Thyroid Study, 1999, (41)	Adults from Japanese descent living in São Paulo, Brazil	890	56 (30-92)	459 (51.6)	0	NA	1.40 (0.90-2.20)	1.07 ng/dL (0.18)	7.3 (7.1-7.5)	6274
Busselton Health Study, 1981, (25)	Adults in Busselton, Western Australia	1902	50 (18-90)	912 (47.9)	0	11 (0.6)	1.42 (1.00-1.96)	16.35 pmol/L (2.89)	20.0 (19.9-20.0)	33,825
Cardiovascular Health Study, 1989, (17)	CDA's with Medicare eligibility in 4 US communities	2526	71 (64-100)	1488 (58.9)	0	52 (2.1)	2.05 (1.45-2.89)	NA	14.1 (8.6-16.4)	31,099
EPIC-Norfolk Study, 1995, (24)	Adults living in Norfolk, England	11,986	58 (40-78)	6365 (53.1)	0	NA	1.70 (1.20-2.30)	12.58 pmol/L (3.17)	13.4 (12.6-14.3)	153,766
Health ABC Study, 1997, (21)	CDA's with Medicare eligibility, 2 US communities	2170	74 (69-81)	1033 (47.6)	0	37 (1.7)	2.00 (1.37-2.72)	NA	11.8 (7.5-12.2)	21,057

Table 1 Baseline Characteristics of Individuals in the Included Studies (n = 43,598) (continued)

Study, Year (Reference)	Description of Study Sample	No.	Median Age (Range), years*	Women No. (%)	Thyroid Med No. (%) at baseline†	Thyroid Med No. (%) follow up‡	TSH, Median (IQR)	FT4 Mean (SD) §	Follow-up median (IQR)	Person years
InCHIANTI Study, 1998, (35)	Adults aged 20-102 years living in Chianti geographic area, Italy	1049	71 (21-102)	575 (54.8)	11 (1.0)	NA	1.38 (0.96-1.98)	1.42 ng/dL (0.29)	9.1 (8.2-9.2)	8435
Leiden 85-plus Study, 1997, (36)	Adults aged 85 years living in Leiden, The Netherlands	452	85 (NA)	290 (60.4)	0	6 (1.3)	1.65 (1.15-2.31)	14.5 pmol/L (2.26)	5.2 (2.5-8.5)	2555
MrOS Study, 2000, (34)	Community-dwelling U.S. men aged 65 years and older	1410	73 (65-99)	0	83 (5.9)	NA	1.97 (1.36-2.72)	0.99 ng/dL (0.15)	12.0 (8.5-12.7)	14,541
Nagasaki Adult Health Study, 1984, (19)	Atomic bomb survivors in Nagasaki, Japan	2342	57 (38-92)	1419 (60.6)	27 (1.2)	NA	2.60 (2.00-3.40)	1.45 ng/dL (0.46)	13.0 (12.3-13.7)	28,574
Pisa cohort, 2000, (38)	Patients admitted to cardiology department in Pisa, Italy II	2695	63 (19-92)	840 (31.2)	0	0	1.53 (1.02-2.30)	1.19 ng/dL (0.24)	2.6 (1.6-3.8)	7326
PREVEND Study, 1997, (37)	Adults living in Groningen, The Netherlands	2493	46 (28-75)	1255 (50.3)	0	4 (0.2)	1.37 (0.99-1.90)	12.81 pmol/L (2.25)	10.9 (10.6 - 11.1)	24,621
PROSPER trial, 1997, (28)	Trial on the benefits of pravastatin vs placebo in adults	4953	75 (69-83)	2403 (48.5)	0	28 (0.6)	1.80 (1.26-2.51)	NA	3.3 (3.0 - 3.5)	15,937

Table 1 Baseline Characteristics of Individuals in the Included Studies (n = 43,598) (continued)

Study, Year (Reference)	Description of Study Sample	No.	Median Age (Range), years*	Women No. (%)	Thyroid Med No. (%) at baseline†	Thyroid Med No. (%) follow up‡	TSH, Median (IQR)	FT4 Mean (SD) §	Follow-up median (IQR)	Person years
Rotterdam Study, 1989 (40)	Adults ≥55 years living in Rotterdam, The Netherlands	1577	68 (55-93)	934 (59.2)	0	NA	1.54 (1.06-2.26)	16.29 pmol/L (2.93)	17.0 (11.2 - 18.9)	23,217
SHIP Study, 1997 (39)	Adults in West Pomerania, North-East of Germany	2977	47 (20-81)	1476 (49.6)	0	90 (3.0)	0.79 (0.61-1.07)	12.67 pmol/L (3.42)	11.3 (10.6 - 11.8)	32,238
Whickham Survey **, 1974 (27)	Adults living in and near Newcastle upon Tyne, England	2320	46 (18-92)	1213 (52.3)	92 (4.0)	54 (2.3)	2.10 (1.20-3.00)	8.41 pmol/L (1.95)	19.0 (15.8-20.0)	37,252
Overall		43,598	64.9 (18-102)	21,580 (49.6)	213 (0.5)	293 (1.4)	1.65 (1.10-2.40)	13.6 pmol/L (2.6)	11.6 (5.1-13.9)	450,684

Abbreviations: CDA = community-dwelling adult; DM II = type 2 diabetes, IQR = interquartile range (25th-75th percentile); NA = not applicable; FT4 = free thyroxine; TSH = thyroid-stimulating hormone.

* Participants younger than 18 years of age were not included

† Participants with missing information on thyroid medication at baseline: Health ABC Study 7, MrOs Study 59, Rotterdam Study 463, Whickham Survey 3

‡ Participants with missing information on thyroid medication at follow-up: Whickham Survey 1430

§ 1 pmol/L is 0.0777 ng/dL

II Excluded patients with acute coronary syndrome or severe illness

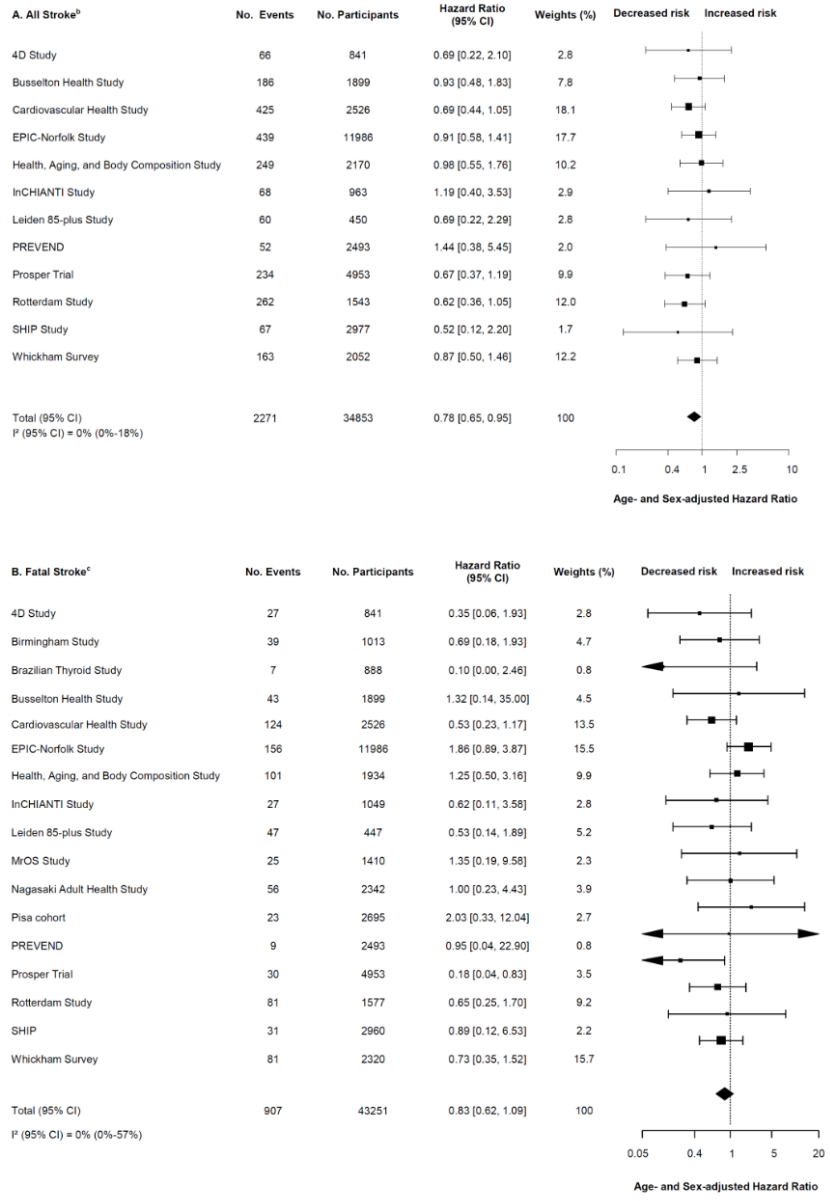
**The Whickham Survey used a first-generation assay for the measurement of TSH and did not measure FT4 but total T4.

The association between TSH and the risk of stroke

The age- and sex-adjusted pooled HR for all stroke was 0.78 (95% CI, 0.65-0.95, across the reference range of TSH mIU/L) and for fatal stroke 0.83 (95% CI, 0.62-1.09) (Figure 1). This corresponds to a 1.28-fold and 1.20-fold increase in all and fatal stroke risk respectively for a participant with a TSH in the lower limit of the reference range (0.45 mIU/L) compared to a participant with a TSH in the upper limit of the reference range (4.49 mIU/L).We found no heterogeneity for the analyses of all stroke or fatal stroke analyses (I^2=0%). Multivariable analyses, adjusting for sex, age, smoking, total cholesterol, systolic blood pressure and history of diabetes yielded similar results with a HR of 0.76 (95% CI, 0.63-0.91) for all stroke and 0.78 (95 % CI, 0.58-1.07) for fatal stroke (Table 2).

Subsequent subgroup analyses did not show a differential risk when stratifying by sex, age groups, history of stroke or race (Table 2). The information on type of stroke was available in a subgroup of 11,192 participants in four studies [17,21,28,40]. Stratifying by type of stroke showed a lower estimate in hemorrhagic fatal stroke compared to ischemic stroke (HR 0.37, 95% CI 0.12-1.12 vs HR 0.78, 95% CI 0.33-1.80), but with an insignificant p for interaction (p= 0.30). Sensitivity analyses excluding specific studies or participants using thyroid hormone replacement therapy did not meaningfully affect the risk estimates (Supplemental Table 2). Additional adjustment for prevalent atrial fibrillation, prevalent cardiovascular disease (defined as previous coronary heart disease or stroke), BMI or lipid-lowering and anti-hypertensive therapy did not attenuate the associations. Estimates derived by the methodological sensitivity analyses were similar to the results of the two-step random-effects model according to DerSimonian and Laird (Supplemental Table 3). We did not find any evidence of publication bias for the TSH analyses, either with visual assessment of age- and sex-adjusted funnel plots or with the Egger test for all stroke (p = 0.75) or fatal stroke (p = 0.29).

Figure 1 The association between TSH and risk of all stroke and fatal stroke*



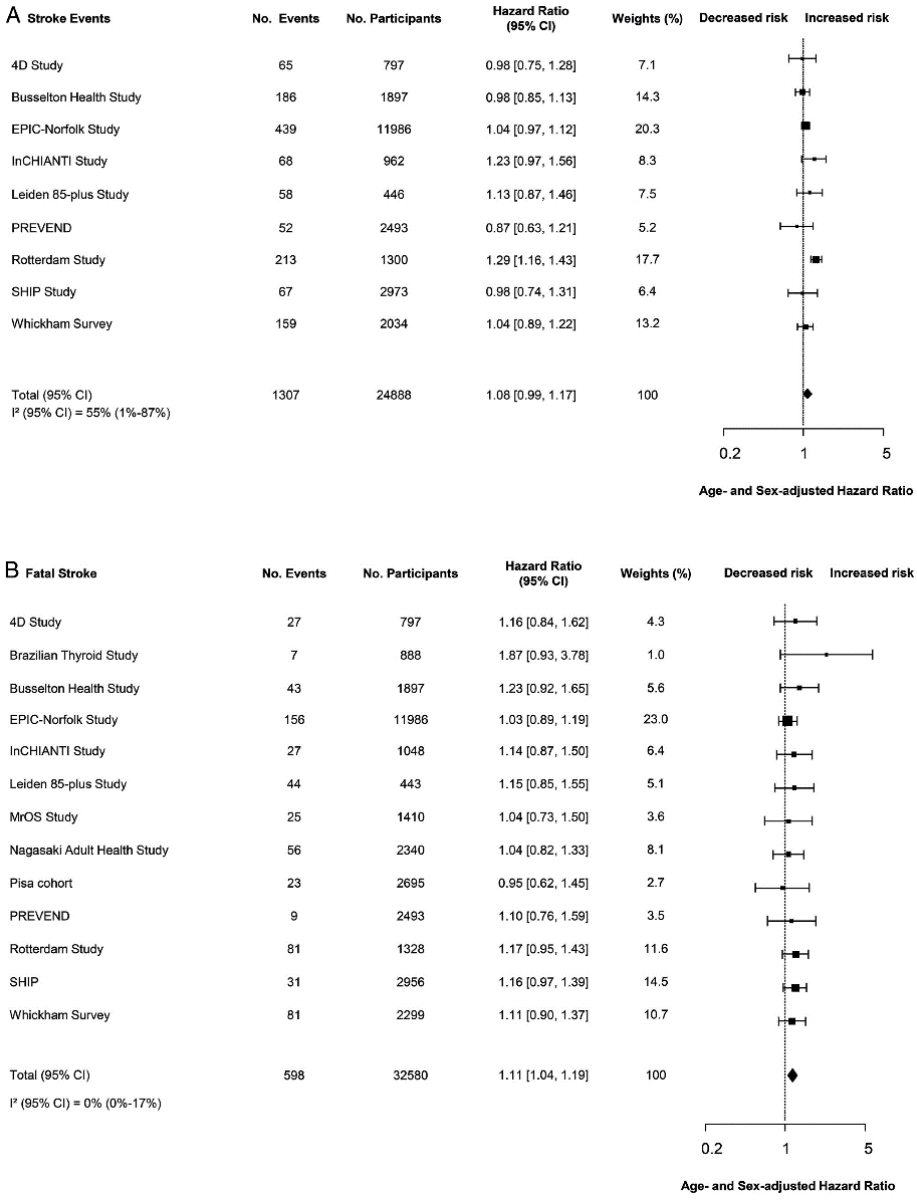
* Hazard ratios (HRs) and their 95% confidence intervals (CIs) are represented by squares across the reference range of TSH (0.45 and 4.49 mIU/L). Sizes of data markers are proportional to the inverse of the variance of the hazard ratios. Data for all stroke were available in 12 studies. Three hundred ninety-three participants were excluded from the analysis of all stroke due to missing follow-up data. Data for fatal stroke were available in 17 studies. Two hundred sixty-five participants were excluded from the analysis of fatal stroke, due to missing cause of death. Abbreviations: TSH = thyroid-stimulating hormone.

Table 2 Stratified analyses for the associations between TSH and the risk of all Stroke and fatal stroke

		All Stroke*				Fatal Stroke†			
		No. events/ Total	Age&sex adjusted HR (95% CI)	Multivariable‡ HR (95% CI)	I^2	No. events/ Total	Age&sex adjusted HR (95% CI)	Multivariable‡ HR (95% CI)	I^2
Total Population		2271/34,853	0.78 (0.65, 0.95)	0.76 (0.63, 0.91)	0%	907/43,333	0.83 (0.62, 1.09)	0.78 (0.58, 1.07)	0%
Sex§	Men	1091/16723	0.80 (0.62, 1.07)	0.78 (0.60, 1.02)	0%	422/21874	0.85 (0.50, 1.41)	0.85 (0.50, 1.35)	0%
	Women	1180/18130	0.78 (0.58, 1.07)	0.75 (0.55, 1.02)	25%	485/21459	0.80 (0.52, 1.25)	0.80 (0.52, 1.22)	12%
	p for interaction		0.90	0.85			0.86	0.85	
Age II	18. – 49	60/8305	0.95 (0.31, 2.86)	1.45 (0.37, 4.17)	0%	12/9,525	0.71 (0.07, 7.47)	1.14 (0.06, 23.85)	0%
	50 – 64	358/9145	0.75 (0.47, 1.19)	0.75 (0.47, 1.22)	0%	104/12,303	1.35 (0.55, 3.25)	1.22 (0.48, 3.16)	0%
	65 -79	1588/15,667	0.83 (0.67, 1.05)	0.80 (0.63, 1.00)	0%	623/19,198	0.89 (0.62, 1.27)	0.95 (0.85, 1.09)	0%
	≥80	265/1736	0.69 (0.40, 1.17)	0.63 (0.36, 1.09)	0%	168/2,307	0.43 (0.22, 0.85)	0.36 (0.17, 0.78)	0%
	p for trend		0.66	0.28			0.61	0.43	
Stroke history¶	No	1875/31,626	0.78 (0.63, 0.98)	0.75 (0.60, 0.93)	0%	710/36,222	0.71 (0.47, 1.07)	0.71 (0.47, 1.05)	24%
	Yes	206/1266	1.00 (0.53, 1.83)	1.14 (0.60, 2.20)	0%	92/1440	0.83 (0.26, 2.50)	1.58 (0.47, 5.27)	20%
	p for interaction		0.47	0.23			0.80	0.22	
Stroke type**	Hemorrhagic	129/11,192	0.47 (0.26, 0.83)	0.47 (0.25, 0.89)	5%	87/11,192	0.38 (0.14, 1.07)	0.37 (0.12, 1.12)	27%
	Ischemic	817/11,192	0.71 (0.50, 1.00)	0.69 (0.48,0.98)	0%	182/11,192	0.69 (0.34, 1.35)	0.78 (0.33-1.80)	0%
	p for interaction		0.24	0.30			0.34	0.30	
Race‡‡	White	1430/19,037	0.76 (0.60, 0.95)	0.73 (0.58, 0.91)	0%	520/23,213	0.71 (0.48, 1.02)	0.67 (0.47 1.00)	0%
	Asian	NA	NA	NA		63/3230	0.48 (0.06, 11.22)	0.62 (0.10, 3.97)	41%
	Black	150/1090	0.85 (0.26, 2.78)	0.91 (0.41, 1.99)	47%	59/1055	0.95 (0.30, 3.12)	0.89 (0.26, 2.91)	0%
	p for interaction		0.88	0.60			0.83	0.91	

Abbreviations: CI, confidence interval; HR, hazard ratio; TSH, thyroid-stimulating hormone. HR's across the reference range of TSH mIU/L (0.45-4.49). *Data available from 12 studies, 393 participants excluded due to missing stroke event data. † 265 participants excluded due to missing data on cause of death. ‡ Adjusted for sex, age, systolic blood pressure, total cholesterol, smoking and prevalent diabetes at baseline. The Birmingham Study excluded due to lack of cardiovascular risk factor data. § Aalyses not adjusted for sex. II HRs adjusted for sex and age as continuous variable to avoid residual confounding within strata. ¶ Information on history of stroke not available for the Pisa cohort, Birmingham Study and Busselton Health Study. Data on history of stroke were missing for 64 participants in total. ** Information on type of stroke was available for the Cardiovascular Health Study, Health ABC Study, PROSPER and the Rotterdam Study. ‡‡Information on was not available for 4D study, Birmingham study. Busselton Health Study and EPIC-Norfolk Study. Excluded 96 participants from MrOs Study due to no events in subgroup.

Figure 2 The association between standardized FT4 and risk of all stroke and fatal stroke*



* Hazard ratios (HRs) and their 95% confidence intervals (CIs) are represented by squares and are per one increase of one standard deviation of FT4. Sizes of data markers are proportional to the inverse of the variance of the hazard ratios. Data for all stroke were available in 9 studies. Three hundred eighty-seven participants were excluded from the analysis of all stroke due to missing follow-up data. Data for fatal stroke were available in 13 studies. Twenty-seven participants were excluded from the analysis of fatal stroke, due to missing cause of death. Abbreviations: FT4 = free thyroxine

Table 3 Stratified analyses for the associations between standardized FT4 and the risk of all stroke and fatal stroke*

		All Stroke†				Fatal Stroke‡			
		No. events/ Total	**Age & Sex adjusted HR (95% CI)**	**Multivariable § HR (95% CI)**	**I^2**	**No. events/ Total**	**Age & Sex adjusted HR (95% CI)**	**Multivariable § HR (95% CI)**	**I^2**
Total		1307/24,888	1.08 (0.99, 1.17)	1.06 (0.99, 1.15)	55%	598/32,580	1.10 (1.04, 1,19)	1.09 (1.02, 1.18)	0%
Sex II	Men	639/11,848	1.02 (0.94, 1.11)	1.00 (0.92, 1.08)	0%	284/16,651	1.10 (0.99, 1.24)	1.08 (0.96, 1.21)	0%
	Women	668/13,040	1.10 (0.99, 1.22)	1.10 (1.01, 1.20)	52%	314/15,929	1.12 (1.03, 1.23)	1.12 (1.01, 1.24)	0%
	p for interaction		0.27	0.12			0.79	0.65	
Age¶	18 – 49y	59/8289	0.81 (0.61, 1.07)	0.75 (0.55, 1.03)	0%	12/9507	1.50 (0.62, 3.67)	0.93 (0.32, 2.71)	36%
	50 – 64y	342/9019	1.03 (0.93, 1.29)	1.03 (0.84, 1.27)	66%	99/11,929	1.09 (0.88, 1.35)	1.06 (0.84, 1.32)	0%
	65 -79y	759/6803	1.12 (1.05, 1.19)	1.10 (1.04, 1.17)	0%	376/9897	1.11 (1.01, 1.22)	1.09 (0.99, 1.21)	0%
	≥80	147/777	1.15 (0.98, 1.35)	1.15 (0.96, 1.38)	0%	111/1247	1.12 (0.94, 1.33)	1.09 (0.89, 1.33)	0%
	p for trend		0.024	0.015			0.54	0.76	
Stroke history**	No	1013/22,446	1.06 (0.95, 1.18)	1.05 (0.95, 1.15)	58%	472/27,256	1.10 (1.02, 1.19)	1.09 (1.00, 1.18)	0%
	Yes	104/483	1.11 (0.95, 1.29)	1.12 (0.95, 1.32)	0%	60/668	1.07 (0.78, 1.45)	1.15 (0.77, 1.73)	26%
	p for interaction		0.64	0.51			0.87	0.80	
Stroke type‡‡	Hemorrhagic	17/1577	1.37 (0.82-2.29)	1.15 (0.64-2.07)	NA	10/1577	1.15 (0.63-2.12)	1.01 (0.49, 2.07)	NA
	Ischemic	157/1577	1.30 (1.14-1.47)	1.20 (1.06-1.37)	NA	39/1577	1.00 (0.71-1.41)	0.90 (0.62-1.28)	NA
	p for interaction		0.84	0.88			0.70	0.77	
Race§§	White	617/10,208	1.12 (0.99, 1.26)	1.11 (0.99 1.23)	51%	319/14,528	1.13 (1.03, 1.23)	1.10 (1.00 1.21)	0%
	Asian	NA	NA	NA	NA	63/3228	1.27 (0.74, 2.18)	1.27 (0.74, 2.18)	58%
	Black	NA	NA	NA	NA	2/48	0.94 (0.23, 3.88)	1.00 (0.14, 7.09)	NA
	p for interaction		NA	NA			0.89	0.87	

Abbreviations: CI, confidence interval; HR, hazard ratio; FT4, free thyroxine. NA, not applicable. HRs are per one increase in FT4 SD. *The Whickham Survey measured total T4. † Data vailable from 12 studies, 384 participants excluded due to missing stroke event data. ‡27 participants excluded due to missing data on death cause. § Adjusted for sex, age, systolic blood pressure, total cholesterol, smoking and prevalent diabetes at baseline. II Not adjusted for sex. ¶ HRs adjusted for sex and age as continuous variable to avoid residual confounding. ** Information on stroke history not available for Pisa cohort, Birmingham Study and Busselton Health Study. Data on stroke history were missing for 64 subjects. ‡‡ Information on type of stroke available for Rotterdam Study. §§Information on race not available for 4D study, Busselton Health Study and EPIC-Norfolk Study. Excluded 96 participants from MrOs Study due to no events in subgroup.

The association between FT4 and the risk of stroke

The age- and sex-adjusted pooled HR for the per SD increase of FT4 and stroke analyses were 1.08 (95% CI, 0.99-1.15) for all stroke and 1.10 (95% CI, 1.04-1.19) for fatal stroke (Table 3, Figure 2). We found substantial heterogeneity for the analyses on all stroke (I^2=55%) but no heterogeneity for fatal stroke (I^2=0%,). When analyzing the association per 1 ng/dL FT4 increase and risk of stroke, the age- and sex-adjusted pooled HRs were 1.40 (95% CI, 0.95-2.05) for all stroke and 1.44 (95% CI, 1.10-1.89) for fatal stroke (Supplemental Table 4). Multivariable analyses, adjusting for sex, age, smoking, total cholesterol, systolic blood pressure and history of diabetes did not change risk estimates substantially (Table 3).

Subsequent subgroup analyses showed a differential risk for the different age categories, where the risk estimates went from protective to deleterious with increasing age (p for trend 0.024, Table 3). When stratifying by sex, history of stroke or race no differential effects were detected. Stratifying for type of stroke also did not show differential risk (Table 3), but this was only possible in one study that was included in the FT4 analyses. We did not find any evidence of publication bias for the FT4 and stroke analyses, either with visual assessment of age- and sex-adjusted funnel plots or with the Egger test for all stroke (p = 0.41) or for fatal stroke (p = 0.28).

DISCUSSION

In the current IPD analysis of 43,598 participants from 17 prospective cohort studies, higher levels of TSH within the reference range of thyroid function were significantly associated with a lower risk of stroke in age- and sex-adjusted and in multivariable analyses. The analyses concerning the association between TSH levels and fatal stroke were qualitatively similar but did not reach statistical significance. The analyses on the association between FT4 and all stroke and fatal stroke support the finding of a higher risk of stroke with differences within the reference range of thyroid function.

Thyroid dysfunction is defined by the biochemical reference ranges for TSH and FT4. These reference ranges, defining the normal range, depend on the assay

used, the distribution of thyroid measurements in the population, or both. A thyroid function within the "normal range" would imply that the levels of circulating thyroid hormone are not accompanied by symptoms, an increased risk of disease or adverse events. In recent years, the applied reference ranges have been debated in the context of mainly the latter two: adverse events and diseases. Higher levels of TSH within the reference range are associated with an increase in systolic and diastolic blood pressure.[44,45] Moreover, increased TSH levels within the reference range are linearly associated with an unfavorable serum lipid profile.[46] On the other hand, lower TSH levels within the reference range are associated with an increased risk of heart failure, coronary heart disease and atrial fibrillation in an elderly population.[7] The arbitrary nature of the cut-offs currently used is an important factor hampering decision making on screening and treatment of thyroid dysfunction.[13] In the context of defining the reference range of thyroid function, our study provides additional evidence that lower levels of TSH and higher levels of FT4 within the reference range are associated with a negative clinical outcome, namely stroke, a major cause of morbidity and mortality. In contrast to blood pressure or cholesterol, reference ranges for thyroid function are currently based on distribution in the population rather than risks of major diseases. It is more challenging to establish reference ranges for thyroid function based on risk of outcomes than for cardiovascular risk factors such as blood pressure and cholesterol, where the increase in risk mainly occurs for values higher than the upper limit. However, both low and high thyroid function is associated with clinical disease, also within the reference range. Furthermore, a previous study from the TSC provided no evidence for a higher risk of coronary heart disease within the normal reference range as currently defined[10]. Also, thyroid function is not solely associated with cardiovascular disease but also a wide variety of clinical outcomes including fracture risk and possibly cognitive function decline.[7,14] Therefore, future research should investigate if re-evaluation of the currently used reference ranges for thyroid function is meaningful, and if so, to what extent this should be done for specific populations or subgroups (e.g. elderly).

Several pathways could explain the relation between thyroid function and stroke. Thyroid hormone has direct effects on the cardiovascular system and is known to decrease systemic vascular resistance[47], increase left ventricular contractile

function and alter systolic and diastolic cardiac function.[48] Differences in thyroid hormone function are associated with the risk of several cardiovascular risk factors including hypertension, [49] dyslipidemia [50] and atherosclerosis.[51] These changes have also been reported in subjects with subclinical thyroid dysfunction [42] and some also with differences of thyroid function within the reference range.[44-46] The fact that adjustment for these cardiovascular risk factors in our multivariable analyses did not substantially alter risk estimates, suggests an effect on the risk of stroke, which is independent of classical risk factors such as hypertension.

Another explanation might be that the lack of effect of multivariable adjustment is due to residual confounding or unmeasured mediators. For example, in the current analysis, additional adjustment for atrial fibrillation, a plausible biological mediator for the association between thyroid function and the risk of stroke [52], did not alter risk estimates substantially. However, detecting an effect may have been hampered by the lack of information on prevalent atrial fibrillation in nine studies and insufficient incidence information. There was no sufficient information available on anti-coagulant medication use of participants, which did not allow for further exploration of possible mediating and confounding effects.

Various abnormalities in the hemostatic system have been reported in overt [53] and subclinical thyroid dysfunction.[54] Hypercoagulability is seen in hyperthyroidism while hypothyroidism has been associated with mainly hypocoagulability.[55,56] Alterations in coagulability and the fibrinolytic system have been linked to a higher risk of cardiovascular disease.[57] Whether hemostasis is also affected within the reference range of thyroid function is not known but might be one of the pathways that play a role in the increased risk of stroke associated with differences in thyroid function within the reference range. Changes in coagulation patterns due to thyroid hormone could imply that thyroid function tending towards hyperthyroidism might increase the risk of ischemic stroke mainly. We only had a small subgroup of studies including information on type of stroke (hemorrhagic vs ischemic), limiting our analysis on type of stroke. The exact mechanism explaining the association between differences in thyroid function within the reference range and the risk of stroke therefore remains to be determined.

Previous studies have reported that the association of thyroid dysfunction with the risk of cardiovascular disease is influenced by age or sex. A study on the

association of thyroid disorders and stroke found a decreased risk of ischemic stroke in treated male patients with thyroid disorders, but not in females.[58] A study level meta-analysis found that subclinical hypothyroidism was associated with increased risk of ischemic heart disease and cardiovascular mortality only in younger populations.[59] In line, a study in participants of 85 years in the general population, revealed no adverse effects of abnormally high levels of TSH.[36] In contrast, an IPD meta-analysis of 55 287 participants did not show significant trend in risk of CHD across different age groups.[1] In our study, stratification by age, sex and race did not reveal differential risk patterns. It should however be noted that no study to date has looked at the association of thyroid function within the reference range and stroke by age or sex and this could be one of the reasons for the discrepancies found between previous literature and our study.

The association of TSH with the risk of stroke in participants without a prior history of stroke was similar to the overall analyses, while in participants with a prior stroke, the association was not present. The total number of participants with a history of stroke was small and therefore, the power to detect a possible differential risk between participants with and without history of stroke could have been limited. The risk of all stroke associated with FT4 levels seemed to increase with older age. However, this finding was not replicated in the TSH or fatal stroke analyses.

Strengths of our study include the ability to perform an IPD analysis including 43,598 participants from 17 studies, based on published and unpublished data. By performing an IPD analysis we were able to standardize the definition of reference range thyroid function and covariates within our study for the analyses. There were differences between the study populations regarding age and sex distribution, amongst others. Nevertheless, there was limited to no heterogeneity of the outcome estimates between the studies. This could indicate the robustness of the findings.

Despite the large number of participants, we had limited numbers of events in those with a history of stroke and only four studies included data on type of stroke. Information needed for certain stratification and sensitivity analyses e.g. by race or prevalent atrial fibrillation was not available for some cohorts. Also, there was no information available on anti-coagulant use or anticoagulant factor levels, hampering analyses concerning possible underlying pathways. Furthermore, TSH

and FT4 measurements were performed only at baseline and data on thyroid medication use during follow-up were not complete, which could change risk over time, in almost all cohorts and therefore it was not possible to take changes of thyroid function over time into account. Residual confounding cannot be excluded, as is the case in all observational studies.

Conclusions

In summary, higher TSH levels within the reference range were associated with a lower risk of all stroke. The analyses for fatal stroke and FT4 were qualitatively similar. These data provide additional evidence that differences within the reference range of thyroid function, as currently defined, are associated with an increased risk of a major adverse event. Future studies should investigate if re-evaluation of the currently used reference ranges for thyroid function, which are based on fixed biochemical parameters instead of health and treatment outcomes and risk of disease and mortality, should be considered. This is pivotal information when designing randomized controlled trials sufficiently equipped to address possible risks and benefits of thyroid function treatment.

Online supplemental material

https://academic.oup.com/jcem/article-abstract/101/11/4270/2765001/Thyroid-Function-Within-the-Reference-Range-and?redirectedFrom=fulltext

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CHAPTER 3.4

THE ASSOCIATION BETWEEN THYROID FUNCTION AND DEMENTIA

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ABSTRACT

BACKGROUND Data are conflicting regarding the association of thyroid function with dementia risk and the possible underlying pathophysiological mechanisms. We therefore aimed to study the role of thyroid function in dementia, cognitive function, and subclinical vascular brain disease using MRI.

METHODS Analyses were performed within the Rotterdam Study (baseline 1997), a prospective population-based cohort. We evaluated the association of thyroid-stimulating hormone (TSH) and free thyroxine (FT4) with incident dementia using Cox models adjusted for age, sex, cardiovascular risk factors, and education. Absolute risks were calculated accounting for death as a competing risk factor. Associations of thyroid function with cognitive test scores and subclinical vascular brain disease (white matter lesions, lacunes, and mircobleeds) were assessed using linear or logistic regression. Additionally, we stratified by sex and restricted analyses to normal thyroid function.

RESULTS We included 9,446 participants with a mean age of 65 years. During follow-up (mean 8.0 years), 601 participants had developed dementia. Higher TSH was associated with lower dementia risk in both the full and normal ranges of thyroid function, Hazard Ratios (HR) 0.90 (95% Confidence Interval [CI], 0.83-0.98) and 0.76 (CI, 0.64-0.91), respectively. This association was independent of cardiovascular risk factors. Dementia risk was higher in individuals with higher FT4 (HR, 1.04; CI, 1.01-1.07). Absolute 10-year dementia risk decreased from 15% to 10% with higher TSH in older women. Higher TSH was associated with better global cognitive scores ($p = 0.021$). Thyroid function was not related to subclinical vascular brain disease as indicated by MRI.

CONCLUSIONS High and high-normal thyroid function are associated with increased dementia risk. Thyroid function is not related to vascular brain disease as assessed by MRI, suggesting a role for thyroid hormone in non-vascular pathways leading to dementia.

INTRODUCTION

Dementia is one of the leading causes of morbidity and mortality and its underlying etiology is multifactorial[1,2]. Vascular disease and vascular risk factors have been of particular interest in recent years as modifiable risk factors for dementia[3].

Thyroid hormone plays key roles in most organs, including the brain, with its most profound effects occurring during perinatal development. However, it also influences neurogenesis during adulthood[4]. Thyroid hormone has well-known effects on the cardiovascular system[5], and both hypothyroidism and hyperthyroidism are risk factors linked to cardiovascular disease[5,6]. These associations have also been reported for variations within the normal range of thyroid function[7].

Several studies have investigated the association between thyroid function and dementia[8-18]. To date, most studies investigating the association between thyroid function and dementia have been cross-sectional[8,9,18], or have had several other limitations, including small sample sizes [10-14,17]. Most importantly, the risk of dementia across the continuous range of thyroid function, including the normal range, has not been investigated. Moreover, the relationship between thyroid function and MRI markers of vascular brain disease, a plausible pathophysiological pathway through which thyroid function may influence dementia [19-21], is still unknown.

The main objective of this study was to determine the association between thyroid function and dementia risk and to investigate the relationship between thyroid function and cognitive function and MRI markers of vascular brain disease.

METHODS

Study participants

All analyses were performed on data from the Rotterdam Study (RS), a prospective population-based cohort study that investigates the determinants and occurrences of cardiovascular, neurological, ophthalmologic, psychiatric, and endocrine diseases in the middle-aged and the elderly. The aims and the design of RS have been described in detail elsewhere[22].

For the current study, we included participants from the third visit of RSI (n = 4,797) and the baseline visits of RSII (n = 3,011) and RSIII (n = 3,932). Baseline TSH or FT4 measurements (from 1997) were available in 9,702 of the above participants. We excluded 116 participants due to prevalent dementia, 133 due to missing information on dementia prevalence, and 7 due to missing follow-up information, leaving a total of 9,446 participants. All study participants were followed up from the day of the baseline laboratory testing to the date of dementia onset, death, or until December 31st, 2013, whichever came first.

Standard protocol approvals, registrations, and patient consents

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

Assessment of thyroid function

Thyroid function was assessed using measurements of TSH and FT4 (using the electrochemiluminescence immunoassay for thyroxine and thyrotropine, "ECLIA", Roche) in serum samples stored at -80°C. We used 0.4-4.0 mIU/L as the reference range for normal TSH levels and 11-25 pmol/L (= 0.85-1.95 ng/dL) as the reference range for normal FT4 levels, according to national guidelines and our previous studies[23,24]. Thyroid peroxidase antibody (TPOAb) levels >35 kU/mL were regarded as positive, as recommended by the assay manufacturer (electrochemiluminescence immunoassay for thyroid perioxidase antibodies, "ECLIA", Roche).

Dementia screening

Participants were screened for dementia at baseline and in follow-up examinations using a three-step protocol[25]. Screening was performed using the Mini-Mental State Examination (MMSE) and the Geriatric Mental Schedule (GMS) organic level. Those with an MMSE score <26 or a GMS >0 subsequently underwent an examination and an informant interview using the Cambridge Examination for Mental Disorders in the Elderly (CAMDEX). Additionally, the total cohort was continuously monitored for dementia through a computerized linkage of medical

records from general practitioners and the regional institute for outpatient mental healthcare with the study database. Available neuroimaging data were used when required for establishing a diagnosis. For all suspected cases of dementia, a consensus panel led by a consultant neurologist (PJK) decided on the final diagnosis in accordance with standard criteria for dementia (DSM-III-R) and Alzheimer's disease (NINCDS-ADRDA).

Cognitive function assessment

Cognitive function was assessed in detail using a test battery comprised of the Stroop test (time in seconds taken for completing each of three tasks: word reading, color naming, and a reading/color naming interference task), the letter-digit substitution task (number of correct digits in 1 minute), the verbal fluency test (number of animal species within 1 minute), the word learning test (recall and recognition of visually-presented words), and the Purdue pegboard test for fine motor skills (pairs of pins placed with both hands in 30 seconds)[26]. For each participant, z-scores were calculated for each test separately by dividing the difference between individual test scores and the mean test score by the standard deviation. We calculated a general cognitive factor (g-factor) by performing principal component analysis incorporating the letter-digit substitution task, the verbal fluency test, the world learning test (average of z-scores of immediate and delayed recall), and the inverse of two components of the Stroop test in a total of 6,685 participants. The median age difference between thyroid function testing and cognitive testing was 3.9 years (interquartile range [IQR] 0.00-4.41 years). The g-factor explains 49.3% of all variance in cognitive tests, which is in accordance with previous literature[26].

MRI acquisition and processing

We obtained brain MRI scans using a 1.5-T scanner (GE Healthcare, Milwaukee, Wisconsin, USA) in RS from 2005 onwards. Details of the MRI protocol have been described extensively before[27]. In short, the protocol included a T1-weighted (T1w) sequence, a proton density-weighted (PDw) sequence, a fluid-attenuated-inversion-recovery (FLAIR) sequence, and a T2*-weighted gradient echo (GRE) sequence. No contrast material was administered. We used an automated brain tissue classification method based on a k-nearest-neighbor-classifier algorithm with an extended white matter lesion segmentation[28,29] to quantify intracranial volume

and white matter lesion volume (in cubic millimeters). We determined the presence of lacunar and cortical infarcts on the FLAIR, PDw, and T1w sequences, and the presence of cerebral microbleeds on the T2*GRE sequence according to our previously described protocol[27,30]. We included a total of 4,178 participants with thyroid function and MRI measurements with a median time between thyroid function testing and MRI assessment of 0.09 years (IQR 0.04-4.52 years).

Assessment of other variables

Blood pressure was measured at the right brachial artery using a random-zero sphygmomanometer after 5 minutes of rest with the participants in a sitting position. The mean of two consecutive measurements was used. Hypertension was defined as a systolic blood pressure ≥140 mmHg, a diastolic blood pressure ≥90 mmHg, or anti-hypertensive medication use at baseline. Total cholesterol was measured in fasting serum using an enzymatic method. Smoking information was derived from baseline questionnaires. The patients were categorized as never, previous, and current smokers. Body-mass index (BMI) was calculated using weight in kilograms divided by height in meters squared.

History of diabetes was defined by repeated impaired fasting glucose levels of ≥7 mmol/L, a non-fasting glucose level of ≥7 mmol/L (when fasting samples were absent), or the use of anti-glycemic medication at baseline. Educational level was assessed during a baseline home interview and the participants were classified into 7 categories ranging from low levels of education (primary only) through high education levels (university). Coronary heart disease at baseline was defined as a history of myocardial infarction, percutaneous coronary intervention, or coronary artery bypass graft or other cardiac revascularization procedures[31]. Atrial fibrillation was assessed using three methods: 1) ECGs at baseline and during follow-up, assessed by a cardiologist, 2) medical information obtained from general practitioners, after ascertainment of ECG, and 3) national registry of hospital discharge diagnosis[32]. Assessment of depressive symptoms was performed using the validated Dutch version of the Center for Epidemiological Studies Depression (CES-D) score[33]. Stroke was defined according to the World Health Organization criteria as a syndrome of rapidly developing clinical signs of focal or global disturbance of cerebral function with symptoms lasting 24 hours or longer or leading to death, with no apparent cause other than that of vascular origin[34].

Incident stroke cases were reviewed and verified by an experienced vascular neurologist (PJK) using hospital letters, and information from practitioners and nursing home physicians.

Statistical analysis

Detailed descriptions of the statistical analyses, including the models used and the procedure of testing assumptions, are shown in Supplemental Methods. We evaluated the association between thyroid function and dementia risk using a Cox proportional hazards model. For the analyses in the normal range of thyroid function, we excluded individuals with abnormal TSH and FT4 levels and those using thyroid hormone replacement therapy. We investigated the association between TSH or FT4 and dementia continuously, across the reference range, and in quartiles within the normal range. The results across the reference range correspond to the HRs when comparing the risks of participants with TSH or FT4 levels in the upper limit of the reference range to those with TSH or FT4 levels in the lower limit of the reference range. Pre-defined stratification by gender, age categories (cutoff age of 65 years), and type of dementia (Alzheimer's vs. other) was performed.

We estimated 10-year absolute risk probabilities using the covariates of the primary Cox proportional hazards model while taking the competing risk of death into account. We performed the following sensitivity analyses: 1) additionally adjusting for CES-D scores at baseline, 2) excluding the first 4 years of follow-up, and 3) censoring participants at time of stroke incidence.

We cross-sectionally assessed the association of TSH and FT4 levels with cognitive function tests, the g-factor, and white matter lesions using linear regression models. The association of thyroid function with lacunar infarcts and microbleeds was assessed using logistic regression. We performed multiple imputations for missing data of the covariates (<5% for all covariates). The proportional hazard and linearity assumptions were met for all analyses. The presented HRs and betas are per one unit increase in logTSH (mIU/L) and per one unit increase in FT4 (pmol/L).

RESULTS

We included a total of 9,446 participants (mean age of 64.9 years) in this study. Baseline characteristics are shown in Table 1. Of the included participants, five lacked TSH data, six lacked FT4 data, and 11 lacked TPO antibody measurements. For the normal range analyses, we only included participants with both TSH and FT4 measurements who were not using thyroid replacement therapy (n = 7,966). At a mean follow-up time of 8.0 years (IQR 5.5-10.7), corresponding to 74,209 person-years, 601 participants developed dementia, of which 487 were of Alzheimer's type. This corresponds to an incidence rate of 8 per 1,000 person-years.

Thyroid function and dementia

Higher levels of TSH were associated with a lower risk of dementia both in the full range (Hazard Ratio [HR] 0.90, 95% Confidence Interval [CI], 0.83-0.98) and the normal range of thyroid function (HR 0.76, 95% CI 0.64-0.91), in age-, sex- and cohort-adjusted models (Table 2).

Table 1 Baseline characteristics of participants in the Rotterdam Study with baseline TSH or FT4 measurements and incident dementia data

Variable	Mean (SD) [a]
Number of participants	9,446
Age, years	64.9 (9.7)
Female (%)	5,358 (56.7)
Hypertension (%)	4,935 (52.2)
Cholesterol, mmol/L	5.7 (1.0)
Smoking (%)	
current	1,911 (20.2)
past	4,567 (48.3)
never	2,968 (31.5)
BMI, kg/m^2	27.3 (4.2)
Diabetes Mellitus (%)	861 (9.1)
Education, median (range)	3 (1-7)
Coronary heart disease (%)	669 (7.1)
Atrial Fibrillation (%)	416 (4.4)
CES-D score, median (IQR)	5.0 (1.0-12.6)
TSH mIU/L, median (IQR)	1.91 (1.29 - 2.77)
FT4 pmol/L	15.7 (2.3)
TPOAb positive, >35 kU/L (%)	1,244 (13.2)
Use of thyroid medication (%)	264 (2.8)

[a] Values are means and standard deviation unless otherwise specified. Abbreviations: CES-D = Center for Epidemiologic Studies Depression Scale; BMI = body mass index; TSH = thyroid-stimulating hormone, FT4 = free thyroxine; SD = Standard deviation; IQR = inter-quartile range; TPOAb = thyroid peroxidase antibodies.

Table 2 Association of thyroid function with incident dementia

Variable	Cases *N*	Total *N*	HR (95% CI) model 1[a]	HR (95% CI) model 2[b]	HR (95% CI) model 3[c]
TSH[d]	601	9,441	0.90 (0.83-0.98)	0.90 (0.84-0.98)	0.90 (0.83-0.97)
FT4[e]	600	9,440	1.04 (1.01-1.07)	1.04 (1.01-1.07)	1.04 (1.01-1.07)
TPOAb positivity[f]	600	9,435	0.72 (0.55-0.93)	0.71 (0.55-0.92)	0.70 (0.54-0.92)
Within normal range of TSH and FT4[g], excluding thyroid medication users					
TSH	496	7,966	0.73 (0.62-0.87)	0.74 (0.62-0.87)	0.73 (0.62-0.87)
FT4	496	7,966	1.04 (1.00-1.09)	1.04 (1.00-1.09)	1.04 (1.00-1.09)
TPOAb positivity	495	7,958	0.71 (0.51-0.99)	0.71 (0.51-0.99)	0.72 (0.51-1.01)
Across the normal range of TSH and FT4[g], excluding thyroid medication users					
TSH[h]	496	7,966	0.49 (0.33-0.72)	0.49 (0.33-0.73)	0.49 (0.33-0.73)
FT4[i]	496	7,966	1.85 (1.01-3.43)	1.78 (0.97-3.26)	1.80 (0.99-3.34)
TSH quartiles					
0.41 - 1.28	166	2,000	1 (REFERENCE)	1 (REFERENCE)	1 (REFERENCE)
1.29 - 1.80	113	1,971	0.79 (0.62-1.00)	0.79 (0.62-0.99)	0.78 (0.62-1.00)
1.81 - 2.48	116	2,004	0.80 (0.63-1.01)	0.79 (0.62-1.00)	0.79 (0.62-1.01)
2.49 - 3.99	101	1,991	0.65 (0.51-0.84)	0.66 (0.52-0.85)	0.66 (0.52-0.85)
p for trend			<0.001	<0.001	0.001
FT4 quartiles					
11.01-14.35	111	1,988	1 (REFERENCE)	1 (REFERENCE)	1 (REFERENCE)
14.36-15.63	113	1,998	1.01 (0.77-1.31)	1.01 (0.78-1.31)	1.04 (0.80-1.35)
15.64-16.99	141	1,989	1.29 (1.01-1.66)	1.28 (0.99-1.64)	1.28 (1.00-1.65)
17.00-24.90	131	1,991	1.16 (0.90-1.50)	1.14 (0.88-1.47)	1.15 (0.89-1.48)
p for trend			0.044	0.062	0.135

[a]Model 1 adjusted for age, sex, and cohort. [b]Model 2 = model 1 + hypertension, cholesterol, smoking, BMI, and diabetes at baseline. [c] Model 3 = model 2 + highest attained education, prevalent coronary heart disease, and prevalent atrial fibrillation. [d] The HRs are per one unit increase in logTSH (mIU/L). Five participants had missing TSH data. [e] The HRs are per one unit increase in FT4 (pmol/L). Six participants had missing FT4 values. [f] TPOAb were regarded positive if >35 kU/mL. Eleven participants had missing TPOAb values. [g] normal range of TSH was 0.4-4.0 mIU/L and the normal range of FT4 was 11-25 pmol/L. [h] This corresponds to the HR when comparing participants with TSH values in the upper limit of the reference range (4.0 mIU/L) to participants with TSH values in the lower limit of the reference range (0.40 mIU/L). [i] This corresponds to the HR when comparing participants with FT4 values in the upper limit of the reference range (25 pmol/L) to participants with FT4 values in the lower limit of the reference range (11 pmol/L).

Abbreviations: FT4 = free thyroxine; TSH = thyroid-stimulating hormone; HR = hazard ratio; CI = confidence interval; TPOAb = thyroid peroxidase antibodies.

Multivariable adjustment did not alter the risk estimates substantially. When comparing participants with TSH levels in the upper limit of the reference range (4.0 mIU/L) to participants with TSH levels in the lower limit of the reference range (0.40 mIU/L), HR was 0.49 (95% CI, 0.33-0.72).

Dementia risk was significantly higher with higher levels of FT4 (HR 1.04, 95% CI 1.01-1.07) and was similarly increased within the normal range of thyroid function (HR 1.03, 95% CI 0.99-1.08). TPOAb positivity was associated with a reduced risk of incident dementia (Table 2), which was attenuated slightly after correcting for thyroid function in the normal range.

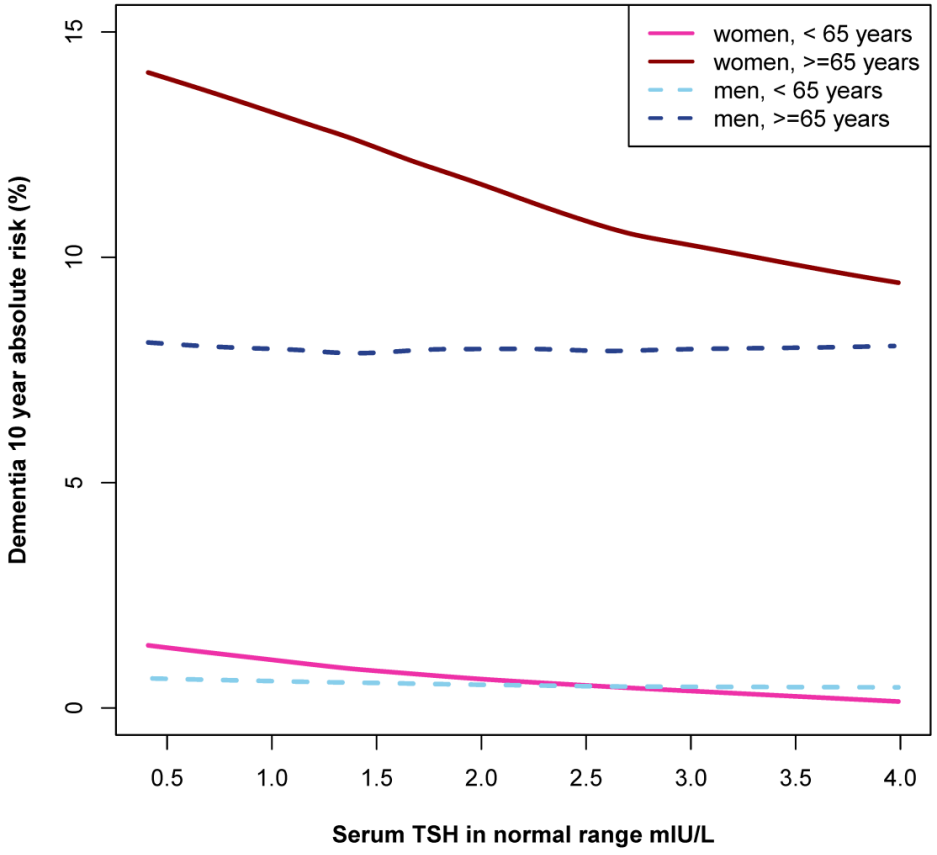
Stratified and sensitivity analyses

Stratification by sex in multivariable TSH analyses suggested a lower risk of dementia in women with higher levels of TSH, with a HR of 0.67 (95% CI, 0.54-0.82). This was not the case for men (HR 0.94, 95% CI 0.69-1.27, Table e-1, p for interaction = 0.074). An apparent differential risk by age was detected for participants younger than 65 when compared to those older than 65 years of age. However, this differential risk was not significant (Table e-1). There were no differences in risk estimates when stratifying for Alzheimer's vs. other types of dementia. We calculated absolute 10-year risks taking the competing risk of death into account, and plotted these absolute risks of dementia against TSH serum values within the normal range for both sexes and age categories separately (Figure 1). In women older than 65, the absolute risk of dementia decreased with higher levels of TSH from almost 15% to less than 10%. Additionally, adjusting for CES-D scores, excluding the first 4 years of follow-up in the analysis, or censoring at time of stroke did not alter the risk estimates (Table e-2).

Cognitive function and MRI

In the multivariable analyses, higher serum TSH levels were associated with a better z-score of global cognition (Beta 0.032 [95% CI 0.005-0.059] for g-factor, Table 3). Higher levels of TSH were also associated with better scores on some of the cognitive function tests, including the Verbal Fluency and Word Learning Tests (Table e-3). There was no association between FT4 levels and any cognitive function test, except for the Word Learning Test (Tables 3 and e-3). There was no association between TSH or FT4 level and white matter lesions, lacunar infarcts, or cerebral microbleeds (Table 4).

Figure 1 Absolute 10-year risk of dementia by TSH values within the normal range.



Absolute 10-years risks of dementia were calculated taking the competing risk of death into account. They are plotted against TSH values within the normal range for both sex and age categories separately.

Table 3 Association of thyroid function in the full range with Z scores of global cognitive function tests

Variable	G-factor Beta (95% CI), N = 6,685	P-val.	MMSE Beta (95% CI), N = 7,782	P-val.
TSH				
Model 1	0.042 (0.015, 0.069)	0.003	0.036 (0.012, 0.069)	0.004
Model 2	0.032 (0.005, 0.059)	0.021	0.033 (0.008, 0.057)	0.009
FT4				
Model 1	-0.003 (-0.013, 0.006)	0.527	0.003 (-0.005, 0.012)	0.455
Model 2	-0.004 (-0.014, 0.005)	0.367	0.002 (-0.006, 0.011)	0.617

[a] Model 1 adjusted for sex, age, cohort, and time between baseline laboratory measurement and cognitive test measurement, adjusted and performed after excluding thyroid hormone medication users and participants with dementia. [b]Model 2 = Model 1 + hypertension, cholesterol, smoking, BMI, diabetes, highest attained education, prevalent coronary heart disease, and prevalent atrial fibrillation.
The G-factor was calculated by incorporating the letter-digit substitution task, verbal fluency test, world learning test (average of z-scores of immediate and delayed recall), and the inverse of two components of the Stroop test. The Betas are per one unit increase in logTSH (mIU/L) and per one unit increase in FT4 (pmol/L). Abbreviations: CI = confidence interval; FT4 = free thyroxine; HR = hazard ratio; MMSE = mini mental state exam; TSH = thyroid-stimulating hormone

Table 4 Association of the full range TSH and FT4 values with MRI brain measurements

MRI Outcome		Total N	TSH, Model 1[a] Beta (95% CI)	TSH, Model 2[b] Beta (95% CI)	FT4, Model 1[a] Beta (95% CI)	FT4, Model 2[b] Beta (95% CI)
White matter lesions[c]		3,574	-0.001 (-0.047,0.028)	0.002 (-0.037,0.038)	0.000 (-0.001, 0.001)	-0.001 (-0.001, 0.001)
	Cases N	**Total N**	**OR (95%CI)**	**OR (95%CI)**	**OR (95%CI)**	**OR (95%CI)**
Lacunar infarcts	192	4,178	1.08 (0.88, 1.34)	1.09 (0.88, 1.35)	1.00 (0.93, 1.08)	1.01 (0.94, 1.08)
Cerebral microbleeds	546	4,178	0.94 (0.83, 1.07)	0.95 (0.83, 1.08)	0.98 (0.94, 1.02)	0.98 (0.94, 1.03)

[a] Model 1 adjusted for age, sex, cohort, and time between thyroid function measurement and MRI, and was conducted after excluding thyroid hormone medication users and participants with prevalent dementia. [b]Model 2 = Model 1 + hypertension, cholesterol, smoking, BMI, and diabetes at baseline, highest attained education, prevalent coronary heart disease, and prevalent atrial fibrillation. [c] The natural logarithm of white matter lesion volume (mm^3) was used to approximate normal distribution. These analyses were additionally adjusted for intracranial volume. The HRs are per one unit increase in logTSH (mIU/L) and per one unit increase in FT4 (pmol/L). Abbreviations: CI = confidence interval; FT4 = free thyroxine; MRI = magnetic resonance imaging; OR = odds ratio; TSH = thyroid-stimulating hormone

DISCUSSION

We report an increased incidence of dementia with high and high-normal thyroid function, while low to low-normal thyroid function is protective. This is confirmed by FT4 analyses, as higher FT4 levels are associated with a higher risk of incident dementia. The absolute 10-year risk of incident dementia decreases up to 5% with increasing TSH levels in older women, while this risk was not altered in men within the normal range of TSH. In cross-sectional analyses, higher TSH levels are associated with better cognitive function, while thyroid function is not associated with vascular damage-related brain features on MRI.

Our study includes detailed descriptions of dementia risk outside and within the normal range of thyroid function, as well as the exploration of vascular pathways as mediators of the relationship. Two prospective studies[10,35] have previously investigated the association of thyroid function within the normal range and dementia risk, and their results conflict with ours. The Framingham Heart Study investigated the association between the normal range of thyroid function and dementia in 1,864 participants, using only TSH levels and reported no significant associations.[10] In a report from the Cardiovascular Health Study, Cappola et al. find that individuals with TSH levels in the fourth quartile had a lower risk of dementia[35] than those with TSH levels in the first quartile. Important differences between these two studies and ours include the average age of the participants (>70 years vs. 65 years for our population) and sample size (<3000 vs. >9000 in our study). In addition, neither of the above studies investigated associations with FT4 levels, evaluated cognitive function, or investigated vascular pathways that might explain the relationship. Our larger sample size specifically enabled us to quantify more precise relative risks as well as absolute risk estimates, particularly within the normal range of TSH levels.

There have been several possible mechanisms described in the literature to explain the relationship between high thyroid function and dementia risk. Thyroid hormone has important effects on the cardiovascular system. In fact, higher thyroid function is associated with several cardiovascular risk factors, such as atrial fibrillation and systolic hypertension. These cardiovascular risk factors may mediate the association between thyroid function and the development of dementia

through vascular brain damage. However, adjusting analyses for various cardiovascular risk factors and assessing stroke as an intermediate for the association did not alter risk estimates. Furthermore, thyroid function was not associated with vascular damage-related brain features on MRI. The similar risk estimates for Alzheimer's disease and other type of dementia on the one hand and the lack of an association between thyroid function and vascular markers on MRI on the other may suggest a specificity of the association of thyroid function with neurodegenerative dementias. However, less than 20% of dementia cases in our population were classified as non-Alzheimer's (i.e. other type). We were therefore not able to investigate this association. Moreover, it is possible that there is a certain degree of mixed pathology even among the Alzheimer's disease cases, given that the diagnoses were mostly made based on clinical evidence.

An alternative explanation of the relationship between high thyroid function and dementia risk is that thyroid hormone excess changes gene expression in relevant pathways[4,36]. Furthermore, excess thyroid hormone may lead to neurotoxicity due to increased oxidative stress and neuronal death[37].

Another possible explanation for the association between thyroid function and dementia is that participants with pre-clinical dementia may have behavioral changes (e.g. malnutrition) leading to changes in thyroid function (i.e. reverse causality). In other words, we may not be observing a true effect of thyroid hormone on dementia risk, but rather the opposite. However, this is highly unlikely, as excluding the first four years of follow-up did not change risk estimates. Furthermore, low thyroid function was associated with better cognitive test scores. More research is needed to unravel the exact causal pathophysiological link between thyroid function and dementia. Defining causal pathophysiological mechanisms not only provides more insight into the development of dementia, but could also uncover potential future screening or novel treatment options. More immediate implications lie in the treatment of (subclinical) thyroid disease. In recent years, clinicians have been prone to start treatment of subclinical and mild hypothyroidism at lower ranges of TSH. Thus, overtreatment is becoming more prevalent[38]. Whether the effects of exogenous thyroid function on the risk of dementia are similar to those of endogenous thyroid still needs to be determined,

but our results may suggest more prudency in the adjustment of levothyroxine treatment in mild hypothyroidism.

The strengths of our study include the large number of participants and the detailed information on the covariates included in the analyses. Furthermore, we were able to assess possible intermediate factors, such as stroke and brain features on MRI related to vascular damage. The long follow-up allowed for the calculation of 10-year absolute risk estimates. A limitation of our study is that thyroid function was measured only once at baseline, which a limitation for most observational cohort studies[10,12]. Compared to other studies investigating incident dementia, our cohort has a younger mean age. However, in the subgroup analysis including only participants over the of age 65 years, we still find a decreased risk of dementia with higher TSH levels. In addition, we were not able to longitudinally investigate the association between thyroid function and vascular brain disease on MRI. Finally, residual confounding cannot be excluded in an observational study, even with adjustments for the large number of potential confounders performed in our analyses.

High and high-normal thyroid function is associated with an increased risk of dementia. This association does not seem to be mediated through vascular pathways. Further research is needed to clarify the pathways involved in the relationship between thyroid function and dementia risk.

Online supplemental material

http://www.neurology.org/content/87/16/1688.long

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CHAPTER 3.5

AGE-DEPENDENT ASSOCIATION OF THYROID FUNCTION WITH BRAIN MORPHOLOGY AND MICROSTRUCTURE

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ABSTRACT

BACKGROUND Thyroid hormone (TH) is crucial during neurodevelopment but high levels of TH have been linked to neurodegenerative disorders. No data on the association of thyroid function with brain imaging in the general population are available.

METHODS We therefore investigated the association of thyroid-stimulating hormone and free thyroxine (FT4) with MRI-derived total intracranial volume, brain tissue volumes and diffusion tensor imaging (DTI) measures of white matter microstructure in 4,683 dementia- and stroke-free participants (mean age 60.2, range 45.6-89.9 years).

RESULTS Higher FT4 levels were associated with larger total intracranial volumes (β=6.73mL, 95% confidence interval 2.94-9.80). Higher FT4 levels were also associated with larger total brain and white matter volumes in younger individuals, but with smaller total brain and white matter volume in older individuals (p-interaction 0.02). There was a similar interaction by age for the association of FT4 with mean diffusivity on DTI (p-interaction 0.026).

CONCLUSIONS These results are in line with differential effects of TH during neurodevelopmental and neurodegenerative processes and can improve understanding of the role of thyroid function in neurodegenerative disorders.

INTRODUCTION

Thyroid hormone impacts different essential neuronal processes including neurogenesis, myelination, and neural differentiation in childhood and throughout adulthood [1,2]. Already during intrauterine neurodevelopment, thyroid hormone impacts on several processes including neuronal cell proliferation, differentiation and migration [1,3]. Suboptimal thyroid hormone availability during early life can have profound impact on brain function later in life. This is illustrated by the link between congenital hypothyroidism and cretinism, a condition characterized by severely impaired physical and mental development. Mild forms of both low and high thyroid function during the gestational period have been associated with a lower child IQ and differences in brain morphology during later life [4].

However, the effects of thyroid hormone on the brain are age dependent because in addition to its effects during development, thyroid hormone is also related to neurodegeneration. In older adults, higher thyroid function has been associated with a higher risk of neurodegenerative disorders and poorer cognition [5,6]. A meta-analysis of cohort studies showed that higher thyroid function is associated with higher risk of cognitive impairment [7]. We previously described an increased risk of dementia with high-normal to high thyroid function and a protective effect of low and low-normal thyroid function [8]. This risk did not seem to be explained by cardiovascular risk factors or subclinical vascular brain damage. The underlying mechanisms explaining the link between thyroid function and dementia are yet unclear, but possible and yet unexplored pathways are through subclinical changes in microstructural organization or brain tissue atrophy.

Due to the link with cognitive impairment, we hypothesized that thyroid hormone could have adverse effects on processes affecting brain volumes and white matter microstructure in older age. We also hypothesized that this effect could be age-dependent, due to the different effects of thyroid hormone during the neurodevelopmental period. Therefore, we investigated the association of thyroid function with intracranial brain volume (as a marker of development), total brain, white matter and grey matter volumes on MRI (as markers of neurodegeneration). Furthermore, we tested whether the association of thyroid function and diffusion

tensor imaging (DTI) measures related to white matter microstructural organization were different in younger versus older participants.

METHODS

Setting

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 [9]. We included participants from three independent cohorts within the Rotterdam Study. The RS Cohort 1 (RSI) includes participants aged 55 years and older and baseline data were collected during 1990-1993. RS Cohort II (RSII) includes participants aged 55 years and older and baseline data were collected from 2000-2001. For the RS Cohort 3 (RSIII), persons included were aged 45 years and over and baseline data were collected from 2006 to 2008. Thyroid function assessment was determined in a random subset of 9,689 participants in all three cohorts and brain MRI was included in the core protocol of the Rotterdam Study since 2005. 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.

Study population

We included all participants from the Rotterdam Study, cohort I wave 3, cohort II wave I and cohort III wave I, with thyroid function measurements, MRI measurements and free of dementia at baseline (n=5104). We excluded 248 participants with prevalent stroke and with MRI-defined cortical infarcts and 173 participants using thyroid function altering medication (levothyroxine, anti-thyroid drugs, amiodarone or corticosteroids). Final study population included 4,683 participants of which 3,852 also had DTI measurements (Figure 1).

Assessment of thyroid function

Thyroid function was measured through thyroid-stimulating hormone (TSH) and free thyroxine (FT4) 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 0.4-4.0 mIU/L and FT4 as 11-25 pmol/L (= 0.85-1.95 ng/dL) according to national guidelines as well as our previous studies [8,10].

MRI acquisition and analysis

Multi-sequence brain MR Imaging was performed on a 1.5 tesla MRI scanner (GE Signa Excite). The imaging protocol and sequence details were described extensively elsewhere [11]. Scans were automatically segmented supra tentorially into grey matter, white matter, cerebrospinal fluid (CSF) and background tissue. Intracranial volume (ICV) (excluding the cerebellum and surrounding CSF) was estimated by summing total grey and white matter and CSF volumes [12]. Total brain volume was estimated by summing total grey and white matter volumes [12]. A post-processing white matter lesion classification, based on the FLAIR image and the tissue segmentation, was used to obtain white matter lesion volumes (natural-log transformed to account for their skewed distribution) [13]. All segmentations were visually inspected and were corrected if needed. Cortical infarcts were visually rated on structural sequences, and were classified as cortical infarcts in case of involvement of cortical grey matter. In a subset of our study population (N=2,449) cerebellar volume was processed automatically using FreeSurfer 4.5. This procedure, based on probabilistic information obtained from a manually labeled training set, assigns a neuroanatomical label to each voxel in an MRI volume. This is explained in more detail elsewhere [14]. In this subset we computed intracranial volume, including cerebellar volume, and total cerebellar volume.

Diffusion-MRI processing

For characterization of white matter microstructural organization with DTI, a single shot, diffusion-weighted spin echo echo-planar imaging sequence was performed. Maximum b-value was 1000 s/mm^2 in 25 non-collinear directions and three volumes were acquired without diffusion weighting (b-value = 0 s/mm^2). For the diffusion acquisition, due to technical issues 1165 participants were scanned with

the phase and frequency encoding directions swapped leading to a mild ghost artifact [15]. We corrected for this potential confounder in our analyses. Diffusion data were pre-processed using a standardized pipeline (including correction for motion and eddy currents) as previously described [16]. A diffusion tensor model was estimated in each voxel, and co-registration between structural imaging and diffusion image space was performed [15]. Through averaging of the diffusion measurements inside the normal appearing white matter (voxels with white matter lesions were excluded from the analysis) we obtained global mean fractional anisotropy (FA), mean diffusivity (MD), radial diffusivity, and axial diffusivity [15]. The median time between thyroid function measurement and MRI scan was 0.21 years (interquartile range: 0.06-10.16).

Assessment of other variables

Blood pressure was measured at the right brachial artery using a random-zero sphygmomanometer after 5 minutes of rest with the participants in sitting position. The mean of two consecutive measurements was used. Information regarding the use of blood pressure lowering medication for the indication of hypertension was derived from structured home interviews and linkage to pharmacy records. Serum total and high density lipoprotein (HDL) cholesterol were measured in fasting serum by enzymatic method. Smoking information was derived from baseline questionnaires and categorized in never, previous and current smokers. Alcohol consumption was documented as intake in grams per day. Body-mass index (BMI) was calculated as weight kilograms divided by height in meters squared. 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 ≥ 11 mmol/L (when fasting samples were absent) or use of anti-glycemic medication at baseline.[17] Educational level was assessed during a baseline home interview and people were classified into 7 categories: from low level of education (primary only) to high (university). Prevalent dementia and clinical stroke were ascertained as previously described.[18,19] In short, participants were evaluated for dementia using a three strep protocol. All participants were screened using the Mini-Mental State Examination (MMSE) [20] and Geriatric Mental State schedule (GMS) [21]. Persons scoring ≤ 25 on the MMSE or >0 on the GMS underwent an examination and informant interview with the Cambridge Examination for Mental Disorders of the

Elderly. Persons suspicious of having dementia underwent further neuropsychological testing if necessary. Furthermore, in addition to the above screening method, persons were continuously monitored for the dementia diagnosis through computerized linkage of the study database and medical records of the general practitioner's office and Regional Institute for Outpatient Mental Health Care (RIAGG). The accepted DSM-III-R criteria were used for the dementia diagnosis[22].

Statistical analysis

We used ordinary least squares linear regression models with restricted cubic splines at three knots [23], which provided the best fit with the data without overfitting, for all analyses, to account for possible non-linear associations. Primary analyses for brain volume measurements were adjusted for age, sex, cohort, time between laboratory measurement and MRI scan and intracranial volume. We included intracranial volume as a covariate in our model to correct for the inter-individual variability in head size [24]. In a second model, we additionally adjusted for several cardiovascular risk factors including total cholesterol, HDL-cholesterol, systolic blood pressure, diastolic blood pressure, smoking, prevalent diabetes mellitus, BMI, alcohol use and educational level. For the analyses of intracranial volume, we did not adjust for the variable itself.

Primary analyses for diffusion measurements adjusted for age, sex, cohort, time between laboratory measurement and MRI scan, intracranial volume, white matter volume and white matter lesion volume. A second model additionally adjusted for total cholesterol, HDL-cholesterol, systolic blood pressure, diastolic blood pressure, smoking, prevalent diabetes, BMI, alcohol use and educational level. TSH was natural log-transformed for all analyses to approximate normality and interaction of TSH or FT4 with age (as a continuous measure) or sex was tested for all analyses. We conducted sensitivity analyses 1) constricting analyses to the reference range of thyroid function (n=4141) and 2) excluding participants with time interval above 1 year between laboratory measurement and MRI scan (n=2527). In order to quantify the effects in different age groups we additionally stratified our main analyses by the mean age of our population (~ 60 years of age). Missing covariates (< 5% for all covariates but alcohol [6.7%]) were imputed using multiple imputations creating 5 data sets according to the Markov Chain Monte Carlo method and pooled

subsequently (IBM SPSS Statistics for Windows, version 21.0. Armonk, NY). Statistical analyses were conducted and plots were produced 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 a total of 4683 participants, with an average age of 60.2 years (range 45.6-89.9) of which 54.9% were female (Table 1). Figure 1 shows the participant selection procedure. As the results of the two tested models are comparable we present both for illustration, but only report the most adjusted model in the manuscript. Linearity assumption was met for all main analyses and indicated in tables if otherwise.

Figure 1 Flow chart of participant selection

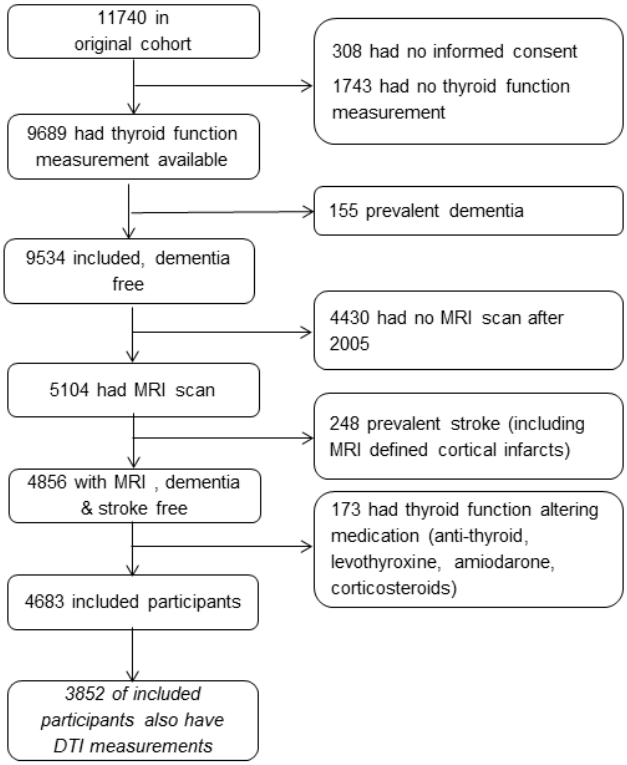


Table 1 Characteristics of the 4,683 study participants

Variable	Mean (SD) [a]
Age, years	60.2 (7.3)
Female sex n, %	2,571 (54.9)
TSH, ImU/L median (IQR)	1.97 (1.36 - 2.78)
FT4, pmol/L	15.5 (2.1)
Intracranial volume, mL	1140.0 (115.5)
Grey matter volume, mL	529.4 (53.6)
White matter volume, mL	409.2 (59.1)
White matter lesion volume, mL, median, IQR	2.9 (1.7-6.0)
Mean fractional anisotropy	0.34 (0.02)
Mean diffusivity, 10^{-3} mm^2/s	0.74 (0.03)
BMI, kg/m^2	27.1 (4.0)
Total cholesterol, mmol/L	5.70 (1.02)
HDL-cholesterol, mmol/L	1.42 (0.41)
Systolic blood pressure, mmHg	134.8 (19.3)
Diastolic blood pressure, mmHg	79.9 (10.9)
Prevalent diabetes n, %	399 (8.5)
Smoking	
Current n, %	1,029 (22.0)
Past n, %	2,211 (47.2)
Never n, %	1,443 (30.8)
Alcohol use, grams per day, median, IQR	15.0 (6.3-21.4)
Time between thyroid function measurement and scan (in years) median, IQR	0.21 (0.06-10.16)

[a] Values are means and (standard deviation) unless otherwise specified.
There were 13 participants with missing values for BMI, 5 total cholesterol, 17 HDL-cholesterol, 14 systolic and diastolic, 17 smoking, 18 for prevalent diabetes and 313 for alcohol use.
Abbreviations: BMI = body-mass index; TSH = thyroid-stimulating hormone, FT4 = free thyroxine; SD = Standard deviation; IQR = inter-quartile range

Brain tissue volumes and intracranial volume

Higher FT4 levels were associated with larger intracranial volume with a beta (β) of 6.23 mL per one unit increase of FT4 pmol/L (95% Confidence Interval [CI], 2.80, 9.66, Table 2), and the association was not different according to age (Figure2, Supplemental Table 1, Supplemental Table 2). There was no association of TSH levels with intracranial volume. Higher FT4 levels were associated with larger brain volume overall (β 2.26, CI, 1.10, 3.43, Table 2) and white matter volume in particular (β 1.43, CI, 0.25, 2.62, Table 2), but not in elderly (Figure 2, Supplemental Table 2). The p for interaction of age with total brain volume was 0.002 and for age with white matter volume was 0.038 (Supplemental Table 1).

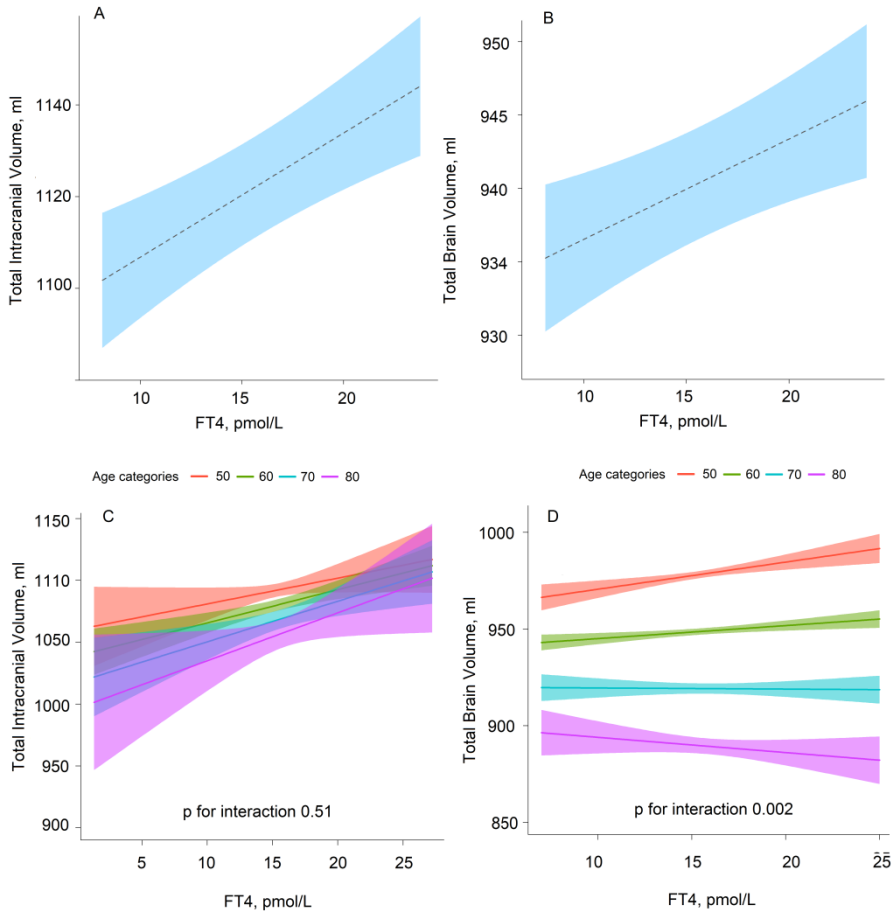
The associations of FT4 and TSH with total brain volume were mainly attributable to white matter and not gray matter volume (Table 2).

Higher levels of TSH were associated with smaller brain volumes (β -1.35, CI, -2.26, -0.45, Table 2). Higher FT4 levels were associated with a larger white matter volume (β 1.43, CI, 0.24, 2.62), but not gray matter volume (β 0.78, CI, -0.25, 1.80). Higher TSH levels were associated with a smaller white matter volume (β -1.58, CI,-2.50, -0.67), but not grey matter volume (β 0.19, CI, -0.61, 0.99). Constricting analyses to the reference range of thyroid function or excluding participants with time interval between laboratory measurement and MRI scan > 1 year did not change effect estimates (Table 3). There was no significant interaction of FT4 or TSH with sex on the association with any of the studied outcomes (p> 0.10). There was no significant interaction of TSH with age on the association with any of the studied outcomes (p> 0.35, Supplemental Table 1).

Total cerebellar volume

The additional analysis using a subgroup of 2249 persons with cerebellar volume data measured with FreeSurfer, yielded a similar albeit a more pronounced association of FT4 with intracranial volume (β 10.13, CI, 3.61, 16.64, Supplemental Table 3). FT4 was positively associated with cerebellar volume (β 0.68.13, CI, 3.61, 16.64, Supplemental table 3). No associations were observed for TSH with intracranial volume or cerebellar volume (Supplemental Table 3).

Figure 2 Total intracranial and brain volume according to FT4 serum values



Plot A) depicts the association of FT4with intracranial volume and plot B) depicts the association of FT4 with total brain volume. Plot C) depicts the interaction of FT4 with age continuously for the intracranial volume analysis and D) depicts interaction of FT4 with age continuously for the total brain volume analysis. All analyses are adjusted for age, sex, cohort and time between laboratory measurements and MRI scan, and the analyses with brain volume were additionally adjusted for intracranial volume.

Table 2 Association of TSH or FT4 with intracranial, total, white and grey matter brain volume measurements

Variable	Total intracranial volume β (95% CI)	Total brain volume β (95% CI)	Total white matter volume β (95% CI)	Total grey matter volume β (95% CI)
TSH				
Model 1	0.39 (-2.32, 3.09)	-1.37 (-2.28, -0.46)	-1.58 (-2.50, -0.67)	0.21 (-0.59, 1.01)
Model 2	0.64 (-2.04, 3.32)	-1.37 (-2.27, -0.47)	-1.55 (-2.47, -0.63)	0.18 (-0.61, 0.98)
FT4				
Model 1	7.23 (3.78, 10.67)	1.95 (0.78, 3.12)*	1.43 (0.25, 2.61)	0.47 (-0.55, 1.49)
Model 2	6.23 (2.80, 9.66)	2.26 (1.10, 3.43)	1.43 (0.25, 2.62)	0.78 (-0.24, 1.81)

Model 1: age, sex, cohort, time between laboratory measurement and MRI scan and intracranial volume Model 2= Model 1 + total cholesterol, HDL-cholesterol, systolic blood pressure, diastolic blood pressure, smoking, prevalent diabetes, BMI, alcohol use and educational level. * p for non-linearity 0.054; Abbreviations: CI = confidence interval; FT4 = free thyroxine; MRI= Magnetic resonance imaging; OR = odds ratio; TSH = thyroid stimulating hormone

Table 3 Sensitivity analyses of association of TSH or FT4 with MRI intracranial and brain volume measurements

Variable		Total intracranial volume β (95% CI)	Total brain volume β (95% CI)	Total white matter volume β (95% CI)	Total grey matter volume β (95% CI)
Reference range (n= 4,141)*					
TSH	Model 1	-0.91 (-5.40, 3.58)	-1.40 (-2.89, 0.09)	-1.88 (-3.40, -0.35)	0.48 (-0.84, 1.81)
	Model 2	-0.87 (-5.34, 3.59)	-1.30 (-2.78, 0.17)	-1.76 (-3.28, -0.22)	0.45 (-0.87, 1.78)
FT4	Model 1	8.31 (4.25, 12.36)	2.32 (0.87, 3.76)**	1.56 (0.07, 3.04)	0.60 (-0.60, 1.80)
	Model 2	7.17 (3.12, 11.22)	2.53 (1.09, 3.97)**	1.41 (-0.08, 2.90)	0.99 (-0.22, 2.20)
Excluding participants with time interval between laboratory measurement and MRI scan > 1 years (n= 2,527)					
TSH	Model 1	1.42 (-2.7, 5.56)	-1.41 (-2.74, -0.08)	-1.22 (-2.55, 0.11)	-0.24 (-1.42, 0.49)
	Model 2	1.54 (-2.57, 5.65)	-1.50 (-2.82, -0.17)	-1.23 (-2.57, 0.11)	-0.30 (-1.48, 0.88)
FT4	Model 1	6.78 (1.95, 11.61)	2.57 (1.01, 4.13)**	2.36 (0.79, 3.93)	0.08 (-1.29, 1.46)
	Model 2	5.40 (0.57, 10.22)	2.95 (1.39, 4.52)**	2.42 (0.83, 4.00)	0.43 (-0.96, 1.82)

Model 1: age, sex, cohort, time between laboratory measurement and MRI scan and intracranial volume Model 2= Model 1 + total cholesterol, HDL-cholesterol, systolic blood pressure, diastolic blood pressure, blood pressure lowering medication with indication of hypertension, smoking, prevalent diabetes, BMI, alcohol use and educational level.

* Reference ranges for TSH were 0.4-4.0 mIU/L and for FT4 were 11-25 pmol/L. ** non-linearity of association $p < 0.05$. Abbreviations: CI = confidence interval; FT4 = free thyroxine; MRI= Magnetic resonance imaging; OR = odds ratio; TSH = thyroid stimulating hormone

White matter microstructural organization

There was no overall association of TSH or FT4 with diffusion properties of white matter, neither FA nor MD (Table 4). However, there was a significant interaction with age for the association of FT4 and MD (p for interaction 0.026, Figure 3, Supplemental Table 1). In older participants, higher FT4 values were associated with a lower FA (albeit not statistically significant interaction, p = 0.052, Supplemental Table 1) and higher MD, generally indicating reduced matter microstructural integrity (Supplemental Table 4). In contrast, in younger participants, higher FT4 levels were associated with a higher FA and lower MD, generally indicating increased white matter integrity. The associations for radial and axial diffusivity followed the same pattern as mean diffusivity (Figure 3).

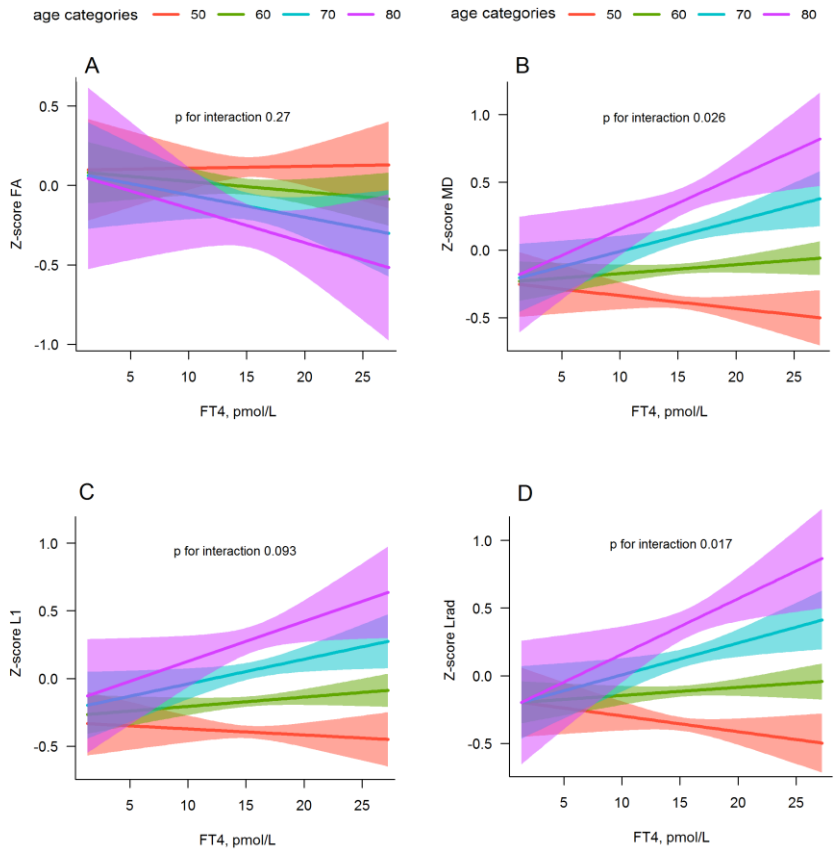
We corrected the p-value (alpha level of 0.05) for multiple comparisons using Šidák correction [25]. The number of independent tests resulted in a threshold for significance of p<0.009. All results survive this threshold except for the association of FT4 in the full range with white matter volume.

Table 4 Association of full range TSH or FT4 with Z-scores of Diffusion Tensor Imaging parameters of white matter (n=3,852)

	FA β (95% CI)	MD β (95% CI)	RD β (95% CI)	AD β (95% CI)
TSH				
Model 1	-0.00(-0.03, 0.03)	0.01(-0.01, 0.03)	0.01(-0.01, 0.03)	0.01(-0.01, 0.03)
Model 2	-0.00(-0.03, 0.03)	0.01(-0.01, 0.03)	0.01(-0.01, 0.03)	0.01(-0.01, 0.03)
FT4				
Model 1	-0.01(-0.05, 0.02)	0.02(-0.01, 0.04)	0.01(-0.01, 0.04)	0.02(-0.01, 0.04)
Model 2	-0.01(-0.04, 0.03)	0.01(-0.02, 0.04)	0.01(-0.02, 0.04)	0.01(-0.01, 0.04)

Model 1: age, sex, cohort, time between laboratory measurement and MRI scan, intracranial volume, white matter volume and white matter lesions. Model 2= Model 1 + total cholesterol, HDL-cholesterol, systolic blood pressure, diastolic blood pressure, blood pressure lowering medication with indication of hypertension, smoking, prevalent diabetes, BMI, alcohol use and educational level. Abbreviations: CI = confidence interval; FA = fractional anisotropy; FT4 = free thyroxine; MD = mean diffusivity; RD = radial diffusivity; AD = axial diffusivityTSH = thyroid stimulating hormone

Figure 3 DTI measurements according to FT4 serum values



Plot depicting the interaction of FT4 with age for z-scores of A) fractional anisotropy, B) mean diffusivity, C) radial diffusivity and D) axial diffusivity . All analyses are adjusted for age, sex, cohort, time between laboratory measurements and MRI scan, intracranial volume, white matter and white matter lesion volume.

DISCUSSION

We report an association of higher FT4 with larger intracranial volume, independent of age. Higher FT4 levels are also associated with a larger total brain volume, mainly attributable to white matter volume. However this association was age-dependent. In older participants (over ~70 years old) higher FT4 levels were associated with a smaller total brain volume, primarily white matter. We also found a differential effect of age on the association of FT4 with DTI measures that can be

linked to white matter integrity. In older participants, higher FT4 were associated with DTI measures reflecting poorer white matter integrity. In younger participants (especially those <50 years), higher FT4 levels were associated with DTI measures reflecting better white matter integrity, primarily lower MD. These findings could indicate an age-dependent effect of thyroid hormone on brain morphology and microstructural organization, beneficial in younger age and deleterious in older age. To our knowledge, there are no previous studies assessing the association of thyroid function with brain volumes and white matter microstructure in the general population. Based on our results, we hypothesize that the findings in younger participants could reflect a positive role of thyroid hormone balance in myelination, sustainability and protection of neurons during early stages of life. In contrast, in older age, high thyroid function could be deleterious by causing neuronal damage and in turn neurodegeneration.

Thyroid hormone is important for growth, development and metabolism in virtually all organs and effects on the brain are numerous. Fetal neurogenesis is thyroid hormone dependent and both lack and excess of thyroid hormone availability can hamper brain development and has deleterious effects on brain morphology [1,2,26]. In older age, mainly high thyroid function has been linked to neurodegeneration and cognitive impairment [6,7,10]. Higher thyroid hormone levels are related to a higher basal metabolic rate and oxygen consumption. In turn, this can affect oxidative stress, either due to increased reactive oxygen species production or lower activity of antioxidants, potentially leading to oxidative damage [27,28]. These effects are reported to be tissue specific, but oxidative damage may be most pronounced in metabolic active organs such as the brain possibly leading to negative effects on neuronal integrity[28]. Free radical injury has been suggested to associate with white matter changes on DTI [29]. With microstructural changes thought to accumulate to macrostructural tissue change, this could be one of the pathways explaining the association of thyroid function with lower white matter volume and poorer integrity in elderly, potentially also the previously described relation with the risk of dementia. The pathophysiology of dementia is multifactorial, but implication of oxidative stress has also been proposed [30,31]. Alternatively, a common genetic predisposition could underlie the association of thyroid function with dementia and white matter integrity.

Thyroid function is known to affect several cerebrovascular risk factors. However, deleterious cardiovascular risk factors, such as dyslipidemia and increased blood pressure, are mainly consequences observed in hypothyroidism. Furthermore, we previously described lack of association of thyroid function with small vessel disease on MRI, including white matter lesions, lacunar infarcts and cerebral microbleeds [8]. Also, adjusting for various cardiovascular risk factors in the current study did not change the associations meaningfully. This, together with the current results, suggests that the association of thyroid function with measures of white matter microstructure in elderly is independent of these risk factors and mediated through other pathways (e.g. oxidative stress). Thyroid hormone also has distinct effects on myelin formation and regeneration [32,33]. However, thyroid hormone and thyroid hormone repletion is mainly associated with acceleration of myelination and remyelination in different animal studies and patient populations [32-34]. This demonstrates the potential complexity of the pathophysiology [2] and more research is needed to unravel the exact pathophysiological link between thyroid function, white matter microstructure and dementia. Discovery of underlying pathways is not only needed to understand the pathophysiology of dementia, but also to identify persons at risk and determine promising treatment targets.

Another implication of our study may lie in the possible effects of overtreatment of hypothyroidism. In recent years, physicians have commenced levothyroxine treatment in people at lower serum TSH thresholds, i.e. milder cases of hypothyroidism [35]. It was found that in patients treated with levothyroxine for hypothyroidism, a substantial proportion was not within treatment target, with over 10% being overtreated and actually classifying as iatrogenic hyperthyroidism [35]. Our study results suggest that endogenous high FT4 values may negatively affect brain volume and brain tissue in older age. Although we have not investigated whether high thyroid function due to levothyroxine (i.e. exogenous thyroxine) use has comparable effects on brain volumes and DTI measurements as endogenous thyroid hormone, we speculate that this is plausible. Further research is needed to confirm this hypothesis.

Strengths of our study include the large sample size and availability of detailed phenotypical information. Also, we were able to adjust for a wide variety of confounders. Nevertheless, residual confounding cannot be excluded in an

observational study, even with adjustments for the large number of potential confounders performed in our analyses. Another limitation of our study is that thyroid function was measured only once, which is a limitation for most observational cohort studies, and therefore changes over time could not be assessed. A sensitivity analysis limited to participants with thyroid function within the reference range (n=4141), which are known to be relatively stable over time [36,37], yielded similar results. Nevertheless, our results need to be confirmed in study preferably with a longitudinal design. Furthermore; no conclusions can be drawn on the causality of the associations due to the cross-sectional design. We used averaged diffusion parameters, aggregated over all the normal-appearing white matter voxels. This did not allow us to assess brain changes on a more regional level. Finally, the Rotterdam Study constitutes of mainly Caucasian participants of 45 years and older, so our results may be less generalizable to younger or other ethnic populations.

Conclusions

In summary, our study shows that higher FT4 levels are associated with larger brain volumes and higher white matter microstructural integrity in younger individuals, but not in elderly. Furthermore, our results highlight the need for caution in overtreatment in mild hypothyroidism. Thyroid hormone excess is a risk factor for dementia and further studies should evaluate whether this is indeed mediated through poorer white matter microstructural integrity.

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Supplemental Table 1 P-values for interaction with age for TSH or FT4 with brain volumes and DTI measurements

Variable	TSH interaction with age	FT4 interaction with age
Total Intracranial volume	0.38	0.51
Total Brain Volume	0.86	0.002
White matter Volume	0.77	0.038
Grey matter volume	0.59	0.64
Fractional Anisotropy	0.82	0.052
Mean diffusivity	0.93	0.026
Radial diffusivity	0.79	0.017
Axial diffusivity	0.88	0.093

Models are adjusted for age, sex, cohort, time between laboratory measurement and MRI scan and supratentorial intracranial volume (where appropriate).
Abbreviations: DTI = diffusion tensor imaging; FT4 = free thyroxine; TSH = thyroid stimulating hormone

Supplemental Table 2 Association of FT4 with intracranial, total, white and grey matter brain volume measurements

Variable	Total intracranial volume β (95% CI)	Total brain volume β (95% CI)	Total white matter volume β (95% CI)	Total grey matter volume β (95% CI)
FT4				
<60 years (n=2428)	6.60 (1.68,11.53)	2.73 (1.15, 4.31)	1.07 (0.47, 3.67)	1.00 (-0.37, 2.38)
≥60 years (n=2255)	8.08 (3.25, 12.91)	0.77 (-0.91, 2.46)	0.73 (-1.10, 2.48)	0.02 (-1.49, 1.53)

Model adjusted for age, sex, cohort, time between laboratory measurement and MRI scan and intracranial volume Abbreviations: CI = confidence interval; FT4 = free thyroxine; MRI= Magnetic resonance imaging; TSH = thyroid stimulating hormone

Supplemental Table 3 Association of TSH or FT4 with intracranial and cerebellar volume measurements with FreeSurfer

Variable	Total intracranial volume **β (95% CI)**	Total cerebellar volume **β (95% CI)**
TSH		
Model 1	-0.64 (-6.25, 4.97)	-0.26 (-0.69, 0.16)
Model 2	-0.49 (-6.07. 5.09)	-0.30 (-0.72, 0.13)
FT4		
Model 1	12.01 (5.50, 18.52)	0.64 (0.15, 1.14)*
Model 2	10.13 (3.61, 16.64)	0.68 (0.18, 1.18)*

Model 1: age, sex, cohort, time between laboratory measurement and MRI scan, intracranial volume for the cerebellar volume analysis). Model 2= Model 1 + total cholesterol, HDL-cholesterol, systolic blood pressure, diastolic blood pressure, smoking, prevalent diabetes, BMI, alcohol use and educational level. * p for non-linearity <0.05. Abbreviations: CI = confidence interval; FT4 = free thyroxine; MRI= Magnetic resonance imaging; OR = odds ratio; TSH = thyroid stimulating hormone

Supplemental Table 4 Association FT4 with *Z-scores* Diffusion Tensor Imaging parameters of white matter

Variable	Fractional Anisotropy **β (95% CI)**	Mean diffusivity **β (95% CI)**	Radial diffusivity **β (95% CI)**	Axial diffusivity **β (95% CI)**
FT4				
<60 years (n=2079)	0.00 (-0.04, 0.05)	- 0.01 (-0.04, 0.02)	-0.02 (-0.01, 0.04)	0.00 (-0.03, 0.04)
≥60 years (n=1773)	-0.04 (-0.09, 0.02)	0.04 (0.00, 0.09)	0.05 (0.00, 0.09)	0.03 (-0.00, 0.07)

Model adjusted for age, sex, cohort, time between laboratory measurement and MRI scan, intracranial volume, white matter volume and white matter lesions. Abbreviations: CI = confidence interval; FT4 = free thyroxine; TSH = thyroid stimulating hormone

CHAPTER 4

METABOLISM

CHAPTER 4.1

LOW-NORMAL THYROID FUNCTION IS ASSOCIATED WITH INCREASED RISK OF DIABETES TYPE II AND PROGRESSION FROM PREDIABETES TO DIABETES

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Peeters RP, Dehghan A
BMC Med. 2016

ABSTRACT

BACKGROUND The association of thyroid function with risk of type 2 diabetes remains elusive. We aimed to investigate the association of thyroid function with incident prediabetes, diabetes and progression from prediabetes to diabetes in a population-based prospective cohort study.

METHODS We included 8452 participants (mean age 65 years) with thyroid function measurement, defined by thyroid-stimulating hormone (TSH) and free thyroxine (FT4), and longitudinal assessment of prediabetes and diabetes incidence. Cox-models were used to investigate the association of TSH and FT4 with prediabetes, diabetes and progression from prediabetes to diabetes. Multivariable models adjusted for age, sex, HDL cholesterol, and glucose at baseline, amongst others.

RESULTS During a mean follow-up of 7.9 years, 798 diabetes cases occurred. Higher TSH levels were associated with a higher diabetes risk (Hazard Ratio [HR] 1.13, 95% confidence interval [CI], 1.08-1.18, per logTSH), even within reference range of thyroid function (HR 1.24, CI, 1.06-1.45). Higher FT4 levels were associated with a lower diabetes risk amongst all participants (HR 0.96, CI, 0.93-0.99, per 1 pmol/L) and in participants within the reference range of thyroid function (HR 0.96, CI, 0.92-0.99). The risk of progression from prediabetes to diabetes was higher with low-normal thyroid function (HR 1.32, CI, 1.06-1.64 for TSH and HR 0.91, CI, 0.86-0.97 for FT4). Absolute risk of developing diabetes type 2 in participants with prediabetes decreased from 35% to almost 15% with higher FT4 levels within the normal range.

CONCLUSIONS Low and low-normal thyroid function are risk factors for incident diabetes, especially in individuals with prediabetes. Future studies should investigate whether screening for and treatment of (subclinical) hypothyroidism is beneficial in subjects at risk of developing diabetes.

INTRODUCTION

Diabetes mellitus and thyroid disease are the two most common endocrine disorders and not rarely co-exist in patients [1]. The role of auto-immunity has been well-recognized in the link between auto-immune thyroid disease and type 1 diabetes mellitus [2]. A relation between thyroid dysfunction and type 2 diabetes mellitus has also been suggested, but the possible underlying mechanisms are divers and show complex interactions [3].

Thyroid hormone is a major regulator of metabolism and energy expenditure, is directly involved in the control of insulin secretion and glucose homeostasis [3,4] and has been shown to preserve beta-cell viability and proliferation [5,6]. Hyperthyroid individuals have an increased insulin secretion [7] and free triiodothyronine levels are specifically associated with improved insulin secretion in individuals with prediabetes [8]. However, the deleterious effect of thyrotoxicosis on glucose metabolism has also been recognized for decades [9]. Excess thyroid hormone (i.e. hyperthyroidism) causes increased liver gluconeogenesis and peripheral insulin resistance and is associated with glucose intolerance [10-13]. Interestingly, lack of thyroid hormone is also associated with a decrease of peripheral insulin sensitivity and glucose intolerance [14] and treatment of hypothyroidism has been shown to improve insulin sensitivity [14,15].

Results concerning the association between thyroid function and type 2 diabetes are conflicting, with register-based studies reporting an association of hyperthyroid and hypothyroidism with type 2 diabetes [16-18] while a recent cross-sectional large population based study found no association between thyroid dysfunction and type 2 diabetes [19]. As a consequence, there is no consensus on whether patients with type 2 diabetes should be screening for thyroid dysfunction.

To date, there are no prospective population-based cohort studies investigating the association across the full range of thyroid function, including the normal range, with the risk of incident prediabetes and diabetes. Also, to our knowledge, no studies so far have examined the role of thyroid function on the early development of diabetes type 2 (prediabetes). Therefore, we aimed to investigate the association of thyroid function with the incidence of pre-diabetes, type 2 diabetes

and the progression from prediabetes to diabetes in the Rotterdam Study, a large prospective population-based cohort study.

METHODS

The Rotterdam Study

The Rotterdam Study is a prospective population-based cohort study that investigates determinants and occurrence of age related diseases in Ommoord, Rotterdam, the Netherlands. The aims and design of the Rotterdam Study have been described in detail elsewhere [20]. The Rotterdam Study consists of three independent cohorts.: RS Cohort 1 (RSI), including 7,983 participants aged ≥55 (baseline 1990-1993), RS Cohort II (RSII), including 3,011 participants aged ≥55 (baseline 2000-2001) and RS Cohort 3 (RSIII), including 3,932 participants aged ≥45 (baseline 2006-2008).

The Rotterdam Study has been approved by the medical ethics committee according to the Population Screening Act: Rotterdam Study, executed by the Ministry of Health, Welfare and Sports of the Netherlands.

Study population

We selected data from participants from the third visit of the first cohort (1997-1999, n=4797) and the first visit of the second (2000-2001, n=3011) and third cohort (2006-2008, n=3932), if thyroid-stimulating hormone (TSH) or free thyroxine (FT4) measurements were performed and if information on diabetes was available. All participants in the present analysis provided written informed consent to participate and to obtain information from their treating physician. All study participants were followed up from the day of baseline laboratory testing to date of onset of (pre-)diabetes, to death, or to January 1, 2012, whichever came first.

Assessment of thyroid function

Thyroid function was measured using the same methods and assay, and samples were collected between 1997 and 2008, depending on the cohort. TSH and FT4 measurements were performed in serum samples stored at -80°C (The electrochemiluminescence immunoassay for thyroxine and thyrotropin, “ECLIA”, Roche). We determined cut-off values for the reference range of TSH as 0.4-4.0 mIU/L and FT4 as 11-25 pmol/L (0.86-1.94 ng/dL) according to guidelines as well

as our previous studies [21]. Thyroid peroxidase antibodies (TPOAb) levels greater than 35 kU/mL were regarded as positive, as recommended by the assay manufacturer (The electrochemiluminescence immunoassay for thyroid perioxidase antibodies, "ECLIA", Roche).

Ascertainment of prediabetes and type 2 diabetes

The participants were followed from the date of baseline center visit onwards. At baseline and during follow-up, cases of prediabetes and type 2 diabetes were ascertained through active follow-up using general practitioners' records, hospital discharge letters and serum glucose measurements from Rotterdam Study visits which take place approximately every 4 years [22]. Normoglycemia, prediabetes and diabetes were defined according to recent WHO guidelines [23]. Normoglycemia was defined as a fasting serum glucose < 6.0 mmol/L; prediabetes was defined as a fasting serum glucose > 6.0 mmol/L and < 7.0 mmol/L or a non-fasting serum glucose > 7.7 mmol/L and < 11.1 mmol/L (when fasting samples were absent); type 2 diabetes was defined as a fasting serum glucose ≥7.0 mmol/L, a non-fasting serum glucose ≥ 11.1 mmol/L (when fasting samples were absent), or the use of blood glucose lowering medication. Information regarding the use of blood glucose lowering medication was derived from both structured home interviews and linkage to pharmacy records. At baseline, more than 95% of the Rotterdam Study population was covered by the pharmacies in the study area. All potential events of prediabetes and type 2 diabetes were independently adjudicated by two study physicians. In case of disagreement, consensus was sought with an endocrinologist [22].

Baseline measurements

Body mass index was calculated as body mass (kg) divided by the square of the body height (m). Serum HDL cholesterol and glucose were measured using standard laboratory techniques. Information on tobacco smoking was derived from baseline questionnaires. Systolic and diastolic blood pressure was calculated as the average of two consecutive measurements. Insulin was measured using an immunoassay (electrochemiluminscence immunoassay "ECLIA", Roche). Over 95% of participants were in a fasting state when blood was drawn at the Rotterdam Study center visit. Information on medication use was obtained from questionnaires in combination with pharmacy records. Thyroid medication, including thyroid

hormone replacement therapy, was prescribed by participant's own GP or specialist and within the context of regular treatment and blinded to measurements of the Rotterdam Study.

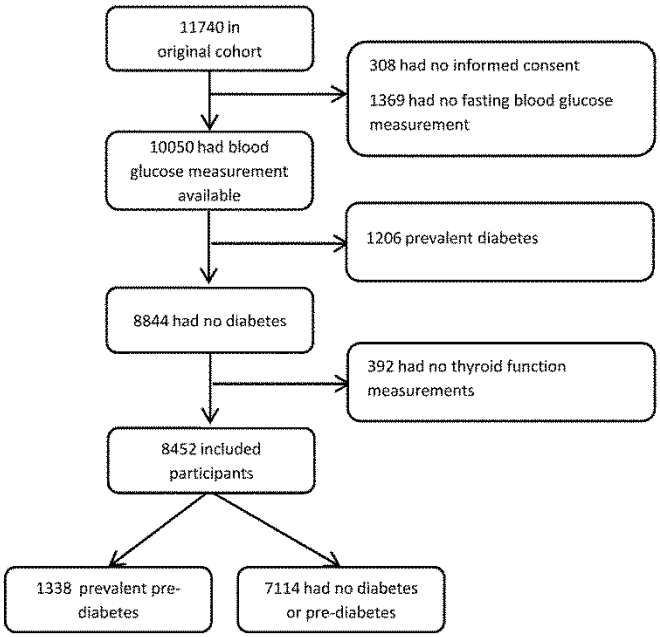
Statistical methods

We used Cox-proportional hazards models to assess the association of TSH or FT4 with incident diabetes or prediabetes. We also assessed the association of thyroid function measurements and incident diabetes in participants with prediabetes separately. We conducted these analyses first in all included participants and then only in those with normal TSH and FT4 values, after excluding levothyroxine users. The primary model, model 1, adjusted for age, sex, cohort, fasting glucose and tobacco smoking. Model 2 additionally adjusted for possible confounders or intermediate factors, including fasting serum insulin, systolic blood pressure, diastolic blood pressure, use of blood pressure lowering medication (diuretics, anti-adrenergic agents, β blockers, calcium channel blockers and RAAS inhibitors), HDL cholesterol and BMI. Furthermore, we assessed the association of TSH and FT4 tertiles in the normal reference range with progression from prediabetes to diabetes and calculated absolute risk estimates for the tertiles, using the covariates of the multivariable model. We performed the following sensitivity analyses 1) excluding participants using levothyroxine at baseline 2) excluding participants using thyroid function altering medication, including levothyroxine, anti-thyroid drugs (e.g. thiamazole), amiodarone and corticosteroids at baseline and follow-up 3) additionally excluding participants with TSH and FT4 values outside the normal range. We stratified by possible effect modifiers including age categories (cut-off of 65 years), and sex. The natural logarithm of TSH was used for the continuous models. The proportional hazards assumption was assessed by performing Schoenfeld tests and plots and was met for all analyses. There was no departure from linearity as assessed by restricted cubic splines or adding quadratic terms of TSH, FT4 or age to the model. Reporting of the results is according to the STROBE statement.

RESULTS

We included a total of 8452 participants with thyroid function measurements and free of diabetes at baseline (Figure 1). The mean age of the included participants was 64.9 years and 58% was female. Baseline characteristics are shown in Table 1. During a mean follow-up of 7.9 years (standard deviation 4.0 years), 1100 participants developed prediabetes (incidence rate [IR] 14 per 1000 person-years) and 798 developed diabetes (IR 12 per 1000 person-years). Completeness of follow-up was 99.4% [24].

Figure 1 Participants selection



Thyroid function and incident diabetes

Higher TSH levels were associated with a higher risk of diabetes with a Hazard Ratio (HR) of 1.13 in model 1 (95% confidence interval [CI], 1.08-1.18, Table 2).Within the normal range the risk of diabetes was 1.24 times higher with higher

TSH levels. In model 2, this association attenuated slightly (HR 1.21, CI, 1.03-1.42, Table 2). In the most adjusted model (model 2), higher FT4 levels were associated with a decreased risk of diabetes (HR 0.96, 95% CI, 0.93-0.99), also within the normal range (HR 0.94, 95% CI, 0.90-0.98). Sensitivity analyses did not change risk estimates meaningfully (Supplemental Table 1). Stratifying the analyses by age category or sex did not show effect modification for incident diabetes (p for interaction > 0.05 for all).

Table 1 Baseline characteristics of included participants

Variable	Mean (SD)*
Number of individuals in the study	8452
Age, in years	64.6 (9.7)
Female, n (%)	4899 (58.0)
BMI, kg/m^2	26.5 (4.05)
Total Cholesterol, mmol/L	5.76 (1.01)
HDL Cholesterol, mmol/L	1.43 (0.41)
Smoking, n (%)	
Current	1742 (20.6)
Former	4020 (47.6)
Never	2691 (31.8)
Systolic blood pressure, mmHg	139 (21)
Diastolic blood pressure, mmHg	79 (11)
Antihypertensive medication use, n (%)	1881 (22.3)
TSH, median (IQR)	1.91 (1.29-2.76)
FT4, pmol/L	15.7 (2.32)
TPOAb positivity, n (%)	1119 (13.2%)
Levothyroxine use, n (%)	233 (2.8)

*unless specified otherwise
TPOAb levels >35 kU/mL were regarded as positive.
Abbreviations: BMI Body mass index, IQR interquartile range, FT4 free thyroxine, SD standard deviation, TPOAb thyroid peroxidase antibodies, TSH Thyroid-Stimulating Hormone, n number

Thyroid function and incident prediabetes

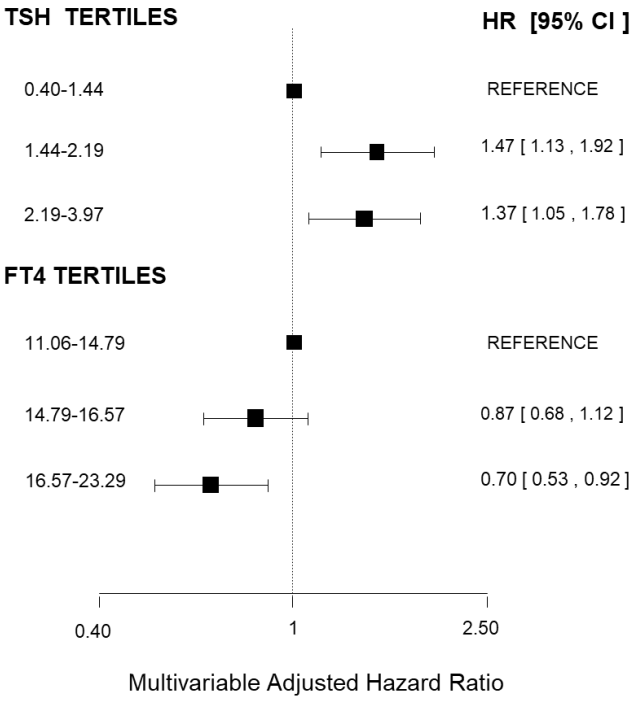
In model 2, the risk of developing prediabetes was higher with higher TSH levels (HR 1.04, 0.97-1.12, Table 2) but not significantly and significantly lower with higher FT4 levels (HR 0.98, 95% CI, 0.95-1.00). When restricting the analyses to the normal range, HR was 1.12 (95% CI, 0.97-1.12) and 0. 96 (95% CI 0.93-0.99) for TSH and FT4 respectively (Table 2).

Table 2 Association between thyroid function and the risk of incident prediabetes and diabetes

Thyroid function measurements	HR (95% CI) Model 1	HR (95% CI) Model 2	Incident Cases	Total participants
Incident Diabetes				
Full range of measurement				
TSH mIU/L	1.13 (1.08-1.18)	1.09 (1.00-1.19)	798	8447
Free T4 pmol/L	0.96 (0.93-0.99)	0.96 (0.93-0.99)	797	8446
Normal TSH and FT4 values				
TSH mIU/L	1.24 (1.06-1.45)	1.21 (1.03-1.42)	685	7188
Free T4 pmol/L	0.96 (0.92-0.99)	0.94 (0.90-0.98)	685	7188
Incident Prediabetes				
Full range of measurement				
TSH mIU/L	1.06 (0.99-1.14)	1.04 (0.97-1.12)	1100	7110
Free T4 pmol/L	0.97 (0.94-0.99)	0.98 (0.95-1.00)	1100	7110
Normal TSH and FT4 values				
TSH mIU/L	1.15 (1.00-1.32)	1.12 (0.98-1.29)	936	6051
Free T4 pmol/L	0.96 (0.92-0.99)	0.96 (0.93-0.99)	936	6051
Progression from Prediabetes to Diabetes				
Full range of measurement				
TSH mIU/L	1.25 (1.11-1.41)	1.19 (1.05-1.36)	412	1337
Free T4 pmol/L	0.92 (0.89-0.97)	0.93 (0.89-0.98)	411	1336
Normal TSH and FT4 values				
TSH mIU/L	1.39 (1.11-1.74)	1.32 (1.06-1.64)	358	1137
Free T4 pmol/L	0.90 (0.85-0.95)	0.91 (0.86-0.97)	358	1137

Model 1: adjusted for sex, age, smoking, fasting serum glucose levels and cohort. Model 2: adjusted for sex, age, smoking, cohort, fasting serum glucose levels, fasting serum insulin measurements, systolic blood pressure, diastolic blood pressure, blood pressure lowering medication, HDL cholesterol and body mass index. Normal range of TSH is defined by 0.4-4.0 mIU/L and normal range FT4 is defined by 11-25 pmol/L and participants not using levothyroxine.
Abbreviations: CI confidence interval, FT4 free thyroxine, HR hazard ratio, TSH thyroid-stimulating hormone.

Figure 2 Association of TSH and FT4 Levels in tertiles within the normal range and Incident Diabetes in individuals with prediabetes



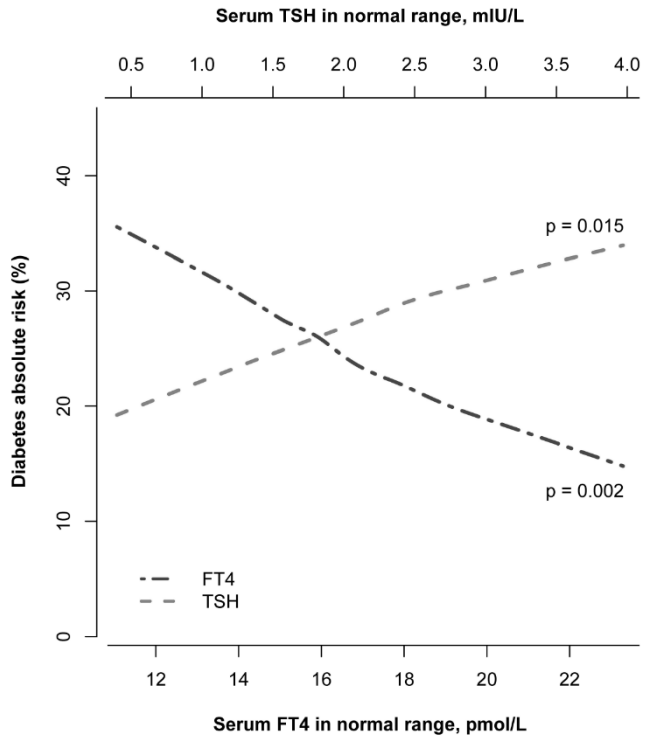
The normal range of TSH defined as 0.4-4.0 mIU/L and of FT4 as 11-25 pmol/, thyroid hormone medication users were excluded. Analyses adjusted for sex, age, smoking, cohort, fasting glucose, serum insulin measurements, systolic blood pressure, diastolic blood pressure, blood pressure lowering medication, cholesterol and body mass index. Abbreviations: AF = atrial fibrillation, TSH = thyroid-stimulating hormone, FT4 = free thyroxine, HR = hazard ratio, CI = confidence interval.

Thyroid function and progression of prediabetes to diabetes

In participants with prediabetes, the risk of developing diabetes was 1.19 times higher per 1 unit of log transformed TSH levels mIU/L (95% CI, 1.05-1.36, Table 2). The risk of incident diabetes in participants with prediabetes is 0.93 times lower with each 1 pmol/L increase of FT4 (95% CI, 0.89-0.98). In the normal range, the risk of developing diabetes was 1.44 times higher (95% CI, 1.13-1.93) when comparing the highest to the lowest tertile of TSH in the normal range in model 1 (Supplemental Table 3). This corresponds to an absolute risk difference of 8.5% for a follow-up of 7 years. Comparing the highest tertile of FT4 to the lowest tertile, the

HR for developing diabetes in individuals with prediabetes was 0.63 (95% CI, 0.48-0.82, Supplemental Table 3). Additionally adjusting analyses for TPOAb positivity did not change risk estimates meaningfully (data not shown). This corresponds to a 1.59 times higher risk and an absolute risk difference of 9.6% of progression to diabetes when comparing the lowest to the highest tertile of FT4 (Supplemental Table 3). These associations attenuated only slightly in model 2 (Figure 2, Supplemental Table 3). Absolute risk of diabetes type 2 in participants with prediabetess decreased from 35% to almost 15% with higher FT4 levels within the normal range (Figure 3).

Figure 3 The 7-year absolute risk of type 2 diabetes is plotted against TSH and FT4 values within the normal range.



These analyses are adjusted for sex, age, smoking, cohort, fasting serum glucose levels, fasting serum insulin measurements, systolic blood pressure, diastolic blood pressure, blood pressure lowering medication, HDL cholesterol and body mass index. Abbreviations: TSH = thyroid-stimulating hormone, FT4 = free thyroxine.

DISCUSSION

To our knowledge, this is the first prospective population-based cohort study describing the relation between thyroid function and the risk of progression from normoglycemia to prediabetes and type 2 diabetes. Higher TSH levels and lower FT4 levels, both outside as well as within the reference range, are associated with an increased risk of diabetes and progression from pre-diabetes to diabetes.

In contrast to the study by Brandt et al., reporting an increased risk of diabetes in hyperthyroid individuals, based on the same Danish nationwide registry data [16], we do not find an increased risk of diabetes in high thyroid function.

There are no other studies addressing the relation between diabetes and thyroid function in the euthyroid range or in individuals with prediabetes. Our results differ from the findings of Fleiner et al. [19] that do not find a higher prevalence of hypothyroidism in type 2 diabetes patients. However the results by Fleiner et al. were cross-sectional and therefore did not address the risk of diabetes development (i.e. temporality). In addition FT4 measurements were not performed in all participants. Our results are in contrast to a Danish nationwide registry study, that reported an increased risk of diabetes in hyperthyroid individuals [16], whereas we do not find an increased risk of diabetes in high thyroid function. However our results are h largely in line with two register-based studies reporting an increased risk of diabetes in hypothyroid individuals [17,18].

There are several pathways that may explain the observed relation between low and low-normal thyroid function and the risk of diabetes. Overt and subclinical hypothyroidism are associated with a decreased insulin sensitivity and glucose tolerance, partially due to a decreased ability of insulin to increase glucose utilization in mainly muscle [14,25]. Other mechanisms, such as downregulation of plasma membrane glucose transporters and direct effects on insulin degradation have also been described [26-28]. Treatment of hypothyroidism has been shown to restore insulin sensitivity and the secretion of glucoregulatory hormones [15]. Furthermore, hypothyroidism is associated with several components of the metabolic syndrome and could therefore indirectly relate to the increased risk of diabetes [29]. However, in our analyses, adjusting for several cardiovascular risk factors and components of the metabolic syndrome did not shift risk estimates

towards the null. Also, excluding participants using thyroid hormone replacement therapy at baseline only slightly altered the results. Even though overt hyperthyroidism is also associated with insulin resistance, our data show that high and high-normal thyroid function are protective for developing of or progressing to diabetes. It could be that insulin resistance in hyperthyroid patients is counterbalanced by other mechanisms associated with prolonged thyroid hormone excess, such as improved beta-cell function and increase insulin secretion [6]. However, the exact pathophysiological mechanisms through which thyroid function could affect diabetes risk in the general population still has to be determined.

The clinical importance of these findings could be several. First of all, the association of thyroid function with development from prediabetes to diabetes is prominent. Thus, individuals with a low-normal thyroid function, which includes a large proportion of the population, are yet at a higher risk of progression from prediabetes to diabetes. Secondly, with aging and increasingly obese populations, there is need for better screening and prevention options for diabetes [30]. One could hypothesize that in individuals with prediabetes with low or low-normal thyroid function (i.e. high TSH and low FT4), lifestyle interventions or diabetes treatment could be prompted in an earlier phase than those with normal or high thyroid function. Alternatively, having prediabetes could be an argument to start treatment of subclinical hypothyroidism to aim for prevention of overt diabetes. Current guidelines do not recommend or specifically address screening of thyroid function or treatment of thyroid dysfunction in individuals with diabetes [31,32].

despite the high prevalence of both conditions in the general population, the relation between thyroid dysfunction and diabetes had been largely unexplored. Further research is needed to determine to what extend this is driven by thyroid hormone related acceleration of development of diabetes or perhaps by other mechanisms such as a common genetic predisposition. Subsequent studies should focus on screening and prevention strategies as well as questions concerning treatment of patients with subclinical hypothyroidism in patients at risk for diabetes.

Strengths of our study include the large number of individuals, the variety of available confounders adjusted for and the long follow-up. Furthermore, we were able to investigate both diabetes and prediabetes. Limitations of our study should also be acknowledged. Residual confounding cannot be excluded in an

observational study, even with the large number of potential confounders adjusted for in our analyses. Furthermore, the Rotterdam Study constitutes of dominantly white participants of 45 years and older and results may therefore not be generalizable to other populations.

Conclusions

In conclusion, low and low-normal thyroid function are related to an increased risk of diabetes and prediabetes. In individuals with prediabetes, the risk of progression to diabetes with low and low-normal thyroid function is more prominent. These data provide new insights into the magnitude of the risk of diabetes and prediabetes associated with variations of thyroid function. More research is needed confirming these current findings in additional populations and addressing possible screening and treatment modalities for both diabetes and thyroid dysfunction.

Online supplemental material

https://bmcmedicine.biomedcentral.com/articles/10.1186/s12916-016-0693-4

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CHAPTER 4.2

THYROID FUNCTION AND THE RISK OF NON-ALCOHOLIC FATTY LIVER DISEASE

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ABSTRACT

BACKGROUND Although thyroid function is associated with several risk factors of non-alcoholic fatty liver disease (NAFLD), its role in NAFLD development remains unclear. We aimed to prospectively investigate the association between variations in thyroid function and NAFLD.

METHODS The Rotterdam Study, a large population-based, prospective cohort study.
Participants and main outcome measures: Participants with thyroid function measurements at baseline and NAFLD data (i.e. 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 cut-off 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: 5.70-10.88 years). After adjusting for age, sex, cohort, follow-up time, use of hypolipidemic drugs and cardiovascular risk factors, higher free thyroxine levels were associated with a decreased risk of NAFLD (Odds ratio [OR], 0.42; 95% confidence interval [CI], 0.28-0.63). In line, higher thyroid-stimulating hormone levels were associated with an increased risk of having clinically relevant fibrosis in NAFLD (OR, 1.49; CI, 1.04-2.15). Compared to euthyroidism, hypothyroidism was associated with a 1.24-fold higher NAFLD risk (CI, 1.01-1.53). Moreover, NAFLD risk decreased gradually from hypothyroidism to hyperthyroidism (p-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

Non-alcoholic fatty liver disease (NAFLD) is one of the the most common chronic liver conditions worldwide.[1] It comprises a broad spectrum ranging from simple steatosis to non-alcoholic steatohepatitis (NASH) with fibrosis, which can eventually progress to cirrhosis and hepatocellular carcinoma.[2,3] NASH-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 (e.g. diabetes 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 FT4 alone,[12] 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.

MATERIALS AND 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 (RSI). In 2000, the study was extended with a second cohort of 3011 subjects (RSII). In 2006, a third cohort of 3932 subjects aged 45 years and over was added (RSIII), and thereafter the study population comprised a total of 14926 subjects.Participants from study cohorts RSI visit 3 (RSI-3), RSII visit 1 (RSII-1) and RSIII visit 1 (RSIII-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 three independent RS cohorts using the same method and assay. Thyroid function assessment was performed for thyroid-stimulating hormone (TSH), free thyroxine (FT4) and thyroid peroxidase antibodies (TPOAb) in baseline serum samples stored at -80°C (The electrochemiluminescence immunoassay for thyroxine, thyrotropin and thyroid peroxidase antibodies, “ECLIA”, Roche). We determined the reference range of TSH (0.4-4.0 mIU/L) and FT4 (0.85-1.95 ng/dl [to convert to picomoles per liter, multiply by 12.871] [alternatively 11-25 pmol/L]), 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 FT4 levels within the reference range. Overt hypothyroidism was defined as serum TSH >4.0 mIU/L and FT4 levels <0.85 ng/dl. Subclinical hyperthyroidism was defined as serum TSH <0.4 mIU/L and FT4 levels within the reference range. Overt hyperthyroidism was defined as serum TSH <0.4 mIU/L and FT4 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 (i.e. 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 (GGT), body mass index (BMI) and waist circumference (WC), was calculated by the formula previously described by Bedogni et al.[19] The accuracy of FLI in the detection 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.[21] Therefore, we used a cut-off 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, Paris, France). 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]

Assessment of other baseline measurement

Information was obtained from each participant through a home questionnaire concerning demographics, medical history, alcohol intake, tobacco smoking and medication use. Blood lipids, glucose, GGT, were measured using automatic enzymatic procedures (Roche Diagnostics GmbH, Mannheim, Germany). BMI was calculated as weight in kilograms divided by height in meters squared. WC 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 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 medication.

Statistical analysis

We prospectively assessed the association between thyroid parameters (TSH, FT4 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 FT4, taking the highest tertile as reference. After our primary analyses, we performed sensitivity analyses, restricting to subjects with TSH and FT4 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 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. Herein, 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 hypolipidemic drugs, total cholesterol, triglycerides, BMI, hypertension, diabetes (model 2). Lipids, BMI, hypertension, diabetes 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 $[(OR_{con\ adj} - OR_{con+med\ adj})/(OR_{con\ adj} - 1)] \times 100\%$, where $OR_{con\ adj}$ is the confounder-adjusted OR and $OR_{con+med\ adj}$ 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 WC 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 FT4) with covariates of the multivariable model, but none of the interaction terms were significant. There was no departure from linearity for the TSH and FT4 analyses, assessed by adding quadratic terms of covariates in the multivariable model. Multiple imputations were performed in case of missing covariates (less than 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), Vienna, Austria, version 3.0.2). Reporting is done according to the STROBE statement.

RESULTS

We included 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.

Table 1 Baseline characteristics of 9419 participants

Characteristics	**Mean (SD)***
Age, years	64.7 (9.7)
Female, n (%)	5321 (56.5)
Smoking, n (%)	
Current	1989 (21.1)
Past	4490 (47.7)
Never	2940 (31.2)
Use of hypolipidemic medication, n (%)	1508 (16.0)
Use of thyroid medication, n (%)	296 (3.1)
Total cholesterol, mmol/l	5.7 (1.0)
HDL-C, mmol/l	1.4 (0.4)
Triglycerides, mmol/l	1.5 (0.8)
Body-mass index, kg/m2	27.2 (4.2)
Waist circumference, cm	93.7 (12.1)
Hypertension, n (%)	5881 (62.4)
Diabetes, n (%)	1073 (11.4)
TSH, mIU/L, median (IQR)	1.9 (1.3-2.8)
FT4, ng/dl	1.2 (0.1)
TPOAb positive, n (%)	1240 (13.2)

*Data are mean and standard deviation, unless otherwise specified.
Abbreviations: sd, standard deviation; HDL-C, high density lipoprotein cholesterol; BMI, body-mass index; TSH, thyroid-stimulating hormone; IQR, interquartile range; FT4, free thyroxine; TPOAb, thyroid peroxidase antibodies.

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 5.7-10.9 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.

Thyroid parameters / status and the risk of NAFLD

The risk of NAFLD decreased gradually with higher FT4 levels (Odds ratio [OR], 0.33; 95% confidence interval [CI], 0.22-0.48 per 1ng/dl) (Table 2). These results remained similar after further adjustments for cardiovascular risk factors (OR, 0.42; CI, 0.28-0.63), and also after restricting the analyses to participants with baseline FLI <60 (OR, 0.42; CI, 0.24-0.74). In the multivariable adjusted model, participants in the lowest FT4 tertile had a 1.31 times higher risk of NAFLD, compared to those in the highest tertile (CI, 1.11-1.56) (Supplemental Table 2). There was a positive linear association between TSH levels and NAFLD risk (OR, 1.09; CI, 1.01-1.19 per 1 logTSH), which was attenuated after additional adjustment for cardiovascular risk factors (OR, 1.07; CI, 0.98-1.17) (Table 2).

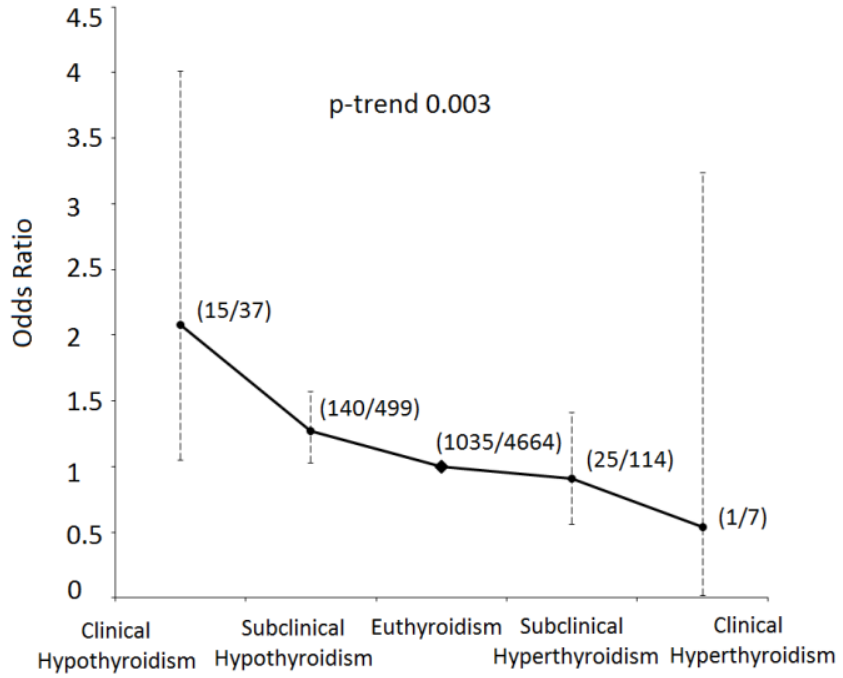
Table 2 Longitudinal association between thyroid function and NAFLD risk

	NAFLD events/ total number	**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)
FT4	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)
FT4	553/3376	0.42 (0.24; 0.74)	0.52 (0.29; 0.92)

Model 1: age, sex, cohort, alcohol intake, smoking, follow-up time.
Model 2: predictors in model 1, use of hypolipidemic drugs, total cholesterol, triglycerides, body mass index, hypertension, diabetes. Abbreviations: NAFLD, non-alcoholic fatty liver disease; OR, odds ratio; CI, confidence interval; TSH, thyroid-stimulating hormone, is per one unit increase of log transformed TSH (mIU/L); FT4, free thyroxine, is per one unit increase of FT4 (ng/dl); FLI, fatty liver index.

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 NAFLD and 13.4% in the association of FT4 with NAFLD; that is 22.2% and 13.4% of the respective associated effect size of TSH and FT4 on NAFLD is explained by BMI and triglycerides. No significant association was observed for TPOAb and NAFLD risk (OR, 1.09; CI, 0.89-1.32) (Supplemental Table 2). There was a significant trend (p for trend 0.003) in the decrease of NAFLD risk (OR from 2.08 to 0.54), across categories of thyroid function from clinical hypothyroidism to clinical hyperthyroidism (Figure 1, Supplemental Table 4).

Figure 1 Longitudinal association between thyroid status and NAFLD



Point estimates for NAFLD (non-alcoholic 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 (0.4-4.0 mIU/l); overt hypothyroidism as TSH>4.0 mU/L and FT4 (free thyroxine)<0.85 ng/dl; subclinical hypothyroidism as TSH>4.0 mU/L and FT4 0.85-1.95 ng/dl; overt hyperthyroidism as TSH<0.4 mU/L and FT4>1.95 ng/dl; subclinical hyperthyroidism as TSH<0.4 mU/L and FT4 0.85-1.95 ng/dl. Dashed lines represent confidence intervals. Within brackets: NAFLD events/Total number.

Compared to euthyroidism, hypothyroidism was associated with a 1.24-fold (CI, 1.01-1.53) higher risk of NAFLD (Table 3). Cross-sectional analyses, based on categorized FLI, demonstrated a significant association of TSH (OR, 1.11; CI, 1.04-1.18) and FT4 (OR, 0.45; CI, 0.34-0.60) 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, Supplemental Table 5).

Table 3 Longitudinal association between thyroid status and NAFLD risk

	NAFLD events/ total number	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, follow-up time.
Model 2: predictors in model 1, use of hypolipidemic drugs, total cholesterol, triglycerides, body mass index, hypertension, diabetes. * includes subclinical and clinical range.
Abbreviations: NAFLD, non-alcoholic fatty liver disease; OR, odds ratio; CI, confidence interval.

Table 4 Longitudinal association between thyroid function/ status and the risk of having a combination of NAFLD and LS ≥8 kPa*#

	NAFLD with LS ≥8.0 kPa/ Total number	OR (95% CI) Model 1	OR (95% CI) Model 2
Longitudinal association between 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)
FT4	69/4762	0.41 (0.09; 1.73)	0.59 (0.13; 2.59)
Longitudinal association between 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, follow-up time.
Model 2: predictors in model 1, use of hypolipidemic drugs, total cholesterol, triglycerides, body mass index, hypertension, diabetes. #For this analysis, we excluded thyroid medication users and participants with previous thyroid surgery. *LS ≥8.0 kilopascals suggests clinically relevant fibrosis.Abbreviations: NAFLD, non-alcoholic fatty liver disease; LS, Liver stiffness; kPa, kilopascals, OR, odds ratio; CI, confidence interval; TSH, thyroid-stimulating hormone, is per one unit increase of log transformed TSH (mIU/L); FT4, free thyroxine, is per one unit increase of FT4 (ng/dl); NA, not applicable.

Thyroid parameters / status 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; CI, 1.09-2.20). In line, higher FT4 levels were associated with a lower risk of having a combination of NAFLD

and LS ≥8.0 kPa, but not significantly (OR 0.41; CI, 0.09-1.73) (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 to euthyroidism, subclinical hypothyroidism was associated with a 2.30-fold (CI, 1.12-4.31) higher risk of having a combination of NAFLD and LS ≥8 kPa (Table 4). Results remained similar after further adjustments for cardiovascular risk factors (Table 4). In euthyroid subjects, higher TSH and lower FT4 concentrations were associated with an increased risk of having a combination of NAFLD and LS ≥8 kPa, but not significantly (OR, 1.13; CI, 0.63-2.03 for TSH) (OR, 0.81; CI, 0.11-5.75 for FT4).

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 FT4 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 (e.g. 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 (TR) agonists.[26-28] Thyroid hormone induces intrahepatic lipolysis through lipophagy, that involves the

sequestration and degradation of lipid droplets within hepatic lysosomes.[29] Moreover, TR-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, since various autoantibodies such as antinuclear antibodies and anti-smooth 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 FT4 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 endpoints have shown that FT4, 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 aging can modify the TSH-FT4 set point of the feedback mechanism, accounting for the weaker TSH-FT4 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 to 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 FT4 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

In summary, individuals with hypothyroidism are at increased risk of NAFLD compared to euthyroid subjects. The current study also reveals a negative linear association between FT4 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 (e.g. screening of thyroid function in NAFLD patients) and possible therapeutic interventions (e.g. decision of treatment in subclinical thyroid dysfunction).

Online supplemental material

https://academic.oup.com/jcem/article-abstract/101/8/3204/2835059/Thyroid-Function-and-the-Risk-of-Nonalcoholic?redirectedFrom=fulltext

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CHAPTER 5

AGING

CHAPTER 5.1

THYROID FUNCTION AND CANCER RISK

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ABSTRACT

BACKGROUND In vitro and in vivo experiments have assigned both oncosuppressive and oncogenic properties to thyroid hormones. Population-based studies found inconclusive results. We aimed to prospectively assess the relation between thyroid function and incident cancer in a population based setting.

METHODS The current study is a prospective population-based cohort study including 10,318 participants for whom baseline measurements of free thyroxine (FT4) and/or thyrotropin (TSH) were available. Cox proportional hazards models were used to assess hazard ratios (HRs) of any solid non-skin cancer, as well as lung, breast, prostate and gastrointestinal cancer specifically.

RESULTS Higher FT4 levels were associated with a higher risk of any solid cancer (HR: 1.42; 95% confidence interval (CI): 1.12-1.79), lung cancer (HR: 2.33; 95% CI: 1.39-3.92) and breast (HR: 1.77; 95% CI: 1.10-2.84) cancer. The risk estimates were similar after exclusion of thyroid-altering medication, but the association lost significance for breast cancer. Compared to the lowest FT4 tertile, the highest tertile was associated with a 1.13 fold increased risk of any solid, 1.79 fold increased risk of lung and 1.14 fold increased risk of breast cancer (p for trend < 0.05 for all). For TSH levels we found no associations with cancer risk. There was no differential effect of sex or age on the association between thyroid function and cancer risk.

CONCLUSIONS Higher FT4 levels are significantly associated with an increased risk of any solid, lung and breast cancer. Further research should elucidate the underlying pathophysiological mechanisms.

INTRODUCTION

Thyroid hormone plays an important role in growth, differentiation, development and metabolism. Via binding to nuclear thyroid hormone receptors (TRs), thyroid hormone can induce or inhibit gene transcription.[1,2] Many pathways influenced by thyroid hormone also play a role in tumorigenesis. For example, induction of deiodinase 3 (D3), an enzyme inactivating thyroid hormone, sustains proliferation in colon carcinoma, suggesting a link between local hypothyroidism and tumor growth.[3] In addition, it has been demonstrated that thyroid hormone has a direct effect on oncogenic pathways, such as the PI3K- and ERK1/2-pathways.[4-7] Thyroid hormone and TRs have been linked to increased proliferation in breast, ovarian and prostate cancer cell lines.[2] On the other hand, protective effects of thyroid hormone have also been hypothesized.[1,2] Thyroid hormone suppresses unregulated cell proliferation by inhibiting activating protein 1 (AP1).[1,2] Various tumor types, such as lung, breast and liver cancer, are associated with inactivating mutations in TRs that block access of wild-type TRs to the target genes, yielding lower thyroid hormone action.[1,2] Restoring wild-type expression of TRβ1 in liver and breast cancer cell lines retards tumor growth and suppresses tumor invasiveness and metastasis.[8]

Population-based studies have associated both overt and subclinical hyperthyroidism to cancer.[9,10] Hyperthyroidism has been associated with an increased risk of prostate[11], breast[12-14] and lung cancer[9], whereas hypothyroidism is linked to a decreased risk of prostate cancer[11] and both an increased[15] and decreased [13,16] risk of breast cancer.

However, the data regarding the link between thyroid dysfunction and cancer do not provide conclusive evidence mainly due to differences in study design. Previous reports suffered from a small sample size (< 30)[12] or a retrospective study design and/or unavailability of thyroid function serum measurements [10,13,16]. Also, most studies restricted their analyses to a specific cancer type[11-16] or cancer mortality[17], instead of focusing on any cancer occurrence and did not assess the relation in a time-to-event analysis.[11-16]

There is only one large longitudinal study investigating the association between thyroid function and cancer risk. A study by Hellevik et al.[9], linked low TSH levels

to a higher risk of cancer. However, in this record-linkage study thyroid function of participants was measured only in a subsample of the population and FT4 measurements were only performed in subjects with TSH values suggesting hyperthyroidism. Furthermore, risk estimates were only adjusted for a limited number of potential confounders and no information on thyroid medication use was available.

For this reason, our objective was to assess the association between thyroid function in the full range (i.e. as a continuous variable, comprising both the normal range and values outside the reference interval of thyroid hormone) and incident cancer in general and lung, breast, prostate and gastrointestinal cancer in particular in a large prospective cohort study.

METHODS

Study design and population

The Rotterdam Study is a prospective cohort study that started in 1990. By the end of 2008 14,926 subjects aged 45 years or over were included. The Rotterdam Study consists of three cohorts, RS I, RS II and RS III. RS I started in 1990 and recruited 7,983 subjects aged 55 years and over from Ommoord, Rotterdam. In 2000 RS II started and 3,011 people who had turned 55 years or had moved into the study district were added to the cohort. The third extension, RS III started in 2006 and included 3,392 Ommoord residents of 45 years and over that had not been previously invited to participate. This study was approved by the Erasmus MC Medical Ethics Committee and the Dutch Ministry of Health, Welfare and Sports. Details on the Rotterdam Study can be found elsewhere.[18,19]

We included participants for whom we had measurements of thyrotropin (TSH) and/or free thyroxine (FT4) and who had given informed consent for follow-up (n = 10,318). Patients with a cancer diagnosis before the measurement of thyroid function were excluded from the analyses. We excluded patients with a history of the cancer type specific for that analysis. Based on these criteria, 4,949 subjects were excluded from the analyses. Subjects were followed from their date of laboratory measurements until any cancer diagnosis, death or January 1st 2012, whichever occurred first.

Assessment of thyroid function

For RS I-1, TSH (TSH Lumitest; Henning, Berlin, Germany) and FT4 (FT4; Vitros, ECI Immunodiagnostic System; Ortho-Clinical Diagnostics, Amersham, UK) were assessed in blood samples collected in 1990. For RS I-3, II-1 and III-1, TSH and FT4 (electrochemiluminescence immunoassay for thyroxine and thyrotropin, "ECLIA", Roche) were assessed in blood samples collected between 1997 and 2008, depending on the RS cycle.[20] The moment of blood drawing was considered the study baseline.[21] The normal range of TSH was 0.4-4.0 mU/L, as recommended by national guidelines and our previous reports.[20,21] For FT4, the normal range comprised 0.85-1.94 ng/dL (11-25 pmol/L).[20] The measurements of the two assays were highly correlated (Spearman correlation co-efficient (r) = 0.96 for TSH, $P < 0.0001$ and r = 0.81 for FT4, $P < 0.0001$).

Assessment of cancer outcomes

The primary outcome of interest was the occurrence of any solid cancer, except for non-melanoma skin cancers. Furthermore lung cancer (ICD-10; C34), breast cancer (ICD-10; C50), prostate cancer (ICD-10; C61) and gastrointestinal cancer (ICD-10; C15-C21) were assessed separately.

Occurrence of cancer was determined through information obtained by four-yearly follow-up rounds from the general practitioners (including discharge letters from hospitals) and by linkage with a nationwide registry of histo- and cytopathology in the Netherlands (PALGA).[22] Two research physicians independently assessed the first date and diagnosis of cancer. All events are pathology based and were classified according to the International Classification of Diseases (ICD) tenth edition. In case of discrepancy, consensus was sought or a cancer epidemiologist decided.

Baseline measurements

Highest attained education was taken as a proxy for socioeconomic status (SES) and was derived from questionnaires. Alcohol intake and tobacco smoking were determined by questionnaires. Alcohol intake was measured in grams per day and smoking status was defined as non-smoker, former smoker or current smoker. Body mass index (BMI) was assessed during physical examination by dividing the body weight in kilograms by the squared height in meters. Blood pressure was the average of two measurements during physical examination. Hypertension was

defined as a systolic blood pressure of ≥ 140 mm Hg, a diastolic blood pressure of ≥ 90 mm Hg or the use of antihypertensive drugs. Diabetes mellitus was defined as a fasting plasma glucose level of ≥ 7 mmol/L, a non-fasting plasma glucose level of ≥ 11.1 mmol/L (if fasting glucose was not present) or the use of antidiabetic medication. Serum cholesterol (mmol/L) was measured in laboratories of the Erasmus Medical Center. Furthermore, subjects provided information on breast cancer specific confounders (including number of pregnancies, hormone use, menarche age, menopause age and menopausal status). Hormone use referred to use of female hormones for menopausal complaints or use of oral contraceptives within 7 years of menopause and was categorized as ever or never. The reproductive lifespan was calculated by subtracting the menarche age from the menopause age. Menopausal status was defined as being perimenopausal or postmenopausal.

Statistical analysis

We used Cox proportional hazards models to obtain hazard ratios (HRs) with their 95% confidence interval (95% CI) for the association between thyroid function and cancer. We conducted analyses in the full range of FT4 and TSH and if an association was present, we also assessed the association between thyroid hormone tertiles and incident cancer separately. Furthermore, we calculated the incidence rate per 10,000 person-years per tertile. In our first model, we adjusted for age (as a continuous variable), sex and cohort. In a second model, we additionally adjusted for potential confounders in line with the previously published literature[10,11,14-17]: SES, alcohol consumption, smoking status, BMI, hypertension, diabetes mellitus and serum cholesterol. For the breast cancer analyses, we adjusted for number of pregnancies, hormone use, reproductive life span and menopausal status additional to the second model. To control for missing values of the confounders, we used the Markov Chain Monte Carlo method to create 5 imputed datasets which were pooled for analyses (missingness was < 2% for all covariates, except for alcohol consumption, number of pregnancies and reproductive life span, which was < 15%).

We conducted predefined stratification analyses by age (cut-off of 65 years) and sex. The cut-off value for age is conform previously published literature and guidelines.[20,21] We used interaction terms of age with FT4/TSH and sex with

FT4/TSH to assess effect modification. A sensitivity analysis was performed excluding subjects receiving thyroid-function altering medication. We performed a sensitivity analysis including cancer cases that were not histopathologically confirmed. Furthermore we performed a sensitivity analysis excluding the first 2 years of follow-up to assess the possibility of reverse causality.

We used log-transformed TSH in all continuous analyses, because of the skewed distribution of TSH.

There was no departure from linearity, assessed by using 3 restricted cubic splines (P > 0.30 for all). There was no departure from the proportional hazards assumption, as assessed by the Schoenfeld test. All analyses were conducted in IBM SPSS Statistics 21, except for the linearity assumption which was assessed in R (rms package, R-project, Institute for Statistics and Mathematics, R Core Team (2013), Vienna, Austria, version 3.0.2) and the proportional hazards assumption, which was assessed using the Schoenfeld test in R (survival package, R-project, Institute for Statistics and Mathematics, R Core Team (2013), Vienna, Austria, version 3.0.2).

RESULTS

We included 10,318 participants with a median age of 61.3 years, of whom 57% were female. A total of 1,465 cases of any solid cancer occurred during a median follow-up duration of 10.4 years (interquartile range (IQR): 4.9-15.5) with an incidence rate of 14 per 1,000 person-years. Baseline characteristics of the study population are shown in Table 1.

There was a positive association between FT4 and any solid cancer (HR: 1.42; 95% CI: 1.12-1.79; per one ng/dL of FT4), lung cancer (HR: 2.33; 95% CI: 1.39-3.92) and breast cancer (HR: 1.77; 95% CI: 1.10-2.84) (Table 2). In line with the negative relation between TSH and FT4, higher serum TSH levels were inversely associated with cancer, but only reached statistical significance in the first lung cancer analysis (HR: 0.84; 95% CI: 0.73-0.97).

For any solid cancer, breast cancer and lung cancer, we also assessed the relation with FT4 tertiles, taking the first tertile as reference (Table 3). The HRs for the highest tertile compared to the reference, were 1.13 for any solid cancer, 1.79 for

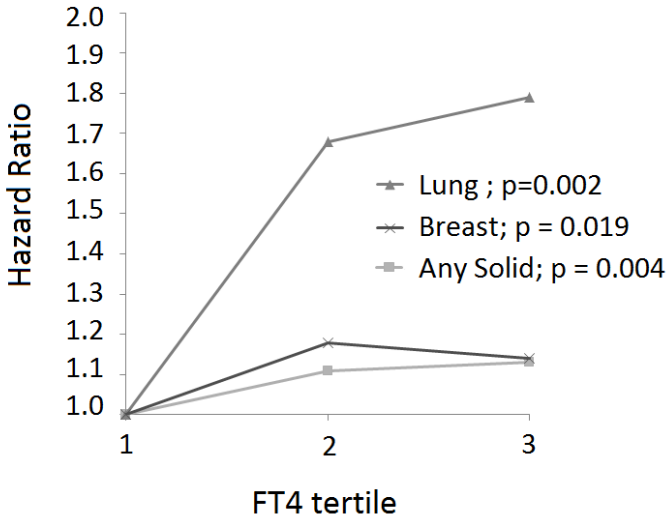
lung cancer and 1.14 for breast cancer but only the HR for lung cancer was significantly increased. However, the HR increased per tertile in all three groups (*P* for trend < 0.05 for all). The incidence rate per 10,000 person-years significantly increased per FT4 tertile for any solid cancer and lung cancer. For breast cancer, the incidence rate in the middle and highest FT4 tertile was similar, but significantly higher than the incidence rate in the first tertile (Table 3). The HRs of any solid cancer, lung cancer and breast cancer are plotted against FT4 tertiles in Figure 1.

Table 1 Baseline characteristics of the 10,318 study participants with FT4 and TSH measurements.

Variable	**N (%)**[a]
Start age, years, median (IQR)	61.3 (57.3-68.4)
Sex, female	5886 (57.0)
FT4, ng/dL, mean (SD)	1.23 (0.2)
TSH, mU/L, median (IQR)	1.9 (1.2-2.7)
Alcohol intake, g/day, median (IQR)	13.8 (1.1-19.9)
Smoking status	
Current	2381 (23.1)
Former	4654 (45.1)
Never	3283 (31.8)
BMI, kg/m^2, median (IQR)	26.5 (24.3-29.2)
Hypertension	5486 (53.2)
Diabetes mellitus	929 (9.0)
Total serum cholesterol, mmol/L, mean (SD)	6.1 (1.2)
Pregnancies, mean (SD)	2.2 (1.4)
Hormone use[b]	1212 (20.6)
Reproductive life span[c], mean (SD)	35.2 (5.5)
Postmenopausal status	5400 (91.7)

[a] Unless stated otherwise;
[b] Ever use of female hormones for menopausal complaints or start oral contraceptives within 7 years of menopause;
[c] The difference between menarche age and menopause age;
FT4 indicates free thyroxine; TSH, thyrotropin; BMI, body mass index; and IQR, interquartile range.

Figure 1 Association between FT4 tertiles and cancer



Hazard ratios for any solid, lung and breast cancer were plotted against FT4 tertiles. The first tertile (0.12-1.14 ng/dL) was taken as the reference category. The second tertile comprised 1.14-1.29 ng/dL and the third tertile 1.29-4.73 ng/dL. Hazard ratios are adjusted for age, sex, cohort, socioeconomic status, alcohol consumption, smoking status, body mass index, hypertension, diabetes mellitus and serum cholesterol; For breast cancer hazard ratios are also adjusted for no. of pregnancies, hormone use (use of female hormones for menopausal complaints or start oral contraceptives within 7 years of menopause), reproductive life span (difference between menarche age and menopause age) and menopausal status.

Excluding participants with thyroid-function altering medication resulted in similar risk estimates, but the association with breast cancer lost statistical significance (Table 4). There was no effect modification by age or sex (P > 0.05 for all interaction terms) (Supplemental Table 1). Risk estimates of the most adjusted models also remained similar when cancer cases that were not pathology based were included as outcome (Supplemental Table 2) and when the first 2 years of follow-up were excluded (Supplemental Table 3).

Table 2 Association between thyroid function and cancer incidence

Cancer type	Events N/ Subjects N[b]	FT4 (ng/dL) Hazard Ratio (95% CI)[a] Model 1[c]	Model 2[d]	Events N / Subjects N[b]	TSH (mU/L) Hazard Ratio (95% CI)[a] Model 1[c]	Model 2[d]
Any Solid	1442/9882	1.48 (1.17-1.86)[e]	1.42 (1.12-1.79)[e]	1465/9972	0.96 (0.91-1.02)	0.97 (0.94-1.00)
Lung	201/10206	2.72 (1.75-4.23)[e]	2.33 (1.39-3.92)[e]	204/10295	0.84 (0.73-0.97)[e]	0.89 (0.77-1.03)
Breast	227/5698	1.73 (1.09-2.74)[e]	1.77 (1.10-2.84)[e]	229/5753	0.97 (0.85-1.11)	0.95 (0.83-1.09)
Prostate	286/4360	1.11 (0.59-2.07)	1.18 (1.01-1.40)[e]	293/4395	1.00 (0.86-1.16)	0.99 (0.92-1.07)
GI	353/10163	1.41 (0.88-2.26)	1.34 (0.84-2.15)	355/10252	1.00 (0.89-1.13)	1.01 (0.89-1.14)

[a] The shown Hazard Ratios are per unit increase of FT4 or log-transformed TSH; [b] Subjects are the study participants with baseline TSH and/or FT4 measurements and no history of that specific cancer type; [c] Model 1 is adjusted for age, sex and cohort; [d] Model 2 = Model 1 + socioeconomic status, alcohol consumption, smoking status, body mass index, hypertension, diabetes mellitus and serum cholesterol; For breast cancer model 2 is also adjusted for no. of pregnancies, hormone use, reproductive life and menopausal status; [e] *P*-value <0.05. FT4 indicates free thyroxine; TSH, thyrotropin.

Table 3 Association between FT4 tertiles and cancer incidence

FT4 tertile (ng/dL)	Any Solid Cancer Events/ Subjects N[a]	Hazard Ratio (95% CI)[b]	IR[c]	Lung Cancer Events/ Subjects N[a]	Hazard Ratio (95% CI)[b]	IR[c]	Breast Cancer Events/ Subjects N[a]	Hazard Ratio (95% CI)[b]	IR[c]
1 (0.12-1.14)	444/3305	REFERENCE	127	42/3409	REFERENCE	11	74/2043	REFERENCE	33
2 (1.14-1.29)	489/3294	1.11 (1.04-1.19)[d]	144	73/3400	1.68 (1.15-2.46)[d]	20	80/1889	1.18 (0.86-1.62)	38
3 (1.29-4.73)	509/3277	1.13 (0.99-1.28)	151	86/3394	1.79 (1.23-2.59)[d]	23	73/1762	1.14 (0.82-1.58)	37
***P* for trend**		0.004			0.002			0.019	

[a] Subjects are the study participants with baseline TSH and/or FT4 measurements and no history of that specific cancer type; [b] Hazard ratios are adjusted for age, sex, cohort, socioeconomic status, alcohol consumption, smoking status, body mass index, hypertension, diabetes mellitus and serum cholesterol; For breast cancer hazard ratios are also adjusted for no. of pregnancies, hormone use, reproductive life span and menopausal status; [c] Incidence Rate per 10,000 person-years; [d] *P*-value <0.05. FT4 indicates free thyroxine; TSH, thyrotropin.

Table 4 Association between thyroid function and cancer incidence after exclusion of thyroid altering medication[a] use

			FT4 (ng/dL) Hazard Ratio (95% CI)[b]				TSH (mU/L) Hazard Ratio (95% CI)[b]	
Cancer Type	**Events (N)**	**Subjects (N)[c]**	**Model 1[d]**	**Model 2[e]**	**Events (N)**	**Subjects (N)[c]**	**Model 1[d]**	**Model 2[e]**
Any Solid	1382	9439	1.59 (1.22-2.07)[f]	1.49 (1.15-1.95)[f]	1405	9523	0.95 (0.89-1.01)	0.96 (0.91-1.01)
Lung	194	9742	3.72 (2.16-6.40)[f]	2.55 (1.46-4.48)[f]	197	9825	0.83 (0.71-0.96)[f]	0.88 (0.75-1.02)
Breast	214	5345	1.72 (0.93-3.17)	1.76 (0.95-3.25)	216	5394	0.95 (0.83-1.10)	0.94 (0.81-1.08)
Prostate	277	4255	1.15 (0.60-2.21)	1.22 (1.00-1.49)	284	4290	0.96 (0.82-1.13)	0.95 (0.88-1.03)
GI	338	9704	1.47 (0.86-2.51)	1.36 (0.80-2.34)	340	9787	1.09 (0.89-1.15)	1.01 (0.89-1.15)

[a] Use of amiodarone, corticosteroids, levothyroxine, propylthiouracil, carbamazole, thiamazole and/or iodine;
[b] The shown Hazard Ratios are per unit increase of FT4 or log-transformed TSH;
[c] Subjects are the study participants with baseline TSH and/or FT4 measurements, no history of that specific cancer type and no use of thyroid-function altering medication;
[d] Model 1 is adjusted for age, sex and cohort;
[e] Model 2 is adjusted for model 1, socioeconomic status, alcohol consumption, smoking status, body mass index, hypertension, diabetes mellitus and serum cholesterol; For breast cancer model 2 is also adjusted for no. of pregnancies, hormone use (use of female hormones for menopausal complaints or start oral contraceptives within 7 years of menopause), reproductive life span (difference between menarche age and menopause age) and menopausal status;
[f] *P*-value <0.05;
FT4 indicates free thyroxine; TSH, thyrotropin.

DISCUSSION

In this prospective population-based cohort study, higher FT4 levels were associated with an increased risk of any solid cancer, lung cancer and breast cancer. Overall, there was a negative association between TSH and cancer incidence, but not significantly. To our knowledge, this is the first prospective cohort study to assess the relation between the full range of thyroid function and cancer incidence.

Our findings differ in some respects from the results of the study by Hellevik et al..[9] This prospective study in nearly 30,000 subjects found that low levels of TSH compared to the euthyroid reference group were associated with higher risk of cancer, mainly lung and prostate cancer.[9] In our study, FT4 and not TSH, was associated with the risk of cancer in general and lung and breast cancer in particular. However, there are several differences between our study and the study by Hellevik et al. First, in the study by Hellevik and colleagues thyrotropin measurements were only performed in specific subsamples of the study population, possibly leading to selection bias. Also, they did not assess thyroid function in the full range and FT4 measurements were only performed in subjects with TSH values suggesting hyperthyroidism. Furthermore risk estimates were adjusted only for age, sex, smoking and BMI and no information on thyroid medication was available.[9]

Based on the hypothalamus-pituitary-thyroid axis, one would expect that our findings on the association of higher FT4 levels and increased cancer risk would also be accompanied by a higher cancer incidence in association with decreasing TSH levels. However, even though lower TSH values were associated with a higher risk of cancer, this only reached significance in the first analysis with lung cancer. An association with FT4, but not with TSH, is in line with previous population based studies investigating the association between thyroid function and clinical outcomes.[20,21,23,24] A potential explanation for the weaker association with TSH might be that, given the mean age of 63 years in our study, our results are due to an altered set point of the hypothalamus-pituitary-thyroid axis. It has been described that serum TSH levels increase with age while FT4 levels remain

unchanged. This might explain the lack of an association with TSH in this elderly population.[25]

There are several pathways that might explain the relation between thyroid hormone and cancer. Firstly, binding of thyroid hormone to TRs can activate the oncogenic phosphatidylinositol-3-kinase (PI3K) pathway, independent of DNA binding.[5] This pathway subsequently induces the expression of the transcription factor hypoxia-inducible factor 1 (HIF1). HIF1 target genes play a prominent role in tumor development, growth, invasion and metastasis. Secondly, thyroid hormone can bind the protein integrin αvβ3. This leads to activation of the PI3K and the ERK1/2 pathway. Via the latter pathway, fibroblast growth factor 2 (FGF2) is induced, which in turn stimulates angiogenesis and thus tumor growth.[5] Also, thyroid hormone can activate mitogen-activated protein kinase (MAPK).[26] In the cell nucleus MAPK leads to serine phosphorylation of thyroid receptors, which induces angiogenesis and tumor proliferation.[26]

Thyroid hormone has also been found to stimulate expression of the programmed death-ligand 1 (*PD-L1*) gene. The PD1/PD-L1 checkpoint plays a key role in protecting cancer cells from T-cell mediated destruction.[27] Other mechanisms of anti-apoptotic effects of thyroid hormone have also been described. Thyroid hormone decreases expression of tumor necrosis factor-α (TNF-α) and FAS ligand, both which are cell surface factors that activate apoptosis.[28] Furthermore, thyroid hormone up regulates various anti-apoptotic genes, while down regulating pro-apoptotic gene expression. An important example of the latter is the decreased activity of caspases, resulting in a reduction of DNA breakdown.[28]

Tumor specific effects of thyroid hormones have been described as well. In prostate cancer cells, thyroid hormone can down regulate B-cell translocation gene 2 (*BTG2*), resulting in increased proliferation.[2] Furthermore, thyroid hormone can alter the expression level of cyclin D1, a protein that regulates the cell cycle, and of cyclin dependent kinases, leading to various tumor types, such as breast cancer and neural cancers.[1] Action of thyroid hormone on estrogen and androgen receptors may also contribute to breast and prostate cancer respectively.[29,30] Also, thyroxine phosphorylates the estrogen receptor α (ERα) and increases the expression of proliferating cell nuclear antigen (PCNA) via induction of αvβ3. These processes are associated with proliferation of ovarian and lung cancer

cells.[31,32] Lastly, the tumor suppressor protein p53 inhibits thyroid hormone-thyroid receptor complexes, while thyroid receptor in turn counteracts stimulation of p53 target genes.[2] Future studies could focus on which of these pathways are involved in the relation between thyroid function and cancer and whether this differs per cancer site and stage.

At baseline, 4.5% of women (n = 263) used levothyroxine and 0.4% (n = 22) used anti-thyroid drugs. In our sensitivity analysis excluding participants with thyroid function-altering medication, the association of FT4 with breast cancer lost statistical significance, although the effect estimates remained similar. It seems that these results became borderline non-significant due to a reduction in sample size, since predominantly women suffer from thyroid disorders and use thyroid-altering medication.[33] Although use of levothyroxine is associated with higher FT4 levels[34], this could not have driven the found associations in our main analyses. In that case, one would expect smaller effect estimates in the sensitivity analysis, whereas we found similar or even strengthened associations. Furthermore, > 97% of the included participants did not use any thyroid-altering medication.

An important strength of our study is the prospective population-based design, large sample size and detailed information on potential confounders. Furthermore, we only looked at the occurrence of pathology confirmed cancer cases and were able to assess the effect of thyroid function in the full range. In contrast to previously conducted studies on this topic[9,10], we have taken thyroid altering medication and a wide range of mediators and confounders into account. Also, risk estimates remained similar after exclusion of the first 2 years of follow-up, making reverse causality unlikely. A limitation of our study is that thyroid function is only measured at baseline; therefore we were unable to assess changes in TSH and FT4 levels in relation to cancer incidence. However, this is a limitation for most population-based cohort studies.[9,17,35,36] Because of a limited number of cases, we were not able to assess the association between thyroid function and thyroid cancer. Also, like most observational studies, we cannot exclude residual or unmeasured confounding. Furthermore, the Rotterdam Study consists of a mainly Caucasian population aged 45 years and over, therefore results might not be generalizable to other populations.

Conclusion

Serum FT4 levels are positively associated with an increased incidence of any solid cancer, lung cancer and breast cancer. Comparing the highest FT4 tertile to the lowest, there was a 1.13 fold increased risk of any solid, a 1.14 fold increased risk of breast cancer, and the risk of lung cancer nearly doubled. Future studies are needed to further elucidate the possible role of thyroid function in the pathogenesis of cancer, mainly lung cancer.

Onine supplemental material

https://academic.oup.com/jcem/article-abstract/101/12/5030/2765090/Thyroid-Function-and-Cancer-Risk-The-Rotterdam?redirectedFrom=fulltext

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CHAPTER 5.2

THE ASSOCIATION BETWEEN THYROID FUNCTION AND THE RISK OF DECLINE IN KIDNEY FUNCTION AND INCIDENT CHRONIC KIDNEY DISEASE

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ABSTRACT

BACKGROUND Thyroid dysfunction has been associated with kidney dysfunction, but mainly in cross-sectional studies. Therefore we aimed to determine the association between thyroid and kidney function in a prospective population-based cohort study longitudinally.

METHODS Participants aged ≥45 years from the Rotterdam Study with thyroid and kidney function assessment were included. Kidney function and new onset chronic kidney disease (CKD) were defined using estimated glomerular filtration ate (eGFR), with CKD defined as eGFR <60 ml/min/1.73 m2 according to the CKD-EPI formula.

RESULTS We included 5103 participants (mean age of 63.6 years) with a mean follow-up of 8.1 years. Cross-sectionally, higher TSH levels were associated with lower eGFR (Beta [β] -1.75 ml/min, 95% confidence interval [CI]; -2.17, -1.33), in multivariable models adjusting for several cardiovascular risk factors including smoking, hypertension and history coronary heart amongst others. In contrast, longitudinally, higher TSH levels were associated with less annual eGFR decline (β -0.06 ml/min, CI; -0.11, -0.01) and lower CKD incidence (Odds Ratio 0.85, CI; 0.75, 0.96). Compared to euthyroid participants, subclinical hyperthyroid individuals had an increased risk for CKD whereas hypothyroid individuals had a decreased risk (p for trend =0.04).

CONCLUSIONS Hyperactive thyroid function is associated with increased risk of kidney function decline while hypothyroidism is associated with a decreased CKD risk. More insight is needed in the pathophysiological pathways connecting high thyroid function and kidney function decline.

INTRODUCTION

Thyroid hormone has an impact on renal tubular function, the renin–angiotensin system and is associated with hemodynamic and cardiovascular alterations that interfere with renal blood flow.[1,2] Hypothyroidism has been associated with a low glomerular filtration rate and chronic kidney disease (CKD).[3,4] On the other hand, chronic kidney disease can also lead to changes in thyroid function for example through non-thyroidal illness, metabolic acidosis or selenium deficiency.[5-7] The co-existence of (subclinical) hypothyroidism and renal dysfunction in patients has prompted investigation into the effects of thyroid hormone replacement therapy on CKD [8,9]. However, most evidence concerning the association between thyroid and kidney function has been based on cross-sectional studies and included mostly patients with chronic kidney disease or thyroid dysfunction.[3,10,11] The understanding of the temporal relationship between thyroid and kidney dysfunction therefore remains largely unexplored. So far, only two prospective studies in specific populations have investigated this association with conflicting results.[4,12] One study was conducted in 555 participants of 85 years-old and found no association between thyroid status and change in renal function.[12] The second study was a large cohort study including only euthyroid subjects and reported an increased CKD risk with low-normal thyroid function.[4] To date, there are no large population-based cohort studies investigating the continuous and full range of thyroid function and the risk of kidney function decline and CKD. Therefore, in the current prospective study, we aimed to investigate the association of thyroid function with the risk of kidney function decline and CKD in the general population.

METHODS

Study population

The Rotterdam Study (RS) is 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 living in Ommoord, a suburb of Rotterdam. The aims and design of the RS have been described in detail elsewhere.[13] For this analysis we included participants

from three independent cohorts within the RS. The RS Cohort 1 (RSI) started in 1990 and included a total of 7,983 participants (response rate 78 percent) aged 55 years and older. RS Cohort II (RSII) includes a total of 3,011 participants (response rate 67 percent) aged 55 years and older and baseline data were collected from 2000-2001. For the RS Cohort 3 (RSIII), all residents of Ommoord aged 45 years and over and who had not been invited before, were asked to participate and baseline data were collected from 2006 to 2008. A total of 3,932 participants entered the study (response rate 65 percent). Participants from study cohorts RSI (RSI-3), RSII (RSII-1) and RSIII (RSIII-1) were eligible for the study if TSH and FT4 measurements as well as serum creatinine were available at baseline and follow-up.

The Medical Ethics Committee of the Erasmus University approved the study protocols 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.

Assessment of thyroid function

We performed thyroid function tests measurements in the three RS cohorts in 9762 subjects. All samples were collected between 1997 and 2008, depending on the cohort, and measured using the same methods and assay. Thyroid function assessment was performed for TSH and FT4 in all three cohorts in serum samples stored at -80°C (The electrochemiluminescence immunoassay for thyroxine, thyrotropin and thyroid perioxidase antibodies, "ECLIA", Roche). We determined the cut-off values for normal range of TSH as 0.4-4.0 mIU/L and FT4 as 11-25 pmol/L (0.86-1.94 ng/dL) according to national guidelines as well as our previous studies. Thyroid function was defined as euthyroid if TSH was in the normal range (0.4-4.0 mIU/L). Overt hyperthyroidism was defined by a TSH level below the normal range (< 0.4 mIU/L) and FT4 above the normal range (> 25 pmol/L), while in subclinical hyperthyroidism FT4 values were within the normal range (11-25 pmol/L). Overt hypothyroidism was defined by TSH levels above the upper range of normal (> 4.0 mIU/L) and FT4 below the normal range (< 11 pmol/L), while in subclinical hypothyroidism FT4 values were within the normal range (11-25

pmol/L). The laboratory measurements of thyroid function were not reported back to the participants and any thyroid therapy prescribed by their own GP or specialist is within the context of regular treatment and blinded to measurements of the RS.

Kidney function and chronic kidney disease

Serum creatinine was determined using an enzymatic assay method and determined using an enzymatic assay method using the same technique at the same laboratory for all three cohorts. Inter-assay and intra-assay coefficient variations were < 0.92% and <1.37%, respectively.[14] Creatinine values were standardized to isotope-dilution mass spectrometry(IDMS)–traceable measurements. In order to calibrate, we aligned the mean values of serum creatinine with serum creatinine values of the participants of the Third National Health and Nutrition Examination Survey (NHANES III) in different gender and age groups (<50,50-59, 60-69, ≥70 years). Estimated Glomerular filtration rate (eGFR) was calculated according to the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula.[15] 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, in years, between the two visits. For these analyses we used all participants with available eGFR measurements. Chronic kidney disease (CKD) was defined as eGFR < 60 ml/min/1.73 m^2. New CKD cases were defined among the individuals free of CKD at baseline (defined by eGFR >60 ml/min/1.73 m^2), who had a decline in eGFR to less than 60 ml/min/1.73 m^2 between the two periodical examinations.

Baseline measurements

Information on smoking was derived from computerized baseline questionnaires and categorized in current, previous and never smokers. Systolic and diastolic blood pressure were calculated as the average of two consecutive measurements, using a random-zero mercury sphygmomanometers. Information on anti-hypertensive medication use was based on home interview at baseline. Serum glucose, total serum cholesterol and high-density lipoprotein cholesterol (HDL-C) were measured using standard laboratory techniques. History of diabetes was defined by a repeated impaired fasting glucose ≥ 7 or use of anti-glycemic medication at baseline. Body-mass index (BMI) was calculated as weight in kilograms divided by height squared in meters.

Statistical analysis

We first jointly analysed RS cohort I and II and then replicated the results in RS cohort III, which provided similar sample sizes for discovery and replication. We present the combined multivariable analyses (RSI, RSII and RSIII) as our primary results. Linear regression models were used to evaluate the association of FT4 or TSH with baseline eGFR (based on creatinine) or decline in eGFR. Logistic regression models were performed to estimate the odds ratio (OR) for the association of FT4 or TSH with incident CKD.

Due to a skewed distribution, TSH was log-transformed for all continuous statistical analyses.

Our primary model included adjustment for age and sex. The multivariable models were additionally adjusted for systolic blood pressure, diastolic blood pressure, smoking, total cholesterol, high density lipoprotein cholesterol, diabetes mellitus, antihypertensive medications, and body mass index.

We performed an analysis of covariance where mean values of decline in eGFR were compared across categories of thyroid function. We also assessed incidence of CKD in categories of thyroid function using logistic regression. For the thyroid function analyses all thyroid hormone users were excluded.

We performed multiple imputation for missing data in the covariates (< 1% for all covariates), using a Markov Chain Monte Carlo method.

We assessed differential risk by age or sex by adding an interaction term with the exposure variable. For the longitudinal TSH and FT4 analyses we performed the following sensitivity analyses: 1) restricting the analyses to participants with TSH and FT4 values within the normal range 2) excluding participants using thyroid function altering medication (including levothyroxine, anti-thyroid drugs, amiodarone and corticosteroids) or prevalent thyroid disease. There was no departure from linearity for the TSH, FT4 or thyroid function analyses. All statistical analyses were performed using SPSS version 21 (SPSS IBM, New York, U.S.A). Reporting of this study is according to the STROBE Statement.

RESULTS

Baseline characteristics of included participants from the Rotterdam Study[13], a population-based prospective cohort study, are shown in Table 1. Out of the 5103 included participants (Figure 1) who had thyroid function and serum creatinine measurements, 43.6% were male (mean age of 64 years), with a mean follow-up of 8.1 years. A total of 4488 participants were euthyroid (87.9%), 467 had subclinical hypothyroidism (9.2%), 32 had hypothyroidism (0.6%), 106 were subclinically hyperthyroid (2.1%), 10 were hyperthyroid (0.2%) at baseline.

Table 1 Baseline characteristics

Variable	**N = 5103**
Age, years	63.6 (9.4)
Men	2227 (43.6)
Systolic blood pressure, mmHg	136.3 (19.8)
Diastolic blood pressure, mmHg	80.3 (11.0)
Smoking	
Current	1057 (20.7)
Former	2419 (47.4)
Total cholesterol, mmol/l	5.7 (1.01)
High densitiy lipoprotein cholesterol, mmol/l	1.4 (0.4)
Body mass index, kg/m^2	27.3 (4.2)
Diabetes mellitus	409 (8.0)
History of coronary heart disease	188 (3.7)
Glomerular filtration rate (creatinine), mL/min/1.73 m^2	84.1 (14.1)
TSH mIU/L	2.0 (1.4, 2.8)
FT4 pmol/L	15.6 (2.2)
Levothyroxine use	150 (2.9)

Categorical variables are presented as numbers (percentages), continuous variables as means (standard deviations) and TSH is presented as median (interquartile range).
Missing data: blood pressure (n=10), smoking (n=19), high density lipoprotein cholesterol (n=14), body mass index (n=21), diabetes (n=40), coronary heart disease (n=30).

Cross-sectional analyses

Compared to euthyroid subjects, hyperthyroid participants had a higher eGFR, while participants with hypothyroidism had a lower eGFR at baseline (Figure 2). There was a significant p for trend across the different thyroid function categories for eGFR at baseline ($p < 0.001$). Higher TSH levels were associated with a lower baseline eGFR (Beta [β] -1.75, 95% confidence interval [CI]; -2.17, -1.33, per one unit of log transformed TSH).The effect estimates for the TSH analyses were similar between primary and multivariable models (Table 2).

Figure 1 Participants inclusion

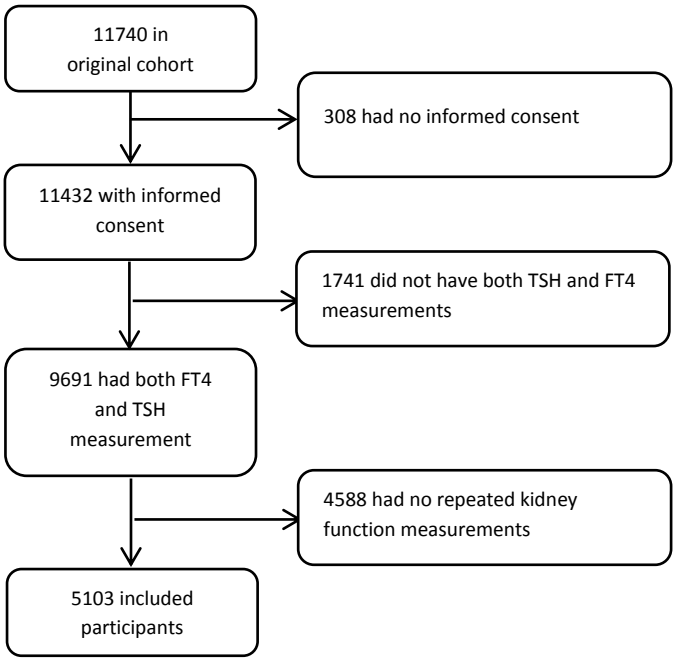
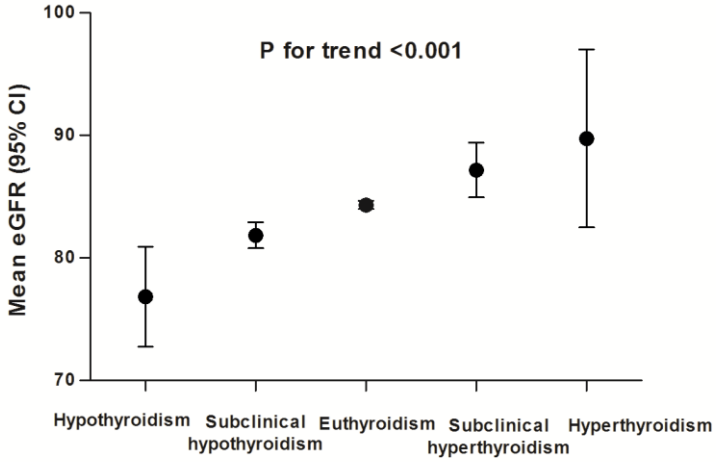


Table 2 Cross-sectional association of thyroid function markers with kidney function

	RS I & II	RS III	Combined
		eGFR	
	Beta(95% CI) N =2524	**Beta(95% CI) N = 2579**	**Beta(95% CI) N =5103**
TSH			
Model I	-2.10 (-2.64, -1.56)	-1.48 (-2.14, -0.81)	-1.83 (-2.25, -1.41)
Model II	-2.00 (-2.53, -1.46)	-1.40 (-2.06, -0.73)	-1.75 (-2.17, -1.33)
FT4			
Model I	0.02 (-0.18, 0.22)	-0.05 (-0.27, 0.17)	-0.02 (-0.17, 0.13)
Model II	-0.01 (-0.21, 0.19)	-0.06 (-0.28, 0.16)	-0.04 (-0.19, 0.10)

Model I: Adjusted for age, sex, and cohort effect.
Model II: Additionally adjusted for systolic blood pressure, diastolic blood pressure, smoking, total cholesterol, high density lipoprotein cholesterol, diabetes mellitus, antihypertensive medications, history of coronary heart disease, and body mass index.
Abbreviations: CI: confidence interval, eGFR: estimated glomerular filtration rate, TSH: thyroid stimulating hormone, FT4: free thyroxine

Figure 2 Mean values of eGFR in categories of thyroid function



From 5013 participants, 4488 were euthyroid, 467 subclinical hypothyroid, 32 hypothyroid, 106 subclinical hyperthyroid, and 10 were hyperthyroid. These analyses were adjusted for age, sex, systolic blood pressure, diastolic blood pressure, antihypertensive medication, smoking, total cholesterol, high density lipoprotein cholesterol, diabetes mellitus, history of coronary heart disease, and body mass index.

Longitudinal analyses

In contrast to the cross-sectional analyses, higher TSH levels were associated with less annual eGFR decline (β -0.06, 95% CI; -0.11, -0.01, Table 3). Also, higher TSH levels were associated with lower incidence of CKD (OR 0.85, 95% CI; (0.75, 0.96, Table 3) There was no association between TSH in the normal range of thyroid function and decline in eGFR or incident CKD (Table 4). When excluding subjects with clinically known thyroid disease or thyroid medication use (Table 4) the estimates were similar, but lost significance. Overall, FT4 values were not associated with eGFR decline or incident CKD (Table 3). There was no differential risk by age or sex (p for interaction > 0.15). Additionally adjusting for BMI change over time, did not change risk estimates (data not shown).

Table 3 Longitudinal association of thyroid function markers with future decline in kidney function and incidence of CKD

		RS I & II		RS III		Combined	
		eGFR decline					
		N = 2524	Beta(95% CI)	N = 2579	Beta(95% CI)	N = 5103	Beta(95% CI)
TSH							
	Model I		-0.06 (-0.11, -0.02)		-0.10 (-0.19, 0.00)		-0.06 (-0.11, -0.01)
	Model II		-0.07 (-0.11, -0.02)		-0.10 (-0.19, 0.00)		-0.06 (-0.11, -0.01)
FT4							
	Model I		-0.00 (-0.02, 0.02)		0.03 (0.00, 0.06)		0.01 (-0.01, 0.03)
	Model II		-0.00 (-0.02, 0.02)		0.03 (0.00, 0.06)		0.01 (-0.01, 0.03)
		Incident CKD					
		N (cases)	OR (95% CI)	N (cases)	OR (95% CI)	N (cases)	OR (95% CI)
TSH							
	Model I	2345 (464)	0.86 (0.75, 0.99)	2513 (129)	0.86 (0.66, 1.12)	4858 (593)	0.86 (0.76, 0.98)
	Model II		0.84 (0.73, 0.98)		0.87 (0.67, 1.14)		0.85 (0.75, 0.96)
FT4							
	Model I		1.02 (0.97, 1.08)		1.02 (0.93, 1.11)		1.02 (0.98, 1.07)
	Model II		1.02 (0.97, 1.08)		1.03 (0.94, 1.12)		1.02 (0.97, 1.07)

Model I: Adjusted for age, sex, and cohort effect.
Model II: Additionally adjusted for baseline eGFR, systolic blood pressure, diastolic blood pressure, antihypertensive medication, smoking, total cholesterol, high density lipoprotein cholesterol, diabetes mellitus, history of coronary heart disease, and body mass index.
All analyses with incident CKD as outcome are adjusted for follow up time.
Abbreviations: CI: confidence interval, eGFR: estimated glomerular filtration rate, CKD: chronic kidney disease, OR: odds ratio, TSH: thyroid stimulating hormone, FT4: free thyroxine

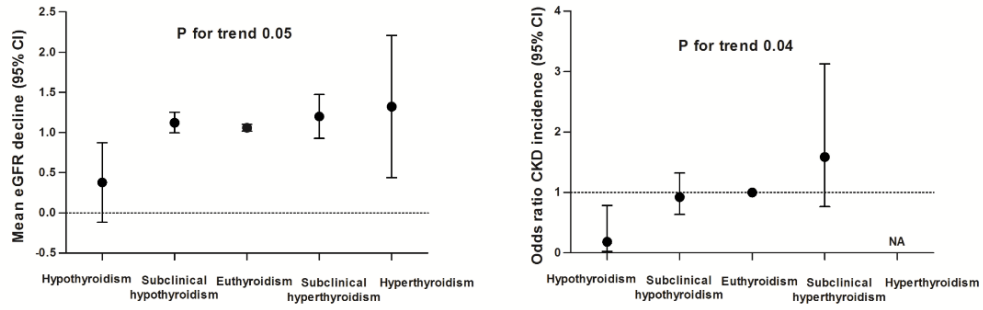
Table 4 Sensitivity analyses for the longitudinal association of thyroid function markers with future decline in kidney function and incidence of CKD

		eGFR decline		CKD incidence
	N	Beta (95% CI)	N(cases)	OR (95% CI)
In subjects with normal range of TSH and FT4				
TSH				
Model I	4459	-0.09 (-0.18, 0.01)	4265 (513)	1.01 (0.80, 1.27)
Model II		-0.09 (-0.19, 0.00)		0.99 (0.77, 1.26)
FT4				
Model I		0.00 (-0.02, 0.02)		1.00 (0.95, 1.06)
Model II		0.00 (-0.02, 0.02)		1.00 (0.95, 1.06)
In individuals without thyroid function altering medication or thyroid disease				
TSH				
Model I	4896	-0.04 (-0.10, 0.01)	4662 (566)	0.88 (0.77, 1.01)
Model II		-0.05 (-0.10, 0.01)		0.87 (0.76, 1.00)
FT4				
Model I		0.01 (-0.01, 0.03)		1.01 (0.97, 1.06)
Model II		0.01 (-0.01, 0.03)		1.01 (0.96, 1.06)

Model I: Adjusted for age, sex, and cohort effect.
Model II: Additionally adjusted for baseline eGFR, follow-up time, systolic blood pressure, diastolic blood pressure, antihypertensive medication, smoking, total cholesterol, high density lipoprotein cholesterol, diabetes mellitus, history of coronary heart disease, and body mass index.
All analyses with CKD incidence as outcome are adjusted for follow up time. We determined the cut-off values for normal range of TSH as 0.4-4.0 mIU/L and FT4 as 11-25 pmol/L (0.86-1.94 ng/dL). Thyroid-altering medication includes levothyroxine, anti-thyroid drugs, amiodarone and corticosteroids
Abbreviations: CI: confidence interval, eGFR: estimated glomerular filtration rate, CKD: chronic kidney disease, OR: odds ratio, TSH: thyroid stimulating hormone, FT4: free thyroxine

Participants with subclinical hyperthyroidism had more eGFR decline and hypothyroid participants had less decline in eGFR (**Figure 3**). For the CKD analyses, the hyperthyroidism category was excluded due to small number of events and therefore infinite estimates. Participants with hypothyroidism had a decreased risk of incident CKD while participants with subclinical hyperthyroidism had an increased risk of CKD. The p for trend was a significant across the different categories of thyroid function for incident CKD ($p = 0.04$) and for mean eGFR decline ($p = 0.05$).

Figure 3 Mean values of eGFR decline and odds ratios of CKD incident in categories of thyroid function



From 5103 participants 4488 were euthyroid, 467 subclinical hypothyroid, 32 hypothyroid, 106 subclinical hyperthyroid, and 10 were hyperthyroid. These analyses were adjusted for age, sex, systolic blood pressure, diastolic blood pressure, antihypertensive medication, smoking, total cholesterol, high density lipoprotein cholesterol, diabetes mellitus, history of coronary heart disease, and body mass index.NA = not available, due to small number of events in this strata.

DISCUSSION

To our knowledge, this is the first prospective population-based study to report an association between high thyroid function and kidney function decline in the general population. We report an increased risk of CKD and eGFR decline in subjects with lower TSH levels or hyperthyroid state, while hypothyroidism seems protective. This is irrespective of age, sex and several cardiovascular risk factors including blood pressure, smoking and cholesterol.

A large study in over 460,000 veterans reports an inverse association between eGFR and the risk of hypothyroidism.[16] This study is however not comparable to ours as it only participants with CKD defined as an eGFR of <60 mL/min/1.73 m2, while our population reflects the general population. Only two previous studies have longitudinally investigated the association between thyroid function and kidney function. However, these studies focused on specific study populations with small sample size, investigated specific thyroid states, instead of the continuous range of thyroid function, and their results were conflicting. While Zhang et al.[4] found a modestly increased risk of CKD with high-normal TSH levels in >100,000

euthyroid subjects, Meuwese et al.[12] found no relation between thyroid function and kidney function in 555 subjects of 85 years old in the longitudinal analyses. The differences between our data and these studies may be explained by various factors.

The Kangbuk Samsung Health (KSH) Study looked specifically in euthyroid subjects, while our results concern the continuous and full spectrum of thyroid function.[4] When we restrict our analyses to subjects with thyroid function within the reference range, we find no association of thyroid function with kidney function decline or CKD. The CKD-EPI equation, which was used in KSH, has shown to introduce inaccuracy among certain racial-ethnic groups such as Asians.[17] In addition, the mean age of the populations and follow-up times differ, since our population is substantially older (mean age of 38 vs 63.6 years), and had a longer follow-up (3.5 vs 8.2 years of follow-up). Finally, selection bias cannot be excluded from the KSH Study due to its design: screening health service of employees. The Leiden 85-plus Study, which focussed on older adults, included a relatively small number of participants (n = 555) and does not report information on CKD at baseline and during follow-up. The mean annual change presented for hyperthyroidism (eGFR -1 ml/min/1.73m^2) and hypothyroidism (eGFR +0.5 ml/min/1.73m^2) compared to euthyroidism are in line with our results, but did not reach statistical significance in that study, possibly due to the lower numbers. Furthermore, in the current study we also conducted analyses with TSH as a continuous variable, where again we observed a protective effect of higher TSH values (i.e. lower thyroid function).

Most, predominantly cross-sectional, studies to date have focused on the hypothesis that hypothyroidism gives rise to several cardiovascular risk factors and circulatory changes through which kidney function could be affected.[4,12,18,19] In our study we confirm the cross-sectional association between (subclinical) hypothyroidism and lower eGFR. However, cross-sectional studies cannot provide information on the temporal relationship between thyroid function and kidney function and are prone to reverse causation.

Therefore, the major aim of this study was to address the issue of temporality by conducting longitudinal analyses. In our study, participants with hypothyroidism had a decreased risk of incident CKD and less eGFR decline while participants

with subclinical hyperthyroidism had an increased risk of CKD and more eGFR decline. No studies to date had focussed on the possible association between a high thyroid function state and renal dysfunction even though both low and high state of thyroid function are associated with cardiovascular risk factors and disease. Effects of subclinical hyperthyroidism include atrial fibrillation, increase in left ventricular mass index, reduced exercise tolerance, reduced heart rate variability and an increase in markers of coagulation.[20-22] Furthermore, thyroid hormone is known to activate the renin–angiotensin system[2], which may cause efferent arteriolar vasoconstriction and lead to glomerular hypertension and hyperfiltration.[23] The opposite relationship on eGFR in our cross-sectional and longitudinal analysis is similar to what has been described for diabetic nephropathy, which is also characterized by a higher eGFR initially (due to hyperfiltration), but by eGFR-loss and CKD in the long run.[24] The seemingly discrepant findings between cross-sectional and prospective analyses on thyroid function and kidney decline could be explained by distinctly different mechanisms. With cross-sectional data we may detect the bi-directional association of kidney and thyroid function, while with longitudinal data we could measure the deleterious effect of long-term exposure to high thyroid function state on kidney function. More research is needed to explore the difference between these two mechanisms to further characterize the long-term effects of variations of thyroid function on kidney function and disease. This is especially important when considering thyroid hormone replacement therapy in subclinical hypothyroidism. Strengths of our study include the large number of participants, detailed information on a wide variety of confounders and long follow-up period. Study limitations include the relatively small number of participants with hyperthyroidism and measuring thyroid function only once at baseline. In addition, information regarding the etiology of CKD cases is not present in the Rotterdam Study. Albuminuria is a strong predictor of kidney function decline, but unfortunately was not available in our cohort at baseline; adjusting our results for albumin-creatinine ratio at follow-up in a subset of participants did not change our findings (data not shown). Also, we were limited by the number of eGFR measurements and were only able to use 2 measurements to define a slope. Furthermore, the Rotterdam Study is constituted of a mainly white population of 45 years and older and might

therefore not be generalizable to other populations. Finally, because we primarily used creatinine-based estimating equations for GFR, we cannot exclude effects of thyroid hormone on the muscle metabolism of creatinine or its tubular secretion.

In conclusion, high thyroid function is associated with an increased risk of incident CKD while hypothyroidism is associated with a decreased CKD risk. Further research should focus on the association between high thyroid function and kidney function and possible pathophysiological mechanisms.

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CHAPTER 5.3

GAIT PATTERNS ASSOCIATED WITH 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), FT4 (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 ($p<0.001$), but no association of FT4 concentrations with Global gait ($p=0.2$). TSH levels were positively associated with Base of support ($p=0.01$) and followed an inverted U-shaped curve with Tandem ($p=0.002$) and velocity ($p=0.02$). Clinical and subclinical hypothyroidism were associated with worse Global gait than euthyroidism ($\beta=-0.61$;CI=-1.03,-0.18; $p=0.004$ and $\beta=-0.13$;CI=-0.26,-0.00; $p=0.04$, 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]. Similar to gait disturbances, thyroid dysfunction becomes more prevalent with advancing age. However, the clinical manifestations of thyroid dysfunction are 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 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 one of the parameters in the Pace domain [16,17]. However, the link of thyroid function with gait and its spatiotemporal aspects remains unknown. 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 (RS) is an ongoing prospective population-based cohort study that investigates chronic diseases in the middle-aged and elderly. The objectives and study design of RS have been described in detail elsewhere[18]. RS 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, RS comprises a total of 14926 participants, who undergo extensive follow-up medical examinations every 2 to 4 years. From 2009 onwards, quantitative gait assessment was included in the study protocol. Between March 2009 and March 2012, 3651 participants of the RS were invited for gait assessment. An overview on the selection of study participants can be found in the flowchart (Supplementary Figure 1).

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.

Population for analysis

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 usage (n=79); 3) history of thyroid disease (n= 192) and 4) previous thyroid surgery (n=33) (Supplementary Figure 1). The remaining 2645 eligible participants were enrolled in the study.

Assessment of thyroid function

Thyroid function tests were performed in study cohorts RS I visit 3 (RS I-3), RS II visit 1 (RS II-1) and RS III visit 1 (RS III-1) using the same method and assay. Concentrations of thyroid-stimulating hormone (TSH), free thyroxine (FT4) and thyroid peroxidase antibodies (TPOAb) were measured on baseline serum samples stored at -80°C using the electrochemiluminescence immunoassay,

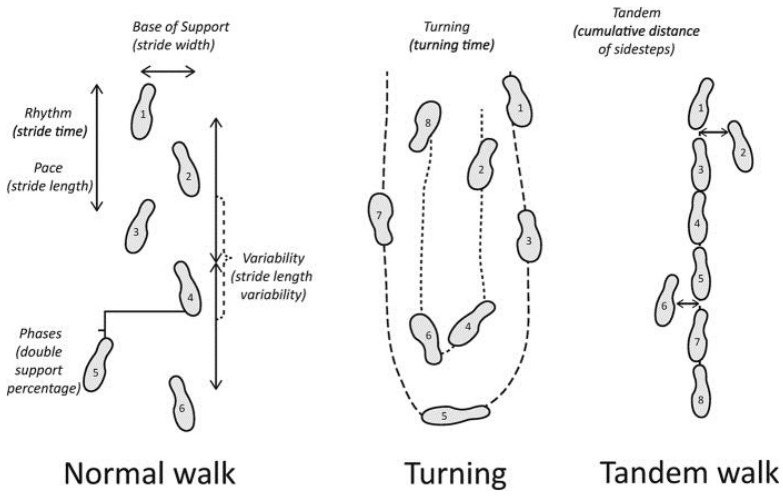
"ECLIA", Roche. We determined the reference range of serum TSH as 0.40–4.0 mIU/L and serum FT_4 as 11–25 pmol/L (alternatively 0.86–1.94 ng/dL), according to national guidelines and our previous studies[19,20]. Euthyroidism was defined as serum TSH within the reference range. Subclinical hypothyroidism was defined as serum TSH >4.0 mIU/L and FT4 levels within the reference range. Overt hypothyroidism was defined as serum TSH >4.0 mIU/L and FT4 levels <11 pmol/L. Subclinical hyperthyroidism was defined as serum TSH <0.40 mIU/L and FT4 levels within the reference range. Overt hyperthyroidism was defined as serum TSH <0.40 mIU/L and FT4 levels >25 pmol/L. TPOAb positivity (reflecting thyroid autoimmunity) was defined as TPOAb levels above the cut-off of 35 kU/ml, in accordance with the recommendations of the assay manufacturer[19,20].

Assessment of gait

Quantitative gait assessment was performed in study cohorts RS I visit 5 (RS I-5), RS II visit 3 (RS II-3) and RS III visit I (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].

Figure 1 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).



Assessment of covariates

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, FT4 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 (i.e. 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 velocity 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 FT4 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, Vienna, Austria, 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 (Supplementary 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).

Table 1 Baseline characteristics of 2645 participants

Characteristics	Mean (SD)*
Age, years	59.6 (6.6)
Female, 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)
FT4, pmol/L	15.5 (2.1)
TPOAb positive, n (%)	312 (11.8)

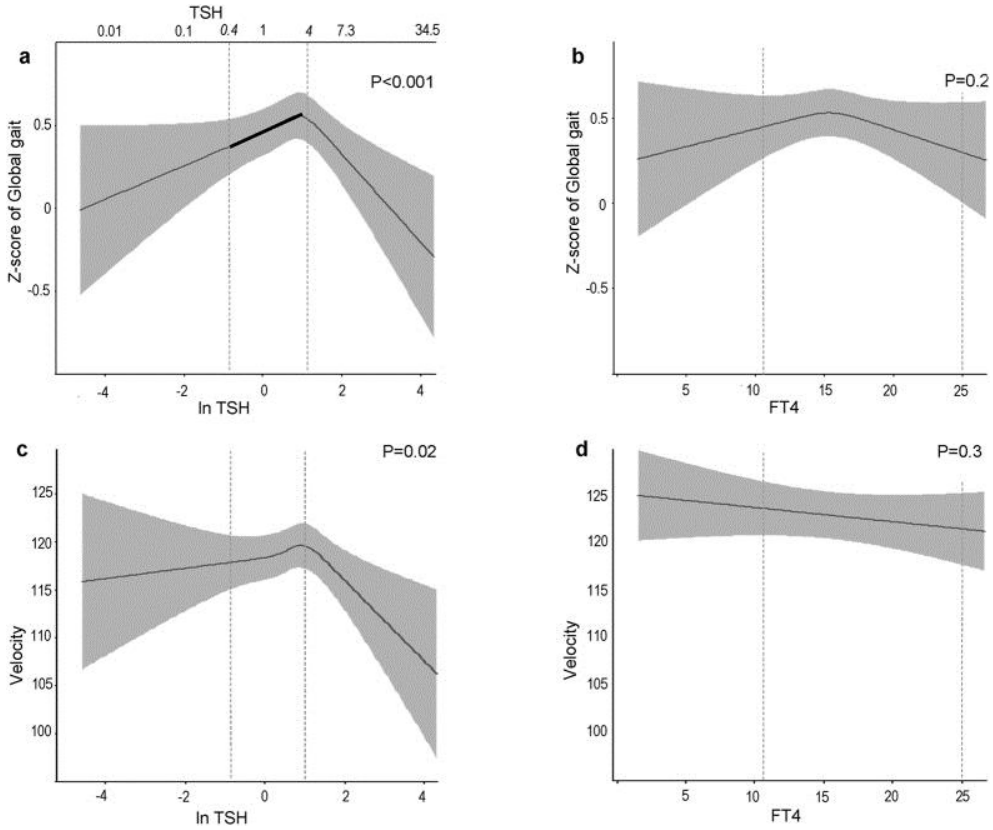
*Data are mean and standard deviation, unless otherwise specified.
Abbreviations: sd, standard deviation ; CESD, Centre for Epidemiological Studies Depression Scale; TSH, thyroid-stimulating hormone; IQR, interquartile range; FT4, free thyroxine; TPOAb, thyroid peroxidase antibodies (cutoff 35 kU/ml).

The association of thyroid function with 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 respect to Global gait (p-value <0.001) (Figure 2 A). However, there was no association of FT4 concentrations with Global gait (p=0.2) (Figure 2 B). When we restricted the analysis to euthyroid participants, higher TSH concentrations were associated with a better Global gait (β, 0.08; 95% confidence interval [CI], 0.02 to

0.13 per 1 unit logTSH; p=0.006). Moreover, there was a borderline statistically significant association between FT4 levels within the normal range and Global gait (β=-0.05; CI=-0.10 to 0.00 per 1 pmol/L FT4; p=0.05) (Figure 2 and Supplementary Table S1).

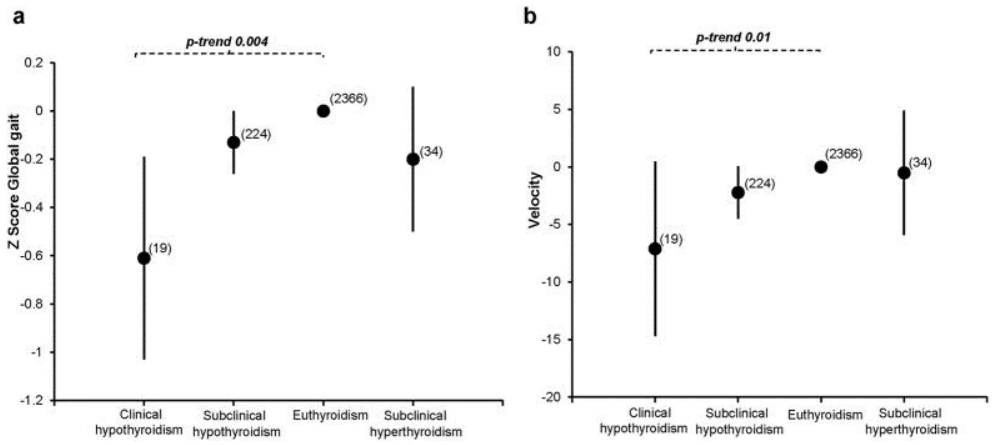
Figure 2 Association of thyroid function with Global gait/Velocity



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, thyroid peroxidase antibodies. We utilized linear regression models with restricted cubic splines. TSH/FT4 concentrations are plotted against predicted means of Z-score Global gait/Velocity (black lines) with 95% CI (gray areas). Dashed lines indicate the limits of TSH or FT4 reference ranges. A higher value of Global gait represents better gait.

Clinical and subclinical hypothyroidism were associated with a worse Global gait than euthyroidism (β=-0.61; CI=-1.03 to -0.18; p=0.004 and β, -0.13; CI, -0.26 to -0.00; p=0.04 respectively) (Figure 3 A). No association was observed between TPOAb and Global gait in the main analysis or after restricting to euthyroid participants (Supplementary Table S2). Results remained similar after excluding participants with prevalent stroke and Parkinson's disease (Supplementary Figure 2).

Figure 3 Association of thyroid status categories with Global gait/Velocity



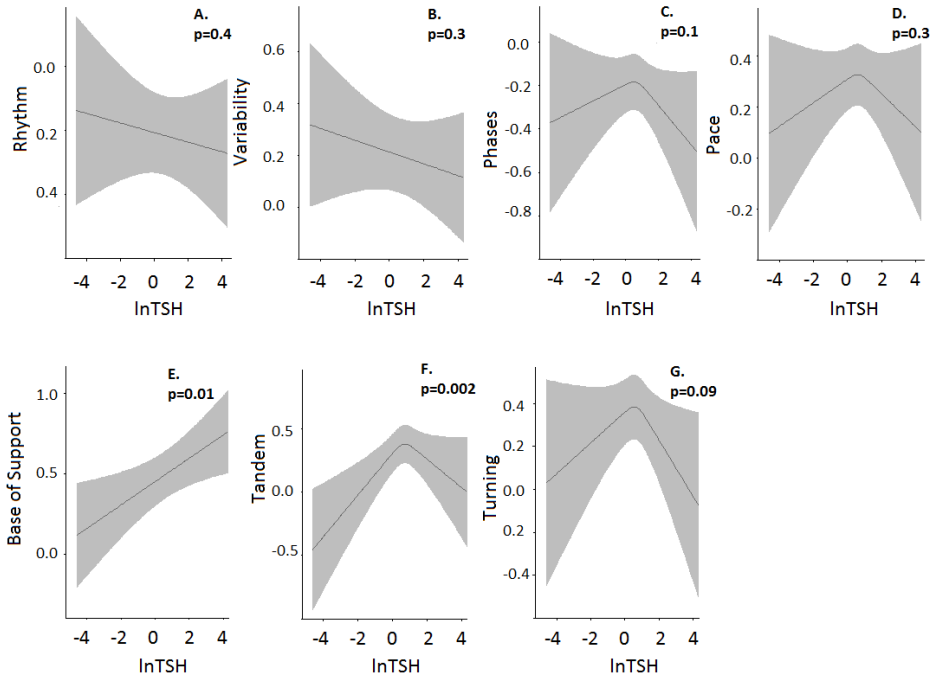
Adjusted for age, sex, cohort, smoking, alcohol intake, education level, height, weight, time interval between thyroid function measurement and gait assessment, knee pain or stiffness, hip pain or stiffness, prevalent stroke, CESD depression score, cerebellar cortical volume, intracranial volume, thyroid peroxidase antibodies. Thyroid status categories are plotted against differences in Z score of Global gait/Velocity, with euthyroid subjects as reference. 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.

The associations of thyroid function with gait domains and gait velocity

TSH levels were positively linearly associated with Base of support (p=0.01) and followed an inverted U-shaped curve with respect to Tandem (p=0.002) (Figure 4) and gait velocity (p=0.02) (Figure 2 C). In euthyroid participants, higher TSH levels were associated with higher Base of support (β=0.07; CI=0.01 to 0.14; p=0.01) and Tandem (β=0.06; CI=0.01 to 0.12; p=0.04), whereas higher FT4 levels were

associated with lower gait velocity (β=-0.96; CI=-1.85 to -0.07; p=0.03) (Supplementary Table S1). Clinical and subclinical hypothyroidism were associated with lower gait velocity than euthyroidism, with borderline statistical significance (β=-7.11; CI=-14.69 to 0.49; p=0.06 and β=-2.22; CI=- 4.50 to 0.05; p=0.05, respectively). Gait velocity decreased gradually from euthyroidism to clinical hypothyroidism (p-trend 0.01) (Figure 3 B).

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, 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.

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 circulating TSH levels may increase the risk of stroke through different pathways such as atrial fibrillation, hypercoagulation or unfavorable cardiovascular risk profile[25,26]. In addition, hypothyroidism can induce immune-mediated cerebellar degeneration[14]. Furthermore, both 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, 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 could explain the association. The most plausible can be peripheral neuropathy, since 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 might 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 velocity[16,17]. A relatively small study (n = 602) reported an association of high-normal FT4 levels with slower walk[17]. A second study reported a faster walk in individuals with mildly elevated TSH levels (4.5-7.0 mU/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 FT4 measurements. Instead, we used both TSH and FT4 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 (i.e. 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 normal TSH and low thyroid hormone levels[39]. Instead, we reported a non-linear association between TSH levels and Global gait. Also, NTIS is typical in critically ill patients, whereas the RS 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 RS does not have data available on serum triiodothyronine levels, which is a limitation for most population-based studies. However, TSH and FT4 concentrations are considered as the most relevant measurements of thyroid function in clinical practice. Moreover, RS 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.

In summary, 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.

Online supplemental material

https://www.nature.com/articles/srep38912

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CHAPTER 5.4

THE RISK OF AMD ACCORDING TO THYROID STATUS

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BMC Med. 2015

**Denotes equal contribution*

ABSTRACT

BACKGROUND In animal models, lack of thyroid hormone is associated with cone photoreceptor preservation while administration of high doses of active thyroid hormone leads to deterioration. The association between thyroid function and age-related macular degeneration (AMD) has not been investigated in the general population.

METHODS Participants ≥55 years from the Rotterdam Study with thyroid-stimulating hormone (TSH) and/or free thyroxine (FT4) measurements and AMD assessment were included. We conducted age- and sex-adjusted Cox-proportional-hazards models to explore the association of TSH or FT4 with AMD, in the full range and in those with TSH (0.4-4.0 mIU/L) and/or FT4 in normal range (11-25 pmol/L). Cox models were performed for the association of TSH or FT4 with Retinal Pigment Alterations (RPA), as an early marker of retinal changes. Multivariable models additionally included cardiovascular risk factors and thyroid peroxidase antibodies positivity. We also performed stratification by age and sex. A bidirectional look-up in Genome-Wide Association Studies (GWAS) data for thyroid parameters and AMD was performed. Single Nucleotide Polymorphisms (SNPs) that are significantly associated with both phenotypes were identified.

RESULTS We included 5573 participants with a median follow-up of 6.9 years (interquartile range 4.4-10.8 years). During follow-up 805 people developed AMD. TSH levels were not associated with increased risk of AMD. Within normal range of FT4, participants in the highest FT4 quintile had a 1.34-fold increased risk of developing AMD, compared to individuals in the middle group (95% confidence interval [CI] 1.07-1.66). Higher FT4 values in the full range were associated with a higher risk of AMD (Hazard Ratio 1.04, CI, 1.01-1.06 per 1 pmol/L increase). Higher FT4 levels were similarly associated with a higher risk of RPA. Restricting analyses to euthyroid individuals, additional multivariable models and stratification did not change estimates. We found a SNP (rs943080) in the *VEGF-A* gene, associated with AMD, to be significant in the TSH GWAS ($p=1.2x10^{-4}$). Adding this SNP to multivariable models did not change estimates.

CONCLUSIONS Higher FT4 values are associated with increased risk of AMD - even in euthyroid individuals- and increased risk of RPA. Our data suggest an important role of thyroid hormone in pathways leading to AMD.

INTRODUCTION

Age-related macular degeneration (AMD) is a disease of the retina in the elderly which can lead to irreversible blindness and is characterized by drusen, pigmentary changes, choroidal neovascularization and geographic atrophy. While AMD is one of the leading causes of visual impairment worldwide and increasing in prevalence [1-7], the exact pathophysiology and pathways leading to AMD are not entirely understood.

Thyroid hormones are known to regulate various visual functions in experimental and human studies [8-10]. Human retinal pigment epithelial (RPE) cells express thyroid hormone receptors and seem to be a direct target for thyroid hormones [11]. Recently it has been shown that suppression of thyroid hormone signaling resulted in preservation of cone photoreceptors in mouse models of retinal degeneration [12]. In contrast, administration of active thyroid hormone leads to deterioration of cones. Thyroid dysfunction and subclinical thyroid dysfunction are common in the general population, with a prevalence up to 10% [13-16]. These thyroid disorders are associated with various cardiovascular risk factors, including alterations in lipid levels, atherosclerosis and hypertension [17-19], which are known predisposing factors for development and progression of AMD [20,21]. However, there are no studies in the general population assessing the association between thyroid function and the risk of AMD. Therefore, we aimed to assess the relation between thyroid-stimulation hormone (TSH), free thyroxine (FT4) and the risk of incident AMD in a prospective population-based cohort study and to study possible underlying genetic pathways through investigating an overlap in genome-wide significant hits (i.e. bidirectional genetic look-up).

METHODS

The Rotterdam Study

The Rotterdam Study is a prospective population-based cohort study that addresses determinants and occurrence of cardiovascular, neurological, ophthalmologic, psychiatric, and endocrine diseases in the elderly living in Ommoord, a suburb of Rotterdam. The aims and design of the Rotterdam study

have been described elaborately elsewhere [22]. For this analysis we included participants from two independent cohorts from the Rotterdam Study. The Rotterdam Study Cohort 1 (RSI) started in 1989 and included a total of 7,983 participants (response rate 78 percent) aged 55 years and older. Baseline data were collected from 1990 until 1993 and four follow-up examinations were performed in 1993-1995, 1997-1999, 2002-2004 and 2009-2011.

The second cohort is the Rotterdam Study Cohort II (RSII) and includes a total of 3,011 participants (response rate 67 percent) aged 55 years and older. Baseline data were collected from 2000-2001 and follow-up examinations were performed in 2004-2005 and 2011-2012.

Study population

Participants from baseline study cohorts RSI (RSI-1) and RSII (RSII-1) were eligible for these analyses if they had TSH and/or FT4 measurements and had gradable fundus photographs at baseline and at least one follow-up eye examination. Since not all participants from RSI had thyroid measurements at baseline, additional baseline samples were drawn from RSI visit 3 (RSI-3). Participants with AMD at baseline (N=567) were excluded from further analyses. In total 5573 participants from these two cohorts were eligible to be included in our analyses (Additional Figure 1). The Medical Ethics Committee of the Erasmus University had approved the study protocols, and participants had given a written informed consent in accordance with the Declaration of Helsinki.

Assessment of thyroid function

For RSI-1, serum TSH (TSH Lumitest; Henning, Berlin, Germany), anti-TPOAb (ELISA; Milenia; Diagnostic Products Corp, Los Angeles, CA, USA) and free T4 levels (FT4; Vitros, ECI Immunodiagnostic System; Ortho-Clinical Diagnostics, Amersham, UK) were determined in a random subset of the baseline serum samples (n=1855). Thyroid function assessment was also performed in baseline serum samples for TSH and FT4 (The electrochemiluminescence immunoassay for thyroxine and thyrotropine, “ECLIA”, Roche) for RSI-3 and RSII-1. The tests’ TSH reference ranges did not differ substantially and had a good Spearman correlation co-efficient (0.96 for TSH, $p < 0.0001$and 0.81 for FT4, $p < 0.0001$). We determined the cut-off values for normal range TSH as 0.4-4.0 mIU/L according to national

guidelines. The reference range for FT4 was 11-25 pmol/L and anti-TPOAb levels greater than 60 kU/mL were regarded as positive.

Diagnosis of age-related macular degeneration

All eligible participants underwent fundus photography after pharmacologic mydriasis. For visits RSI-1 to RSI-3 and RSII-1 a 35° film fundus camera was used (Topcon TRV-50VT, Topcon Optical Company, Tokyo, Japan) after which a 35° digital color fundus camera (Topcon TRC-50EX, Topcon Optical Company, Tokyo, Japan with a Sony DXC-950P digital camera; 0.44 megapixel, Sony Corporation, Tokyo, Japan) followed for visits RSI-4, RSI-5, RSII-2 and RSII-3. Fundus transparencies were graded according to the Wisconsin Age-Related Maculopathy Grading [23] and the modified International Classification System [24] by trained graders under the supervision of senior retinal specialists (J.R.V., C.C.W.K.). The eyes of each participant were graded and classified separately, and the eye with the more severe grade was used to classify the person. In the analyses incident early and late AMD combined was used as outcome variable. In the manuscript this is referred to as AMD. Besides AMD we also investigated AMD specific lesions as a separate outcome variable. These lesions included retinal pigmentary alterations, large drusen (≥125µm) and large drusen area (≥ 5331,820 µm2) [25].

Baseline measurements

Smoking was derived from computerized baseline questionnaires and categorized in current or non-current smokers. Blood pressure, systolic and diastolic, was calculated as the average of two consecutive measurements, using random-zero mercury sphygmomanometers. Hypertension was defined as having a systolic blood pressure ≥ 140 mmHg or a diastolic blood pressure ≥ 90mmHg or using anti-hypertensive medication at baseline. Cholesterol was measured at baseline by the CKCL (Centra Clinical Chemical Laboratory) of the Erasmus University Medical Center. A subgroup of measurements was carried out in the laboratory of the Department of Epidemiology & Biostatistics (Erasmus University Medical School). History of diabetes was defined by a repeated impaired fasting glucose ≥ 7 or use of anti-glycemic medication at baseline. Body-mass index (BMI) was calculated as weight kilograms divided by height squared in meters.

Statistical analysis

Participant baseline characteristics were compared using a χ^2 or t-test. Due to a skewed distribution, TSH was log-transformed for the statistical analyses. We used cox-proportional hazards model to calculate the relationship between TSH and FT4 at baseline and the risk of incident AMD, first including all participants and then including only those with normal range TSH and/or FT4 values. We performed a crude cox-model including only thyroid parameters after which we also included quadratic and cubic terms to explore possible non-linear relationships. We then performed additional models adjusting first for age and sex and second also adding smoking, hypertension, cholesterol, diabetes and BMI to the model. Hypertension, cholesterol, diabetes and BMI could act as confounders and possible mediators depending on the presumed pathway through which thyroid function is related to AMD. These variables were included in the multivariable model as possible confounders of non-vascular pathways. We looked at the association between AMD and TSH or FT4 both continuously and in quintiles, as well as overall and within the normal range of TSH. The middle quintile was used as reference group as biologically it is expected to represent the subgroup with the most normal thyroid function within the euthyroid group. We performed pre-defined stratification by sex and age categories, using a cutoff 65 years as this is the median of the current population and the treatment threshold for subclinical thyroid dysfunction according to the European guidelines [26]. Further interaction terms were introduced to the model to explore possible differential risk patterns. We performed a sensitivity analysis excluding those using thyroid medication at baseline (levothyroxine and anti-thyroid drugs) and those with prior self-reported thyroid disease at baseline. We also performed FT4 and TSH analyses with specific AMD lesions defined as retinal pigment alteration, large drusen and large drusen area as separate outcome variable to examine possible early changes in underlying pathways. To address the issue of drop-out of individualsduring follow-up that could possibly be not completely at random, we adjusted the model for inverse probability weights (IPW"s). These were calculated using possible baseline explanatory variables for drop-out such as smoking, BMI and medication use. Proportional hazards assumption was checked statistically using the Schoenfeld test and assessing the Schoenfeld plot. All statistical analyses were performed

using SPSS version 21 (SPSS IBM, New York, U.S.A) except for the Schoenfeld tests and (Schoenfeld) plots which were performed in R (survival package, R-project, Institute for Statistics and Mathematics, R Core Team (2013), Vienna, Austria, version 3.0.2).

Bidirectional genetic look-up

Genome-Wide Association Studies (GWAS) have been performed for AMD [27] and thyroid function (TSH and FT4) [28,29]. These studies identified several single-nucleotide polymorphisms (SNPs) associated to these two phenotypes. Some of the genome-wide significant SNPs in the AMD GWAS might also play a role in thyroid function and vice versa. Overlap between common genetic polymorphisms can provide insight into possible shared genetic pathways. It might also elucidate a mediation effect between the two phenotypes, i.e. identify and explicate the process that underlies a possible observed relationship between thyroid function and AMD. To evaluate these potential genetic pathways, we conducted a bidirectional genetic look-up using the results of the above mentioned GWA Studies for AMD and thyroid function. We first extracted SNPs that reached genome-wide significance from the AMD GWAS performed by the AMD Gene consortium [27]. We then checked whether these were significantly associated with TSH or FT4 in the thyroid function GWAS performed by Porcu et al. [28]. Hereafter we extracted the genome-wide significant SNPs for TSH or FT4 from the thyroid function GWAS and checked whether they were associated with AMD in the AMD GWAS. For the significance level, we applied a multiple testing correction (Bonferroni Correction), using a p-value threshold of 0.05 divided by the amount of significant SNPs per GWAS. In case of a significant finding, we added the SNP to the multivariable model to evaluate a possible mediation effect.

RESULTS

We included 5573 participants with TSH and/or FT4 measurements at baseline and incident AMD data, with a median follow-up of 6.9 years (interquartile range [IQR] range of 4.4-10.8 years). Of these, 5572 had TSH and 5504 had FT4 baseline measurements. A total of 805 people developed AMD (Early AMD N=725, Late AMD N=80) during follow-up with an incidence rate of 18 per 1000 person-

years. The baseline characteristics for those with and without incident AMD during follow-up were comparable, except for proportion of diabetes **(Table 1)**.

Table 1 Baseline characteristics of included participants from the Rotterdam Study evaluating the association between thyroid function and AMD*

Variable	No incident AMD N=4768	Incident AMD N=805	P-value**
Age, years	67.6 (7.6)	67.9 (7.1)	0.29
Sex % female	57.6	57.8	0.94
History of Diabetes %	10.8	8.4	**0.04**
BMI kg/m^2	26.9 (3.9)	26.6 (3.7)	0.07
Cholesterol mmol/L	6.1 (1.2)	6.1 (1.1)	0.23
Smoking % current	20.7	21.0	0.85
Hypertension %	63.0	58.7	0.17
TSH mIU/L median (IQR)	1.78 (1.15-2.69)	1.73 (1.17-2.67)	0.78
FT4 pmol/L	15.8 (2.6)	16.0 (3.2)	0.13
TPOAb kU/L	30.5 (95.1)	30.8 (96.2)	0.93

*Values are means and SD unless otherwise specified.
** For comparison a t-test was conducted, for TSH the log-transformed values were used.
Abbreviations: AMD = Age-related Macular Degeneration; BMI = body-mass index; TSH = thyroid-stimulating hormone, FT4 = free thyroxine; SD = Standard deviation; IQR = inter-quartile range; TPOAb = thyroid peroxidase antibodies.

Association between thyroid function and AMD

Although there was no association between TSH and AMD (hazard ratio [HR] 0.99; 95% confidence interval [CI] 0.91-1.07, Table 2), the risk of AMD was significantly increased in those with higher FT4 levels (Table 2). When categorizing the FT4 values within normal range quintiles, those in the highest FT4 quintile had an increased risk compared to the middle group with a HR of 1.34 (95% CI, 1.07-1.66) and a non-significant p for interaction (p=0.066) (Table 2). This association remained similar after additional adjustments for smoking, diabetes, hypertension, cholesterol, BMI, and TPOAb positivity (Figure 1). This association also remained similar after analyzing only those within the normal range of TSH and FT4 i.e. normal thyroid function. Excluding those with thyroid medication or thyroid disease at baseline as a sensitivity analysis, did not alter the association (Table 3). Stratifying for age and sex did not reveal any significant differential risk (Additional Table 1).

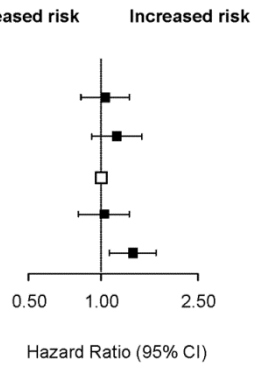
Table 2 Association between TSH, FT4 and risk of AMD

Incident AMD vs no AMD	AMD *N*	Total *N*	HR (95% CI), model 1	HR (95% CI), model 2	HR (95% CI), model 3
TSH mIU/L	805	5572	0.99 (0.91-1.07)	0.99 (0.91-1.07)	0.99 (0.91-1.07)
TSH in normal range[a]	696	4756	1.06 (0.91-1.23)	1.09 (0.93-1.27)	1.08 (0.93-1.26)
Normal range TSH[a]					
Q1 0.40-1.10	148	1082	1.04 (0.82-1.32)	1.00 (0.79-1.28)	1.00 (0.79-1.28)
Q2 1.11-1.54	167	990	1.32 (1.04-1.66)	1.29 (1.02-1.62)	1.29 (1.02-1.62)
Q3 1.55-1.99	128	962	1	1	1
Q4 2.00-2.61	117	851	1.09 (0.85-1.40)	1.07 (0.83-1.37)	1.07 (0.83-1.38)
Q5 2.62-3.97	136	871	1.22 (0.96-1.56)	1.22 (0.95-1.55)	1.21 (0.94-1.54)
P interaction			*0.648*	*0.485*	*0.517*
Total	*696*	*4756*			
FT4 pmol/L	791	5504	1.04 (1.01-1.06)	1.04 (1.01-1.06)	1.04 (1.01-1.06)
FT4 in normal range[b]	765	5382	1.04 (1.01-1.07)	1.04 (1.01-1.07)	1.04 (1.01-1.07)
Normal range FT4[b]					
Q1 11.0-14.0	149	1090	1.03 (0.82-1.29)	1.04 (0.82-1.31)	1.04 (0.82-1.31)
Q2 14.0-15.1	152	1001	1.12 (0.89-1.41)	1.17 (0.92-1.47)	1.16 (0.92-1.47)
Q3 15.1-16.2	144	1094	1	1	1
Q4 16.2-17.5	134	1060	1.01 (0.80-1.28)	1.03 (0.81-1.30)	1.03 (0.81-1.31)
Q5 17.5-24.9	186	1137	1.34 (1.07-1.66)	1.35 (1.08-1.69)	1.35 (1.09-1.69)
P interaction			*0.066*	*0.088*	*0.080*
Total	*765*	*5382*			
Normal range FT4[b] *in normal range TSH*[a]	673	*4658*	1.04 (1.01-1.08)	1.04 (1.01-1.08)	1.04 (1.01-1.07)

[a] normal range of TSH defined as 0.4-4.0 mIU/L[b] normal range of FT4 defined as 11-25 pmol/L
Model 1: Adjusted for sex and age. Model 2: Model 1 + smoking, hypertension, cholesterol, diabetes, BMI. Model 3: Model 2 + thyroid peroxidase antibodies positivity
Abbreviations: AMD Age-related Macular Degeneration ; BMI body-mass index; CI confidence interval; FT4 free T4; HR hazard ratio; Q quintile; TSH Thyroid-Stimulating Hormone

Figure 1 Quintiles of FT4 within the normal range and risk of AMD

FT4 pmol/L quintiles	AMD N	Total N	HR Ratio (95% CI)[a]
11.0 - 14.0	149	1090	1.04 (0.82-1.31)
14.0 - 15.1	152	1001	1.16 (0.92-1.47)
15.1 - 16.2	144	1094	[REFERENCE]
16.2 - 17.5	134	1060	1.03 (0.81-1.31)
17.5 - 24.9	186	1137	1.35 (1.09-1.69)



[††] The normal range of FT4 was defined as 11-25 pmol/L (Conversion 1 pmol/L=0.0777 ng/dL).
[a] Analyses were adjusted for sex, age, smoking, hypertension, cholesterol, diabetes, body-mass index and thyroid peroxidase antibodies positivity
Abbreviation: Age-related Macular Degeneration; FT4 free thyroxine; HR hazard ratio

Table 3 Sensitivity analyses excluding participants with thyroid medication or thyroid disease at baseline

Incident AMD vs no AMD	AMD N/ Total N	HR (95% CI), model 1	HR (95% CI), model 2	HR (95% CI), model 3
Excluded medication thyroid[a]				
Free T4	752/5225	1.03 (1.01-1.06)	1.03 (1.01-1.06)	1.03 (1.01-1.06)
TSH mIU/L	778/5417	0.99 (0.91-1.08)	1.00 (0.92-1.09)	1.00 (0.91-1.09)
Excluding baseline thyroid disease[b]				
Free T4	751/5237	1.04 (1.01-1.08)	1.04 (1.01-1.07)	1.04 (1.01-1.08)
TSH mIU/L	764/5300	0.98 (0.89-1.07)	0.98 (0.89-1.07)	0.97 (0.89-1.07)

[a] 155 participants had thyroid medication (ie. thyroid hormone use) at baseline
[b] 272 participants had self-reported thyroid disease at baseline
Model 1: Adjusted for sex and age. Model 2: Model 1 + smoking, hypertension, cholesterol, diabetes, BMI. Model 3: Model 2 + thyroid peroxidase antibodies positivity
Abbreviations: BMI body-mass index; CI confidence interval; FT4 free thyroxine; HR hazard ratio; TSH thyroid-stimulating hormone

The association between thyroid function and retinal pigment alterations for FT4 showed similar significant hazard ratios, with the exception of the risk estimates when looking at FT4 only in the normal range of TSH (Table 4). TSH and FT4 were not associated with large drusen or large drusen area (data not shown).

Introducing quadratic and cubic terms for TSH and FT4 to the crude model, as an exploration of non-linearity, did not improve model performance. Taking possible non-random follow-up using IPW's did not change risk estimates. The proportional hazards assumption was checked statistically with the Schoenfeld test and Schoenfeld plot and met for both the TSH (p = 0.232) and FT4 (p = 0.154) analyses.

Table 4 Association between FT4 and TSH with retinal pigment alterations[a]

Incident pigment alterations vs no pigment alterations	Cases N/ Total N	HR (95% CI), model 1	HR (95% CI), model 2	HR (95% CI), model 3
TSH mIU/L	729/5401	0.98 (0.90-1.06)	0.97 (0.90-1.06)	0.96 (0.88-1.04)
Normal range TSH[b]	618/4591	1.02 (0.87-1.20)	1.05 (0.89-1.23)	1.04 (0.88-1.22)
FT4 pmol/L	720/538	1.04 (1.01-1.07)	1.04 (1.01-1.07)	1.04 (1.01-1.07)
Normal range FT4[c]	697/5226	1.04 (1.01-1.07)	1.04 (1.01-1.07)	1.04 (1.01-1.07)
Normal range FT4[c] in normal range TSH[b]	601/4500	1.03 (1.00-1.07)	1.03 (0.99-1.06)	1.03 (0.99-1.06)

[a] participants with late AMD were excluded from this analysis
[b] normal range of TSH defined as 0.4-4.0 mIU/L.
[c] normal range of FT4 defined as 11-25 pmol/L.
Model 1: Adjusted for sex and age. Model 2: Model 1 + smoking, hypertension, cholesterol, diabetes, BMI. Model 3: Model 2 + thyroid peroxidase antibodies positivity
Abbreviations: BMI body-mass index; CI confidence interval; FT4 free thyroxine; HR hazard ratio; TSH thyroid-stimulating hormone

Bidirectional genetic look-up

In the thyroid function GWAS, 20 SNPs were associated with TSH and 6 with FT4 [28]. The AMD GWAS revealed 19 genome-wide significant SNPs related to the phenotype. None of the SNPs from the thyroid function GWAS were significant in the AMD GWAS. One SNP (rs943080) in the Vascular Endothelial Growth Factor A (*VEGFA*) gene that is related to AMD, was also significantly associated with TSH ($p=1.2x10^{-4}$, significance threshold =0.0026) (Additional Table 2). Within our study population, GWAS data were available for a total 4646 participants. Additionally correcting for the rs943080 SNP in the most adjusted model in these participants,

resulted in similar risk estimates for the FT4 analysis (HR 1.04, CI 95% 1.01-1.07). Stratifying for this SNP did show risk differences between the different genotypes but not significantly (Additional Table 1).

DISCUSSION

In this prospective cohort study we investigated the association between thyroid function and incidence of AMD. Higher FT4 values were associated with an increased risk of developing AMD, even within the normal range of TSH and FT4 (i.e. euthyroid subjects), while there was no association between TSH and AMD. The similar findings between higher FT4 levels and retinal pigment alterations might suggest that thyroid hormone plays a role in the development of AMD rather than just act as a promoter of disease. To our knowledge, this is the first prospective population-based cohort study to look at the association between thyroid function and AMD.

A limited number of studies investigating thyroid disease and AMD have been published, all lacking laboratory assessment of thyroid function. Bromfield et al. reported an increased risk of AMD in subjects with self-reported hypothyroidism [30]. A case-control study by Anand et al. reported an association between thyroid hormone use and a higher risk of AMD with geographic atrophy [31], but no data were reported on the number of patients that were over- or undertreated. Similarly, the Beaver Dam Eye study also reported an association between thyroid hormone use and early AMD [32], but this was not confirmed by Douglas et al. [33]. As mentioned previously, none of these studies had laboratory assessment of thyroid function nor did they investigate the association in a time-to-event analysis. In our study, excluding all subjects using thyroid medication did not alter risk estimates, supporting a potential intrinsic effect of thyroid hormone.

There are several pathophysiological explanations for the relationship between thyroid hormones and AMD. In a mouse model of retinal degeneration, suppression of thyroid hormone signaling resulted in preservation of cone photoreceptors [12]. The same study found that stimulating thyroid hormone signaling, by administering the active thyroid hormone triiodothyronine, deteriorates cones in mouse models with a slow progressive and moderate degeneration

phenotype [12]. In addition, mice lacking type 3 deiodinase, the enzyme responsible for the degradation of thyroid hormones, have decreased survival and disturbed maturation of cone photoreceptors [34]. The findings of these studies suggest that thyroid hormone may lead to a higher turnover of photoreceptors and in retinal degeneration this leads to deterioration of photoreceptors. Beside photoreceptors, thyroid hormone might also have an influence on the retinal pigment epithelial cells [11]. In the healthy retina the turnover of photoreceptors is extremely high. Every day the photoreceptors shed the ends of their outer segments resulting in full renewal every ten days. These shedded parts of the outer segments are fagocytosed by the retinal pigment epithelium (RPE) cell [35]. Increase of the turnover of the photoreceptors by thyroid hormone may bring additional stress to the process. RPE cells at distress may change resulting in pigmentary alterations in the macular area. The RPE cells may also be targeted directly by the thyroid hormone resulting in these changes [11]. These results may provide an explanation for the findings in our study.

Thyroid dysfunction has been linked to cardiovascular risk factors and disease, including effects on the vascular function, lipids and atherosclerosis [36]. As some of these risk factors are also linked to AMD [20,21], one could speculate about a joint vascular pathway leading to both thyroid dysfunction and AMD or perhaps that the relation between thyroid dysfunction and AMD could be mediated through this pathway. We were not able to confirm these hypotheses. First of all, these cardiovascular risk factors are mainly seen in hypothyroidism, (i.e. high TSH and low FT4), whereas our data show an association between high FT4 and AMD. Also, correcting for some of these risk factors (e.g. hypertension), that could act as confounders and possible mediators, did not change risk estimates suggesting that the effect of thyroid function is not through these pathways. Lastly, *VEGFA* gene was found to be significant in the look-up for the TSH GWAS and not the FT4 GWAS. However, our results suggest a higher risk of AMD in higher levels of FT4 and not in TSH. Furthermore, the association did not change by adding this SNP to the multivariable model.

We find an effect with FT4 but not with TS, which seems to be in line with previous literature from cohort studies in elderly populations investigating the relation between thyroid function and several other endpoints [37,38]. Regulation of serum

thyroid hormone levels is controlled by the hypothalamus-pituitary-thyroid axis. The set point of this feedback mechanism is defined individually, with thyroid hormone levels showing a much greater inter-individual than intra-individual variability [39]. The individual set point can be modulated by several pathophysiological (e.g. critical illness) and physiological (e.g. aging) mechanisms[40]. This could be an explanation why in this elderly and aging population we do find an association with FT4 but not with TSH, especially in the euthyroid range. Furthermore, previous literature showed an increase in TSH with increasing age, suggesting higher TSH levels are needed to keep thyroid hormone levels within the desired range[38]. We only have thyroid function measures at baseline and are therefore not able to investigate whether changes in thyroid function over time is an explanation for the discordant association between TSH, FT4 and AMD.

Important strengths of our study are the assessment of thyroid function at baseline through laboratory testing as well as the elaborate assessment of AMD at baseline and follow-up. Also, we were also able to investigate the association between thyroid function and specific AMD lesions like retinal pigment alterations to examine possible early changes in underlying pathways. The availability of genetic data gave us the opportunity to explore possible genetic pathways. The bidirectional genetic look-up, revealed one SNP in the *VEGFA* gene to be significant in the TSH GWAS but not for FT4. Adding this SNP to the multivariate model did not alter risk estimates. An explanation for the absence of overlapping genome-wide significant SNP's could be that these GWA studies were underpowered for this association.

A limitation of our study is that thyroid parameters were measured once at baseline. Therefore, the evolution of thyroid hormone levels could not be taken into account. Also, residual confounding cannot be excluded, even with the large number of covariates included in these analyses. Lastly, this study is conducted in a mainly Caucasian population of 55 years and older and may not be generalizable to other populations.

Conclusions

We find an increased risk of incident AMD in subjects with higher FT4 levels, even in those with a normal thyroid function and when excluding thyroid medication users. This implies an intrinsic (i.e. not exogenous) deleterious effect of thyroid

hormone on AMD. We also find an association between higher FT4 levels and retinal pigment alterations, suggesting that thyroid hormone could even play a role in the early stage of development of AMD. Functional and clinical studies could provide more evidence for a true causal relationship.

Supplemental online material

https://bmcmedicine.biomedcentral.com/articles/10.1186/s12916-015-0329-0

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CHAPTER 5.5

CHARACTERISTICS AND DETERMINANTS OF THYROID FUNCTION

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Thyroid 2016

ABSTRACT

BACKGROUND Information on determinants and change of thyroid function over time is sparse and conflicting but crucial for clinical interpretation and research. Therefore, our aim was to systematically investigate determinants of thyroid-stimulating hormone (TSH), free thyroxine (FT4) (as markers of thyroid function), their mutual relation (as marker of thyroid function set point) and changes in thyroid function over time in the Rotterdam Study, a population-based cohort study.

METHODS We included 9402 participants not taking thyroid medication and with available thyroid function measurements. Repeated measurements (6.5 year-interval) were available for 1225 participants. The association of selected determinants with TSH, FT4 and their mutual relation (reflecting thyroid function setpoint) was estimated using linear regression models using restricted cubic splines with three knots. The factors investigated were age, gender, body-mass index (BMI), tobacco smoking, alcohol use, thyroperoxidase antibodies (TPOAb), and common genetic factors.

RESULTS Most influential determinants of TSH were age, smoking, genetic determinants and TPOAb levels ($p < 0.001$). For FT4, most influential determinants were age, BMI, gender, genetic determinants and TPOAb levels ($p < 0.001$). Older age, female gender and increased TPOAb levels were associated with a stronger relation between TSH and FT4. TSH levels did not change over time, irrespective of age. FT4 levels increased over time, most prominently those older than 65 years of age (mean increase of 4.5 pmol/L).

CONCLUSIONS The main factors that influence the relationship between thyroid hormone and molar concentrations of TSH in study are age, smoking, BMI and common genetic variants. The set point that determines TSH secretion as it relates to negative thyroid hormone feedback is modified by age, gender and TPOAb positivity. FT4 levels increase over time, with a more pronounced increase in the elderly, while TSH values seem stable over time. Our results question the current notion of a TSH increase with increasing age.

INTRODUCTION

Thyroid function is determined by genetic and environmental factors, including life-style related influences. Clinically, thyroid function is defined by laboratory measurement of thyroid-stimulating hormone (TSH) and free thyroxine (FT4). Twin studies have estimated a heritability of 49-65% for TSH and 40%-90% for FT4, suggesting a strong influence of genetic factors on thyroid function[1-4]. Nevertheless, genome-wide association studies (GWAS), designed to identify common single nucleotide polymorphisms (SNPs) relevant to thyroid function, have so far only explained a small proportion of thyroid function variability[5,6]. Genetic variants analyzed by a large multi-center GWAS in over 25,000 participants explain 5.6% and 2.3% of total TSH and FT4 trait variance, respectively, leaving a large proportion of genetic determinants undiscovered[6]. In addition to genetic predisposition, several environmental and existential factors have been implied to influence thyroid function, including age, gender, body-mass index (BMI), tobacco smoking, alcohol use and thyroperoxidase antibodies (TPOAb), reflecting thyroid autoimmunity[7-14]. The association of thyroid function with age has been investigated in several studies with conflicting results, ranging from positive to negative associations for both TSH and FT4 values [9,13-16]. The between-study differences can at least partially be explained by variability in iodine status between populations, but other determinants might also contribute to these discrepancies[13]. For other determinants (e.g. BMI and smoking), literature has been less controversial on the direction of association. However, to date, no study has investigated the effect of these different characteristics and genetics factors on the mutual relationship between FT4 and TSH (as a marker of thyroid function set point) [4,17-22].

The identification of thyroid function determinants and the quantification of the extent of potential effects is important for clinical interpretation and research, yet the evidence on determinants of thyroid function is insufficient and conflicting. Therefore, we aimed to investigate if and to what extent age, gender, BMI, smoking, alcohol use, TPOAb and common genetic variants are could be determinants of TSH, FT4 and their mutual relationship in a large prospective

population-based study. Furthermore, we set out to describe changes in thyroid function over time using repeated measurements.

METHODS

The Rotterdam Study

All analyses were performed in 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 RS have been described in detail elsewhere[23]. We included participants from three independent cohorts of the RS. The RS Cohort 1 (RSI) includes participants aged 55 years and older and baseline data were collected during 1990-1993. RS Cohort II (RSII) includes participants aged 55 years and older and baseline data were collected from 2000-2001. For the RS Cohort 3 (RSIII), all residents aged 45 years and over who had not been invited before were asked to participate and baseline data were collected from 2006 to 2008. The Medical Ethics Committee of the Erasmus University approved the study protocols 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.

Study population

Participants from study cohorts RSI visit 3 (RSI-3), RSII visit 1 (RSII-1) and RSIII visit 1 (RSIII-1) were eligible for the cross-sectional analyses if TSH or FT4 measurements were available (Supplemental Figure 1A). For the longitudinal analyses we included participants from RSI that had two measurements of TSH and/or FT4 at different visits (RSI-1 and RSI-3, Supplemental Figure 1B). For all analyses, we excluded participants using any thyroid medication (i.e. levothyroxine, thiamazole, carbamazole or propylthiouracil) at baseline.

Thyroid function measurements

TSH, FT4 and/or TPOAb assessment for the cross-sectional analyses (1997 - 2007, Supplemental Figure 1A, Assay 1) was performed at the same time in all three cohorts in serum sample stored at -80 degrees Celsius of 9,402 participants not using thyroid medication (The electrochemiluminescence immunoassay for thyroxine, thyrotropine and thyroid peroxidase antibodies, "ECLIA", Roche, Mannheim, Germany). TPOAb levels greater than 35 kU/mL were regarded as positive, according to assay manufacturer recommendations.

For the longitudinal analyses thyroid function measurements were performed in participants of RSI (not using thyroid medication) using a different assay (TSH Lumitest; Henning, Berlin, Germany, TPOAb with ELISA; Milenia; Diagnostic Products Corp, Los Angeles, CA, USA, FT4; Vitros, ECI Immunodiagnostic System; Ortho-Clinical Diagnostics, Amersham, UK) and were conducted at two different time points between 1990 and 2000 with this same assay (n= 1255, Supplemental Figure 1B, Assay 2). In the assay used for the longitudinal analyses, TPOAb levels greater than 60 kU/mL were regarded as positive, according to assay manufacturer recommendations. The measurements performed with the two different assays were highly correlated (Spearman correlation co-efficient (r) =0.96 for TSH, $p < 0.0001$, r=0.81 for FT4, $p < 0.0001$ and r=0.68 for TPOAb, $p < 0.0001$).

Other variables

Based on previous studies, biological plausibility and data availability [4,6-8,19,24], we selected age, gender, tobacco smoking, alcohol intake, BMI, TPOAb and a genetic risk score (GRS) of TSH or FT4 as potential determinants for thyroid function. Information on smoking was derived from baseline questionnaires and categorized in current, previous and never smokers. Information on alcohol consumption was acquired from questionnaires where participants were asked for the average daily consumption of alcohol. Alcohol intake was recorded in grams per day and then divided in quintiles of daily intake. BMI was calculated as weight in kilograms divided by height in meters squared.

Genotyping and Genetic Risk Score

Genotyping was conducted, in self-reported white participants, using the Illumina 550K and 610k arrays and details are described elsewhere [23]. Participants were excluded if they had excess autosomal heterozygosity, mismatch between called

and phenotypic sex, or recognized as being outlier with identical-by-state clustering analysis. We used MACH to impute SNP dosages based on the phase I version 3 reference panel from the 1000 Genomes Project [25,26]. For this study, we selected common SNPs previously reported to have an association with TSH or FT4, from the largest multi-center GWAS of >25,000 individuals [6]. A GRS was compiled using 20 SNPs that have been associated with TSH and six SNPs that have been associated with FT4 (Supplemental Table 1). We calculated a weighted GRS by multiplying the number of risk alleles at each locus by the corresponding reported β coefficient from the abovementioned GWAS and then summing the products. The total score was then divided by twice the average effect size multiplied by 100 to rescale the scores to a range between 0 and 100. A GRS for TSH and FT4 was available for a total of 7125 participants.

Statistical analysis

We assessed the relation and explained variability (r^2) of the environmental and common genetic determinants with TSH or FT4 and their mutual relation, in univariable and multivariable ordinary least squares linear regression models. Non-linearity was assessed with restricted cubic splines using three knots at the 10th, 50th and 90th percentile and we additionally adjusted the multivariable analyses for cohort. Effect estimates are reported as standardized β (β per SD of the determinant). As a sensitivity analysis, we repeated these models after exclusion of participants within the lowest and highest 2.5-percentile of TSH and FT4. To account for multiple testing we applied a Bonferroni correction, considering 0.05 divided by the number of tested variables multiplied by the number of outcomes as significant (0.05/ (7*2) = 0.0036). Furthermore, we assessed changes in TSH or FT4 over time using ordinary least squares linear regression models. For these analyses, we added time between the two measurements and FT4 or TSH at baseline to the multivariable models. Also, we compared participant baseline characteristics for subjects with and subjects without follow-up measurements of TSH and FT4. As a sensitivity analysis, we repeated these models after exclusion of participants receiving thyroid medication during follow-up. Additional sensitivity analyses performed were: 1) excluding participants with TPOAb positivity, as defined by the assay manufacturer, and 2) excluding those with self-reported thyroid disease. TSH and TPOAb levels were log transformed (natural logarithm)

for the continuous analyses to approximate a normal distribution and denoted as lnTSH and lnTPO. Multiple imputations were performed in case of missing variables (less than 2% for all variables). 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) or SPSS version 21 (SPSS IBM, New York, U.S.A).

RESULTS

Environmental characteristics as determinants of TSH and FT4

The final study population consisted of 9402 participants for the cross-sectional analyses with a mean age of 65.1 years of which 55.9% were female (Table 1). The associations of the potential determinants with TSH are shown in Figure 1 and Supplemental Table 2. For TSH levels the most influential non-genetic factors were age (β -0.07), smoking (β -0.07) and TPOAb levels ($p < 0.001$). For FT4, the environmental determinants with the largest influence were age (β 0.07), BMI (β -0.10), gender (-0.09) and TPOAb levels ($p < 0.001$) (Figure 2, Supplemental Table 2).

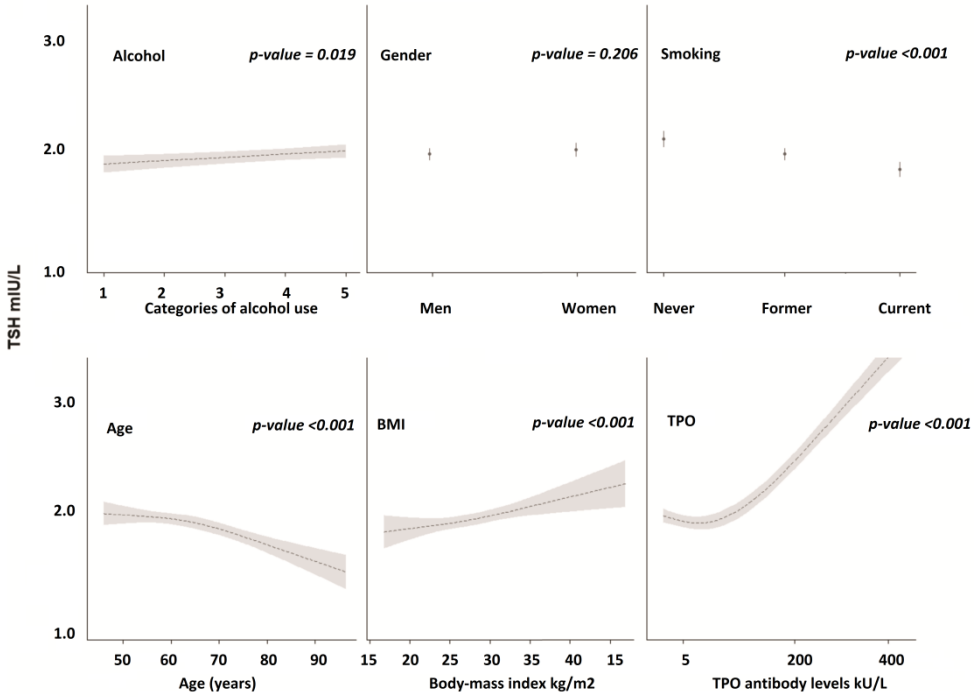
Table 1 Baseline characteristics of included participants in the Rotterdam Study with TSH or FT4 measurements

Variable	Mean (SD)[a]
Number of participants[b]	9402
Age, years	65.1 (9.9)
Female (%)	5253 (55.9)
BMI kg/m2	27.2 (4.2)
Smoking, N (%)	
current	1998 (21.2)
past	4474 (47.6)
never	2930 (31.2)
TSH ImU/L median (IQR)	1.90 (1.29-2.75)
Alcohol grams/day, median (IQR)	14.6 (1.4-20.0)
FT4 pmol/L	15.6 (2.2)
TSH ImU/L (2.5%-97.5%)	0.40-6.64
FT4 ImU/ L (2.5%-97.5%)	11.6-20.2
TPOAb positive[c], N (%)	1136 (12.1)
Natural logarithm of TPOAb	2.38 (1.07)

[a] Values are means and SD unless otherwise specified. [b] Of which 9392 had both FT4 and TSH measurements. A total of 9391 participants had TPO measurements. [c] TPOAb positivity was defined as >35 kU/L. Abbreviations: BMI body-mass index; TSH thyroid-stimulating hormone, FT4 free thyroxine; SD Standard deviation; IQR inter-quartile range; TPOAb thyroperoxidase antibodies

Repeating the analyses after exclusion of participants in the highest and lowest 2.5th percentile did not change the associations meaningfully except for gender and age (Supplemental Table 3). In these analyses the association between gender and thyroid function remained significant in all models while the association between age and TSH lost statistical significance after correction for multiple testing.

Figure 1 The association of environmental determinants with TSH levels

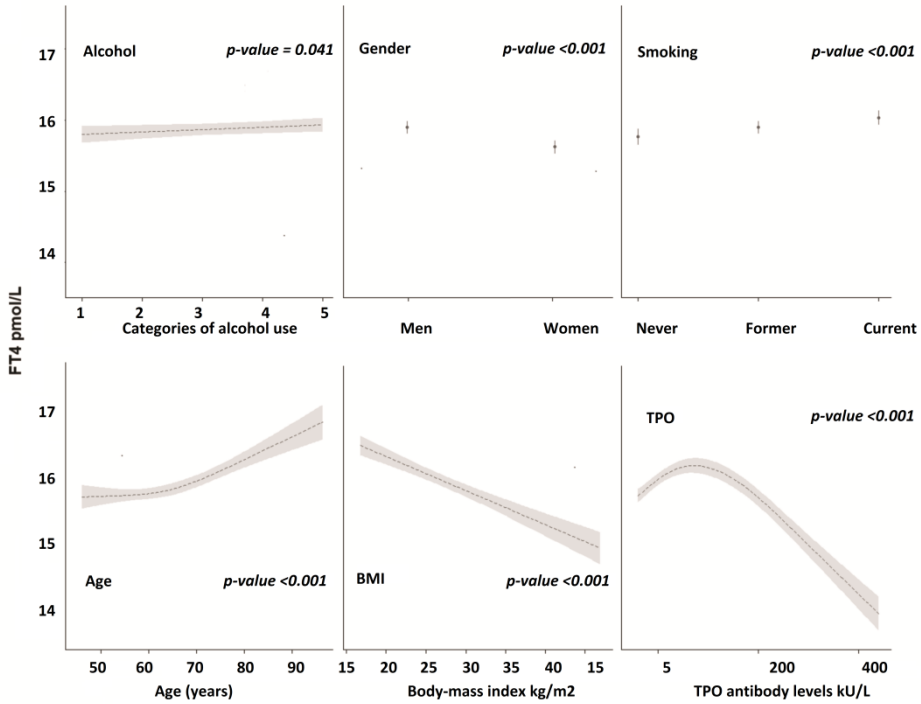


The association of each determinant with TSH is depicted as the regression line using ordinary least square restricted cubic splines regression with 3 knots or logistic regression and the accompanying 95% CI interval for the entire population. P-values are for the plotted association. These associations were evaluated adjusting each determinant for all other determinants. Alcohol categories represent quintiles of alcohol use (grams/day) in the population, where quintile one has an average use of 0.06 grams of alcohol a day, quintile two 3.5, quintile three 11.8, quintile four 16.3 and quintile five 32.9. Abbreviation: CI confidence interval, TSH thyroid-stimulating hormone

Genetic risk score as determinant of TSH and FT4

The GRS for TSH explained 4.3% of variance in TSH levels in the population (Supplemental Table 4). The GRS for FT4 explained 2.3% of population variance in FT4 levels. After exclusion of participants in the highest and lowest 2.5th percentile, the r^2 of the GRS for TSH and FT4 was 5.6% and 2.5% respectively. Including both common the GRS and environmental determinants in the model, the r^2 increased to 10.3% (+4.7%) for TSH and 5.1% (+2.6%) for FT4.

Figure 2 The association of environmental determinants with FT4 levels

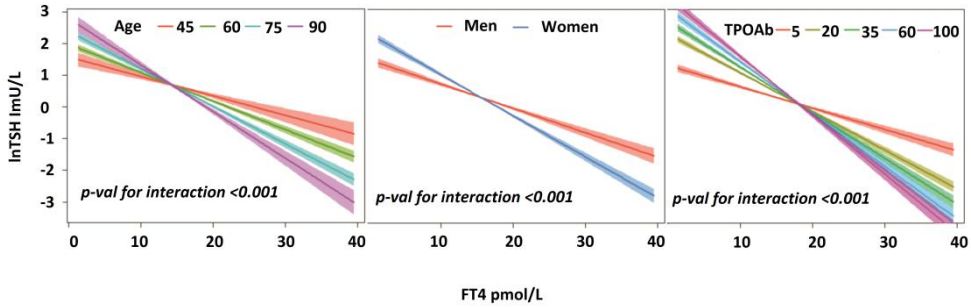


The association of each determinant with TSH is depicted as the regression line using ordinary least square restricted cubic splines regression with 3 knots or logistic regression and the accompanying 95% CI interval for the entire population. P-values are for the plotted association. These associations were evaluated adjusting each determinant for all other determinants. Alcohol categories represent quintiles of alcohol use (grams/day) in the population, where quintile one has an average use of 0.06 grams of alcohol a day, quintile two 3.5, quintile three 11.8, quintile four 16.3 and quintile five 32.9. Abbreviation: FT4 free thyroxine

Determinants of the thyroid function set point

There was a negative log-linear association between TSH and FT4 (Supplemental Figure 2). The association between TSH and FT4 differed according to age, gender and TPOAb levels (Figure 3). Subjects with higher age, women and subjects with higher TPOAb levels had a stronger log-linear relationship between TSH and FT4 (p for interaction <0.001). As a consequence, for women with TSH levels in the lower range, FT4 levels were higher than in men with the same level of TSH values, whereas in the higher range of TSH women had lower FT4 levels than men. The association of FT4 with TSH was not modified by BMI, smoking or alcohol (p for interaction > 0.50). The association and effect modification remained similar after the exclusion of participants in the highest and lowest 2.5th percentile.

Figure 3 The association of age, gender and TPOAb on the relation between TSH and FT4



The relation of TSH with FT4 according to age, gender and TPOAb are depicted. The p-value for interaction are for the continuous variables of age and TPOAb levels and 95% CI per group is depicted. TThese analyses were adjusted for age, gender, BMI, smoking, TPOAb levels and alcohol use. The natural logarithm of TPOAb and TSH levels were used for these analyses. Abbreviations: BMI body mass index, TPOAb thyroperoxidase antibodies, TSH thyroid stimulating hormone

TSH and FT4 changes over time

We included 1225 participants for the longitudinal analyses of which all participants had two TSH measurements and 1002 participants also had two FT4 measurements (Supplemental Table 5). The median time between measurements was 6.5 years (range 6.01- 9.71 years). The time between the two measurements was not associated with age of subjects at first measurements. Participants who had a second measurement were on average younger, had lower blood pressure,

and were less often diabetics or current smokers. Nevertheless, the TSH and FT4 values were not significantly different between participants who did and those who did not have two measurements (Supplemental Table 5).

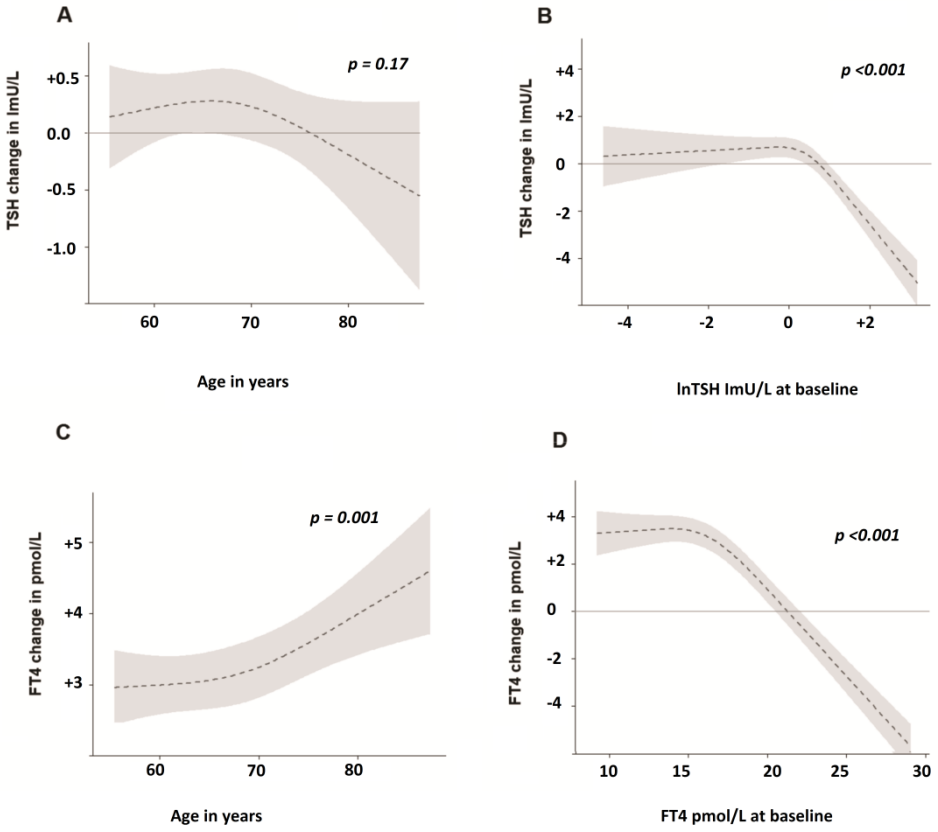
Overall, there was no change in TSH levels over time, irrespective of age at first measurement (Figure 4A). However, changes of TSH in time did depend on the TSH levels at baseline. If TSH levels at baseline were higher than ~TSH 2.72 mIU/L, TSH decreased over time (Figure 4B). FT4 levels increased over time where the increase was more prominent in the elderly (increase of 4.5 pmol/L, Figure 4C) and those with lower FT4 levels (increase of 4 pmol/L, Figure 4D). In participants with baseline FT4 values higher than ~22 pmol/L, FT4 levels decreased over time (Figure 4D). Excluding participants in the lowest or highest 2.5th percentile did not change the association of thyroid function and age. Sensitivity analyses excluding participants using thyroid medication during follow-up, excluding participants with a self-reported history of thyroid disease or excluding participants with TPOAb positivity, did not change the associations meaningfully.

DISCUSSION

In the current study, the main determinants of thyroid function were age, smoking, BMI, TPOAb levels and common genetic determinants. The mutual relation between TSH and FT4 (i.e. thyroid function set point), was modified by age, gender and TPOAb positivity. TSH values were fairly stable over time, except in those with higher baseline levels of TSH, where a decrease over time is seen. FT4 levels increase over time, with a more pronounced increase in older subjects and in those with low to average FT4 levels at baseline.

The heritability of thyroid function has been reported to be high, while the GRS for TSH and FT4 in our study only explained 5.6% and 2.5 % of the variance respectively. This highlights the need for identification of additional genetic determinants [27]. However, the large variability that is left unexplained is not solely due to yet unknown genetic factors but also dependents on environmental determinants.

Figure 4. The change in TSH and FT4 levels over time



Panel A. The association of change of TSH levels over time plotted against the age of participants at baseline. Panel B. The association of change of TSH levels over time plotted against the TSH levels of participants at baseline. Panel C. The association of change of FT4 levels over time plotted against the age of participants at baseline. Panel D. The association of change of FT4 levels over time plotted against the FT4 levels of participants at baseline. These analyses were adjusted for age, gender, BMI, smoking, TPOAb and time between measurements. The median time between measurements was 6.5 years (range 6.01- 9.71 years). For all panels the regression line and accompanying 95% confidence intervals are depicted. Abbreviations: BMI body mass index, TPOAb thyroperoxidase antibodies, TSH thyroid stimulating hormone

Most studies to date have focused on a specific determinant (e.g. smoking) in relation to thyroid function [7]. In contrast, in the current study we assess several important environmental as well as genetic determinants in relation to thyroid function. Only two previous smaller cross-sectional studies have analyzed several determinants of thyroid function in the general population, but the direction and extent of the associations differed from ours [3,4]. For example, Roef et al. [4] report

that a lower TSH as well as FT4 with higher BMI, while in our population we find a positive association of BMI with TSH and a negative association of BMI with FT4. Differences between our results and results from these studies could be due to several reasons including; the large sample size of our population compared to previous studies (>9,000 vs <1,000), differences in mean age, iodine status and ethnical backgrounds. Also, the number of genetic variants included in these previous analyses was smaller than in the current study. To our knowledge, no population-based study to date has reported on the association of environmental factors with the relation between TSH and FT4. We could therefore not compare our results with previous literature.

Regarding the association of age with thyroid function, results from cross-sectional studies are conflicting. Data from the National Health and Nutrition Examination Survey (NHANES) show a shift of the TSH distribution toward higher levels with older age, but provided no information on FT4 concentrations in different age groups [12]. A study from Scotland reports a significantly higher median and 97.5th centile TSH with higher age [24]. However, in contrast to these studies and in line with our results, two studies report a lower TSH and higher FT4 with higher age, of which the degree is partially dependent on historical iodine intake [13,28]. Nevertheless, these are all cross-sectional reports on the association of age with thyroid function. Cross-sectional analyses can provide information on differences in different age-groups but cannot provide insight on dynamic changes with aging.

To date, only two prospective population-based cohort studies with longitudinal thyroid function measurements have studied the relation of thyroid function with aging. Although both studies had similar iodine status, their results on the relation of thyroid function with aging (i.e. over time) differ from ours. Whereas both Waring et al (Cardiovascular Health Study [CHS], USA) and Bremner et al (Busselton Health Survey, Australia) find an increase in TSH over time and with increasing age, TSH levels did not change over time in our study. The lack of change in TSH in our study was independent of age at baseline. In participants with the highest levels of TSH at baseline, TSH even decreased over time. This decrease in TSH in the opposite direction of previous reports from CHS and the Busselton Health Survey could reflect a regression to the mean (i.e. normalization). We do not observe this phenomenon in participants with TSH values below 2.72 mU/L; the

TSH levels in these participants remain stable over time. Our data show an increase in FT4 values with age, which is in line with the previous report from Waring et al. but in contrast to Bremner et al, who report no change in FT4 levels with age.

There are different possible explanations for the differences between our results and results from the two before mentioned studies. Most importantly, the mean age of the studies differs notably, varying between 49 and 85 years for the Busselton and CHS cohorts respectively, whereas the mean age was 67 years for our longitudinal analyses. Furthermore, even though the current iodine status between the three cohorts of Busselton, CHS and RS is comparable and can be classified as sufficient on average, the intra-study variability could still be large. For example, the RS and Busselton cohorts include mainly white participants from one specific community, while CHS is a multicenter cohort study including multi-ethnic participants from several regions from the USA. This could lead to large variability in iodine intake and status between participants [29-32]. In addition, historical iodine intake is different between all three cohorts. This has been shown to result in differences in age-related thyroid function changes, which is irrespective to current iodine status [13,33].

The observed increase of FT4 with age in our study could represent an increase in autonomous thyroid hormone excretion. In elderly, hyperthyroidism is more prevalent [15,34,35], with Graves' disease and toxic (multi)nodular struma as the leading cause of hyperthyroidism in elderly living in iodine sufficient regions [34,36,37]. The increase of FT4 levels with aging would physiologically be expected to result in a concordant decrease in TSH levels. However, the observation that TSH levels remain stable over time in our study, irrespective of age at baseline, suggests a change in the physiological set point of the hypothalamic-pituitary-thyroid (HPT) axis.

Several age-related changes in pituitary sensitivity to TRH as well as pituitary TSH secretion capacity have been described, which could lead to alterations in the relation between TSH and FT4 [38,39]. However, even though we observe effect modification of the TSH-FT4 relation by age, the association is opposite to what we would expect. With older age there seems to be a stronger relation between TSH and FT4 (effect modification) suggesting an increased "sensitivity" of the HPT-axis

set point. This is in line with studies demonstrating that lower doses of LT4 are needed to suppress TSH secretion in elderly subjects [40-43]. Alternatively, and biologically more plausible, this effect modification by age could mainly reflect a marked increase in autonomous thyroid hormone secretion in the elderly, as previously mentioned, with a relative mild change in set point. Independent of the mechanisms that lead to these changes, the increase in FT4 without a concomitant change in TSH with increasing age could explain several study results from population-based cohorts, including the Rotterdam Study, reporting on the association of FT4 with different clinical outcomes, while there is no significant association with TSH [44-51]. Lastly, another explanation for changes during aging is a change in TSH bioactivity [52], which was not measured in our study. To date, there are no studies specifically addressing changes in TSH bioactivity and aging in the general population and thus this topic merits further research.

Strengths of our study include the large number of participants, providing adequate statistical power to investigate the relations of interest. Furthermore, through longitudinal assessment of TSH and FT4, we were able to investigate the changes of thyroid function over time. Also, by evaluating several of the determinants in one model, we were able to investigate the independent effect of each determinant. A limitation of our study is the lack of information on iodine status, TSH bioactivity and T3 measurements in our participants, which is a limitation in most population-based cohort studies [9,14]. Another limitation could be the use of immunoassays for FT4. Studies thus far have shown immunoassays of FT4 to sometimes correlate poorly and equilibrium dialysis or ultrafiltration LC-MS/MS seem to correlate better, especially in certain circumstances such as pregnancy and illness[53,54]. However, our population consists of community-dwelling elderly, the correlation of the two immunoassays used in our population was good and therefore we think this limitation is less applicable to our study. Lastly, the RS consists of a mainly white population of 45 years and older and results from this study may therefore not be well-generalizable to other populations.

Conclusions

Age, smoking, BMI, TPOAb and genetic influences have the largest impact on TSH and FT4, while age, gender and TPOAb had the greatest influence on the relation between TSH and FT4. With the included common genetic and environmental

factors we were able to explain 10.3 % of TSH and 5.1% of FT4 variability. Our data show that with age, TSH levels remain stable whereas FT4 increases substantially. This could explain why several studies report associations between clinical outcomes and FT4 but not TSH. Differences in findings with other populations could be due to differences in (historical) iodine status or other factors (e.g. ethnicity) and should be investigated. Further research should examine the influence of changes in thyroid function over time on health and disease.

Online supplemental material

http://online.liebertpub.com/doi/10.1089/thy.2016.0133

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CHAPTER 6

GENERAL DISCUSSION

MAIN FINDINGS AND INTERPRETATION

THE HEART

Thyroid hormone is important for development and growth in nearly all organs and tissues throughout life. The role of thyroid hormone on the cardiovascular system has been long recognized[1] and the heart is by far the most rigorously studied target organ of thyroid hormone in basic, epidemiological and translational research.[2] Previous epidemiological studies have focused mainly on the association of subclinical thyroid dysfunction and cardiovascular disease.[3-5] Our work has shown that the association between thyroid function and diseases of the heart also extends within the currently defined reference range. In **Chapters 2.1, 2.2 and 2.4**, we show that higher free thyroxine (FT4), and lower thyroid-stimulating hormone (TSH) to a lesser extent, is associated with an increased risk of atrial fibrillation (AF), sudden cardiac death (SCD) and atherosclerotic disease and mortality. These associations extend both within as well as outside of the reference range, with most prominent increased risk within the reference range. Within the reference range of FT4, the absolute risk of atrial fibrillation in elderly (defined as participants 65 years and older) increased from 6% to almost 12% from low-normal to high-normal FT4. For sudden cardiac death the 10-year risk increases from 1% to 4% for euthyroid participants with higher FT4 levels. We describe a 3.64-fold increased risk of atherosclerotic cardiovascular mortality with higher FT4 levels within the reference range. This was more marked in but not restricted to participants with previous atherosclerotic disease (3.26-fold vs 4.84-fold within the reference range). Also, we show a 2.42-fold increased risk of having high coronary artery calcifications scores, a marker of subclinical atherosclerosis, with higher FT4 levels, again, within the reference range. Interestingly, these associations were independent from cardiovascular risk factors such as dyslipidemia and hypertension, suggesting alternative mediating factors. In **Chapter 2.5** we provide an alternative approach to defining reference ranges. We present a proof of principle of how to define ‘optimal health ranges’ for thyroid function based on cardiovascular risk and these results will be discussed further under gaps in knowledge and future directions.

THE BRAIN

Stroke and other cardiovascular disease share many of the same risk factors including dyslipidemia and hypertension.[6] In turn, these risk factors are affected by thyroid hormone action and thyroid dysfunction.[2] Nevertheless, in contrast to an abundance of studies on ischemic heart disease and heart failure, we identified paucity in literature on the association of thyroid function with stroke (**Chapter 3.1**). In a published study meta-analysis we report no association of subclinical hypo-or hyperthyroidism on the risk of stroke, but we were able to identify no more than 6 published studies. Therefore, we performed a large individual participant-based (IPD) meta-analysis, including 17 cohorts and over 45,000 participants, to investigate the association of subclinical hypothyroidism and variations of thyroid function within the reference range with stroke risk (**Chapters 3.2 and 3.3**). We report on an increased risk of stroke with subclinical hypothyroidism, but mainly in participants younger than 65 years of age (up to 4.22-fold). Furthermore, we conclude that within the reference range, high-normal thyroid function is associated with an increased risk of stroke (approximately 1.3-fold). Even though within the Rotterdam study we mainly find an association of high and high-normal thyroid function with stroke (absolute risk difference of ~ 3% with increasing levels of FT4), the findings of the IPD meta-analysis on subclinical hypothyroidism do elude to a possible non-linear association. However, this has to be investigated in several populations with different backgrounds, in order to explain the possible discrepancies.

Neurocognitive and cerebrovascular disease have gained interest as important targets of thyroid hormone disturbances in recent years.[7] We identified higher levels of FT4 and lower levels of TSH as a risk factor for dementia, both within as well as outside of the reference range (**Chapter 3.4**), independent of cardiovascular risk factors. This association was most pronounced in older women, with a risk difference of 4% for the 10-year absolute risk of dementia, perhaps partially due to the higher baseline risk in the Rotterdam Study as compared to men. There was no association of thyroid function parameters with subclinical vascular disease on MRI and we therefore concluded that cardiovascular risk factors and disease are unlikely a part of the underlying pathophysiology.

METABOLISM

The recent findings of the association of thyroid function with diseases of older age show a deleterious effect of high thyroid function, most commonly high FT4. An exception to this rule seems to be metabolism-related disorders. In **Chapter 4.1**, we show an increased risk of type 2 diabetes with higher TSH and lower FT4 levels, most prominently in those with prediabetes at baseline and thyroid function within the reference range. The drop of type 2 diabetes risk in this specific subgroup was dramatic, going from a risk of 35% of developing diabetes to less than 15% when going from low-normal to high-normal FT4 levels. This is not only etiologically interesting, but could also candidate people with prediabetes as a specific subgroup to target for diabetes prevention in the context of thyroid function. Screening efforts, mainly targeting people with prediabetes, could be one of the first steps for research concerning the association of thyroid function and diabetes.

Non-alcoholic fatty liver disease (NAFLD) is the one of the most common chronic liver conditions worldwide and thyroid hormone affects numerous risk factors that can lead to NAFLD.[8] In **Chapter 4.2** we report an increased risk of NAFLD with lower FT4 levels (2.38-fold) and higher TSH levels. In line, higher TSH levels were associated with an increased risk of having clinically relevant fibrosis in NAFLD. A post-hoc analysis in a subgroup of participants from a randomized controlled trial (RCT) showed improvement of NAFLD with levothyroxine therapy in patients with subclinical hypothyroidism.[9] However, these results need to be verified in a larger RCT and with special attention to possible overtreatment and adverse short-term and long-term effects.

AGING

Cancer is one of the leading causes of morbidity and mortality around the world and increasingly becoming the leading cause in the industrialized world (Table 1).[10] Literature findings on the association of thyroid function with cancer have been controversial ranging from an association of hypothyroidism or hyperthyroidism with cancer risk, to no association.[11-14] Most studies to date have lacked a sufficiently large sample size or prospective study design. In **Chapter 5.1** we identify higher FT4 levels to be significantly associated with an increased risk of

any solid (1.42-fold), lung (2.33-fold), and breast cancer (1.77-fold). The exact mechanisms that link thyroid hormone with cancer risk need to be investigated in greater depth, but could vary according to certain characteristics. Thyroid hormone might well have pro-oncogenic and antioncogenic activities depending on cancer type (e.g. breast cancer vs lung cancer or small cell vs non-small cell lung cancer), site and stage. Further epidemiological studies could give a first clue in this respect.

In **Chapter 5.2**, we describe both the cross-sectional as well as longitudinal association of thyroid function with CKD. Interestingly, higher TSH levels were associated with lower eGFR cross-sectionally. However, longitudinally, higher TSH levels were associated with less annual eGFR decline and lower chronic kidney disease incidence. In the cross-sectional analysis, there is no distinction in temporality and because we know that poor kidney function also affects thyroid function, the cross-sectional results could also point towards reverse causation (i.e. poor kidney function leading to low thyroid function and not vice versa). However, in our prospective analysis temporality is assessed and we conclude that higher thyroid function is a risk factor for kidney function deterioration. Previous studies showing similar trends have been underpowered to report significant findings.[15] Future collaborative efforts could provide replication of our findings.

Disturbances in gait gradually increase with advancing age and perceived as a marker of general health status.[16] Previous studies have suggested an association of subclinical hypothyroidism with increased gait speed.[17] However, information on the association of thyroid function in general with gait velocity and other domains of gait was lacking. In **Chapter 5.3**, we describe a non-linear association of FT4 with global gait, a general marker of gait incorporating several gait domains, and to a lesser extent gait velocity, where both high and low FT4 seem deleterious. Gait is perceived as multifactorial, including musculoskeletal and neurological components amongst others and the underlying mechanism linking thyroid function to gait still need to be determined.

Vision impairment is one of the most burdensome diseases in elderly (Table 2).[10] Age-related macular degeneration (AMD) is the leading cause of loss of vision in people over 65 years of age.[18,19] Animal models have shown cone photoreceptor preservation with lack of thyroid hormone, while administration of high doses of

active thyroid hormone leads to deterioration.[20] In line, we report that high FT4 levels are associated with a higher AMD risk in the general population (**Chapter 5.4**), suggesting an important role of thyroid hormone in pathways leading to AMD, both within as outside of the reference range of thyroid function. The thyroid gland itself is also not resistant to external and existential factors such as aging. In **Chapter 5.5** we investigated determinants of thyroid function and established the trajectory of TSH and FT4 over time. We conclude that most important factors influencing both TSH and FT4 include age, genetic determinants and thyroid peroxidase antibodies. For TSH we additionally identified smoking as an influential factor, while for FT4 we additionally identified BMI and gender. However, the explained variability of thyroid function including environmental, existential and genetic determinants remained low (11.2% for TSH and 7.1% for FT4). Older age, female gender and increased TPOAb levels were associated with a stronger relation between TSH and FT4 (i.e. set point). Unlike previous literature, we report that in our study TSH levels did not change over time, irrespective of age, while FT4 levels did increase over time, most prominently those older than 65 years. Reasons for discrepancy could be differences in age groups studied and historical iodine status. Therefore, these factors should be taken into account when comparing future studies and results.

MEDIATING FACTORS

While establishing an association of thyroid function with hard clinical endpoints is a pivotal start, it is just that; a start. An important next step pertains to the identification of underlying pathways and mediators. Interestingly, the associations studied of thyroid function with clinical end points by our and other groups, ranging from SCD to dementia and from cancer to chronic kidney disease, appear to be independent of cardiovascular risk factors, such as lipids and hypertension. This implies that factors other than cardiovascular risk factors could play a role in the link between thyroid function and diseases of older age. In **Chapter 2.3** we investigated a promising possible mediator of the association between thyroid function and sudden cardiac death; QT variability. We report on a U-shaped association of FT4 with QT variability, implying that QT variability could be a mediator of FT4 with SCD. This probably only holds true in the higher range of

thyroid function, because higher FT4 is linearly associated with SCD risk. Even though we initially speculated that the association of thyroid function with dementia could be through cardiovascular risk factors and possible subclinical vascular brain disease, our results did not provide evidence for this hypothesis. Thyroid hormone impacts different essential neuronal processes including neurogenesis, myelination, and neural differentiation throughout life.[21] In **Chapter 3.5** we investigated the association of thyroid function with brain morphology and microstructural organization and report an age-dependent relation of FT4 with brain volumes and microstructural organization. Higher FT4 levels were associated with larger total brain (mainly white matter) in younger individuals, but with smaller total brain volume in older individuals. There was a similar pattern by age for the association of FT4 with mean diffusivity on diffusion tensor imaging. Our results need to be replicated, but can improve understanding of the role of thyroid function in neurodegenerative disorders.

METHODOLOGICAL CONSIDERATIONS

To answer the research questions in this thesis, we used several study designs and types of analyses. The strengths and limitations thereof are described in the respective chapters within this thesis. A few general considerations regarding systematic reviews and meta-analyses, including IPD meta-analyses, will be discussed here. In general, systematic reviews and meta-analyses are considered the highest form of evidence, whether the research question is etiological, diagnostic, prognostic, or therapeutic in nature.[22] IPD meta-analyses in turn are described as the 'gold standard' of systematic reviews by the Cochrane collaboration.[23] In an IPD meta-analysis, rather than extracting the published summary data from identified literature, researchers aim to collect all individual data available from the researchers of the individual studies. This provides the opportunity for a range of analyses that is usually not possible with so-called published data meta-analyses, such as specific sensitivity and stratified analyses. IPD meta-analyses can also help overcome possible publication bias, because unpublished data is obtained and incorporated in the analyses. Other advantages of IPD meta-analysis over published data meta-analyses include the use of

consistent inclusion and exclusion criteria of participants, possibility to account for missing data, use of the longest possible follow-up, adjustment of analyses for the same set of variables and use of identical statistical analytic approach in each study before aggregation of data amongst others. In this thesis, IPD-meta-analyses have proven to be especially useful in investigating the association of thyroid function with the risk of stroke (**Chapters 3.1, 3.2 and 3.3**).

However, even the 'gold standard' of evidence synthesis had disadvantages. Most importantly, as compared to published studies meta-analysis, it takes time and efforts to identify, obtain and analyze the individual participant data from each study included.[24] Also, in IPD meta-analyses more advanced statistical knowledge may be required when analyzing data from individual studies than when aggregating summary data from published results. Furthermore, even though often overlooked, IPD meta-analyses are prone to some of the same biases as published studies meta-analysis, including publication and selection bias.[25] Lastly, even if all relevant data is rigorously sought, it might not always be found or shared, increasing concerns of possible availability bias. In our IPD meta-analyses, all but one small study agreed to participate and all participating studies eventually shared individual participant data, rendering the possibility of availability bias small.

GAPS IN KNOWLEDGE AND FUTURE DIRECTIONS

Thyroid dysfunction is common in the general population and easily diagnosed through laboratory measurement of thyroid-stimulating hormone (TSH). Furthermore, especially in case of hypothyroidism, treatment is accessible, inexpensive and perceived as safe. All necessary conditions for screening therefore seem to be met; yet, screening is not applied in the general population. In 2015, the U.S. Preventive Services Task Force identified "professional disagreement about the appropriate cut points for the lower and upper boundaries of normal TSH levels in the general population and in subgroups" as one of the issues hampering decision making on screening for thyroid dysfunction[26]. Why does this professional disagreement exist and more importantly, how to resolve this?

Many patients with overt thyroid dysfunction present with non-specific complaints such as fatigue and weight changes.[27] Overt thyroid dysfunction is currently defined by TSH and free thyroxine (FT4) values outside of the reference range. In case of subclinical or mild thyroid dysfunction, the TSH, but not the FT4 value is outside the reference range. These reference ranges are statistically defined by the 2.5th and 97.5th percentile and are usually determined in a seemingly healthy population without taking symptoms or long-term risk of disease into account. However, there is increasing evidence, including evidence from our research efforts, that mild thyroid dysfunction and even variations in thyroid function currently defined as 'normal' are associated with several serious adverse outcomes including cardiovascular disease (CVD) and cognitive decline.[3,4,28,29] Since reference ranges not only define thyroid disease but also provide the basis for treatment decisions, a debate has emerged concerning the current definition of thyroid dysfunction, especially for subclinical hypothyroidism.[30,31]

The definition of reference ranges by taking the 2.5th and 97.5th percentiles is not restricted to thyroid function but is commonly used for serum and clinical measurements, such as heart rate. Because these percentiles for laboratory measurements are calculated from normal or log-normal distribution, they are sometimes also referred to as 'normal ranges' - not to be confused with 'optimal ranges'. The exceptions to this rule include cardiovascular risk factors such as cholesterol and body-mass index (BMI). The reference ranges for BMI and cholesterol do not depend on the distribution in the general population. Instead they are targeted towards prevention of CVD.[32] Could a similar approach also be applied for TSH and FT4 values?

Current American and European Thyroid Association guidelines already partially apply a threshold for treatment of mild hypothyroidism based on CVD risk. Based on individual participant data level meta-analyses showing a higher risk of CVD in people with TSH levels >10mIU/l [4,29], both guidelines recommend to consider treatment in patients with subclinical hypothyroidism if TSH levels are ≥10 mIU/L. In similar analyses, a higher risk of CVD was also shown for subclinical hyperthyroidism, especially in participants with TSH <0.1 mIU/L.[3,4] Thyroid hormone action is however not restricted to the cardiovascular system, but has implications on nearly all tissues and organs. Numerous studies have shown

associations of mild thyroid dysfunction with cognitive and bone health amongst others. [28,33,34] Interestingly, studies also identified relevant associations of variation of thyroid function within the reference ranges with sudden cardiac death, diabetes and dementia, suggesting a continuum of risk as opposed to clear thresholds.[28,30,35]

Another challenge includes the non-linear nature of thyroid function associations. In other words, both high and low thyroid function are associated with adverse clinical outcomes, even though this is not always the case for the same outcome. For example, a higher thyroid function within the reference range has been associated with dementia risk and sudden cardiac death, while lower thyroid function seems protective [28,35]. In contrast, a lower instead of higher thyroid function within the reference range has been associated with the risk of incident diabetes [36]. In case of CVD it is even more complex, since both high and low thyroid function increase the risk of coronary heart disease and heart failure [3,4,29]. This means that when tailoring the thyroid function reference ranges to adverse clinical outcomes, the direction of association has to be taken into account. If not, treatment of subclinical hypothyroidism for prevention of a particular outcome (e.g. diabetes) may lead to an increased risk of another outcome (e.g. dementia). This is even more complex for outcomes showing a non-linear association with thyroid function, such as CVD. In that case, treatment of subclinical hypothyroidism without an accurate target range may easily result in overtreatment and as such increase the risk of CVD.

Randomized trials give more definitive answers concerning treatment risks and benefits. However, they are resource exhaustive, take a long time to complete and are therefore not always able to answer the most pressing or even most relevant questions. For example, there are currently at least four interventional clinical trials being performed worldwide comparing treatment with levothyroxine versus placebo or other therapies for patients with subclinical hypothyroidism[37]. The target populations range from patients after head- and neck surgery to elderly with subclinical hypothyroidism and primary outcomes ranging from post-operative complications to quality of life. All of these trials apply the statistical definition of the reference ranges, none of these trials primarily address relevant hard end-points (e.g. CVD or dementia) and none address the issue of differences between

subgroups (e.g. different targets for women vs men). While the current trials might answer the research questions they have specifically been designed for, most overarching population-wide questions will remain unanswered.

A recent example is the Thyroid hormone Replacement for Untreated older adults with Subclinical hypothyroidism (TRUST) trial, to date the largest randomized controlled trial (RCT) on subclinical hypothyroidism in elderly, showing no effect of levothyroxine use in elderly with subclinical hypothyroidism on thyroid function related quality of life.[38] As previously mentioned, current American Thyroid Association and European Thyroid Association guidelines recommend treatment of subclinical hypothyroidism with TSH levels >10mIU/l but also particularly in patients at high cardiovascular risk and in patients with hypothyroidism-related complaints.[39,40] It seems that those patients were not included in the trial by Stott et al. Due to the initial trial design, where patients were included if their healthcare provider did not start levothyroxine, patients with a high cardiovascular risk and more complaints were probably excluded, as evidenced by the low median thyroid-stimulating hormone levels (~5.7 mIU/L) and lack of symptoms at inclusion. In addition, due to the change in primary outcome from cardiovascular events to quality of life because of difficulties in inclusion, although understandable, the trial was underpowered to show effects of treatment on cardiovascular disease incidence. Other outcomes than cardiovascular disease were not considered in the study design. Although this trial does provide evidence to refrain from treatment in elderly with mild subclinical hypothyroidism that are not being treated by their general practitioner, numerous gaps in knowledge, including treatment effects on risk of hard clinical end-points and appropriate treatment targets, remain unanswered.

One of the challenges in defining the ‘optimal health ranges’ for thyroid function will be to reach a consensus on relevant clinical outcomes that define optimal thyroid function and determine which subgroups need specific reference ranges. But how do we take on these challenges as a scientific community without wasting resources? In this respect, epidemiological observational studies can provide a more tailored direction on thyroid function related outcomes and help to define at which threshold these risks increase. In **Chapter 2.5**, we describe the absolute 10-

year risk of CVD ter mortality according to TSH and FT4 levels. We also propose a proof of principle to define 'optimal health ranges' that can be more meaningful than the current statistical definition based on these disease risks. We also suggest that the 'optimal health ranges' could be age- and sex-dependent; e.g. due to the inherent different risks of disease in different subgroups.

Remarkably, FT4 and not TSH is the better predictor of the 10-year risk of CVD mortality (**Chapter 2.5**). This is not the first study from our and other groups to report an association with FT4 and not or to a lesser extent with TSH with certain outcomes (p could be several explanations for this phenomenon. It has been described that older age is associated with an altered set point of the hypothalamic-pituitary-thyroid axis associated with older age.[41] This could mean that different levels of TSH are needed during aging to maintain the same FT4 levels and might explain the lack of an association with TSH in elderly populations like in the Rotterdam Study. Another explanation could be that TSH predominantly reflects the hypothalamic-pituitary-thyroid axis set point itself rather than disease risk,[42] while, independent of TSH, circulating FT4 (and subsequently triiodothyronine acting intracellular) represents the bioavailable thyroid hormone that can be taken up by cells, thereby leading to clinical consequences of thyroid hormones peripherally. Independent of the underlying mechanism, these findings prompt the question to whether FT4 independently of TSH, should be included in the definition of thyroid function and dysfunction. Our results need to be replicated with sufficient samples and representative populations, both in populations similar to our population as well as complementary populations such as younger participants. Furthermore, a similar approach of defining 'optimal health ranges' can be applied to other hard clinical endpoints deemed relevant and specific for thyroid function. It will be a challenge to decide which outcomes to include and will require international collaboration and discussion to come to a consensus within the clinical and scientific thyroid community.

Table 1 Burdensome chronic non-communicable disease categories in elderly[1]

	TSH	FT4	Chapter(s) of this thesis in RS
CVD	=	↑/ U	2.1-2.5
Cancer	=	↑	5.1
Chronic respiratory diseases	-	-	-
Digestive diseases	=/↑	↓	4.2
MND*	↓	=/↑	3.4; 3.5
Sensory impairment	=	↑	5.4
Musculoskeletal**	U	=	5.3
Genitourinary diseases	↓	=	5.2

CVD = cardiovascular and circulatory diseases, MND = Mental and behavioral, and neurological disorders, RS = Rotterdam Study, U = U-shaped or inverted U-shaped association.
*Association is age-dependent for the brain morphology and microstructural analysis and depicted in this table for elderly. **Chapter 5.3 discusses gait disturbances associated with thyroid function. Gait disturbances are perceived as multifactorial, including musculoskeletal.
[1]Prince et al., "The burden of disease in older people and implications for health policy and practice" Lancet 2015; 385: 549–62

Table 2 Burdensome chronic non-communicable diseases in elderly[1]

	TSH	FT4	Chapter(s) of this thesis in RS
Ischemic heart disease	=	=/↑	2.1; 2.4; 2.5
Cerebrovascular disease	=	↑	2.5
Diabetes Mellitus	↑	↓	4.1
COPD	-	-	-
Dementia	↓	=/↑	3.4
Vision impairment	=	↑	5.4
Hearing impairment	-	-	-

COPD = Chronic obstructive pulmonary disease, RS = Rotterdam Study, U = U-shaped or inverted U-shaped association.
[1]Prince et al., "The burden of disease in older people and implications for health policy and practice" Lancet 2015; 385: 549–62

In practice, IPD meta-analyses, including a large number of participants and a variation in populations will be necessary to provide sufficiently robust evidence on 'optimal health ranges'. IPD-meta-analyses also provide a platform for communication concerning these concepts to reach a general agreement that can influence clinical guidelines globally. Information from these observational studies can then be used to design more comprehensive RCT's that have the potential to answer questions regarding treatment risk-benefits beyond these 'optimal health ranges'. Then, and only then, can the appropriateness of screening for thyroid dysfunction established with meaningful reference ranges be fully addressed.

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CHAPTER 7

SUMMARY & SAMENVATTING

ENGLISH SUMMARY

Chapter 1 of this thesis provides a general introduction and background information on thyroid function and dysfunction (***Chapter 1.1***). Hypothyroidism is more elaborately discussed in ***Chapter 1.2***, with a focus on the current definition of thyroid function in general and hypothyroidism in particular.

Chapter 2 is devoted to the association of thyroid function with diseases of the heart. In ***Chapter 2.1*** we describe that higher free thyroxine (FT4) serum concentrations are associated with a higher 10-year risk of sudden cardiac death (SCD), even within the reference range. In ***Chapter 2.2*** we conclude that FT4 and not thyroid-stimulating hormone (TSH) is a risk factor for atrial fibrillation. In ***Chapter 2.3*** we report on a U-shaped association of FT4 with QT variability, a marker for cardiac repolarization, which could provide a link between thyroid function and cardiovascular disease (CVD) in general and SCD more specifically. In ***Chapter 2.4*** we investigate atherosclerotic CVD (ACVD), subclinical and clinical, and report on an increased risk of ACVD with higher FT4 levels, more marked in people with prevalent ACVD. We also report that higher FT4 levels are associated with a high coronary artery calcification score, which is a marker for subclinical atherosclerosis. In ***Chapter 2.5*** we propose to define 'optimal health ranges' of thyroid function as reference ranges, by defining the risk of disease associated with variations of thyroid function. We identify FT4 as the main predictor of the 10-year risk of CVD mortality. Furthermore, we show that for CVD outcomes these ranges are possibly sex and age-dependent.

Chapter 3 we discuss the relation of thyroid function with the brain. In ***Chapters 3.1*** we identify a lack of literature on the association of thyroid function with the risk of stroke, one of the major causes of mortality and morbidity globally. In ***Chapter 3.2,*** we conduct a large individual participant meta-analysis and show that subclinical hypothyroidism is associated with the risk of stroke, but only in younger participants. However, when we restrict our analyses to the reference range in ***Chapter 3.3*** we identify high-normal thyroid function as a risk factor for stroke in all age-groups. One of the most burdensome diseases of older age is undoubtedly dementia. While hypothyroidism can clinically present with impaired memory, studies on the association of thyroid function and risk of dementia were sparse. In

Chapter 3.4, we identify high and high-normal thyroid function (mainly low TSH) as a risk factor of dementia. Additionally, there was a lack of association of thyroid function with any of the markers of subclinical vascular brain disease and we therefore conclude that this association is unlikely to be mediated through vascular risk factors. In ***Chapter 3.5*** we explore brain volumes and microstructural organization measured on MRI as possible factors influenced by thyroid function. We report that the association of thyroid function with brain morphology and microstructural organization may be age-dependent. In elderly, high thyroid function seems deleterious (smaller brain volumes and indication of reduced matter microstructural integrity), while this was not the case in younger participants.

Chapter 4 includes results from our studies on the possible role of thyroid function with diabetes and non-alcoholic fatty liver disease (NAFLD). In ***Chapter 4.1*** we identify low and low-normal thyroid function as a risk factor for diabetes. This is especially the case in people with prediabetes. This finding can serve as a possible first step into identifying subgroups at risk of developing diabetes in the context of thyroid function. Most studies on the association of thyroid function and NAFLD have been cross-sectional. In ***Chapter 4.2*** we show an increased risk of NAFLD and even fibrosis in participants with low and low-normal thyroid function in a longitudinal study design.

Chapter 5 includes our studies on disorders and diseases of older age that are especially burdensome in elderly populations. Cancer contributes heavily to the global burden of disease, especially in the developed world, and the risk of cancer increases with aging. In ***Chapter 5.1*** we show that higher FT4 levels are associated with a higher risk of all solid cancers but particularly lung cancer and breast cancer. It will be important to replicate these findings, but also to discover the underlying pathophysiological mechanisms. In ***Chapter 5.2*** we describe the association both cross-sectionally as well as longitudinally of thyroid function with kidney function and kidney function decline. Our cross-sectional results are in line with previous studies, which have been mostly cross-sectional, in finding low thyroid function to be associated with lower kidney function. However, our longitudinal analyses, which are less sensitive to reverse causation, show that higher thyroid function is related to higher risk of chronic kidney disease.

Apart from cancer and kidney disease we also studied the association of thyroid function with gait. Gait can be defined as the pattern of limb motion of animals, including humans, when moving from one place to another and has proven an important general health marker. In ***Chapter 5.3***, we identify that TSH is non-linearly associated with global gait, a marker that combines several gait domains, where both high and low TSH seem deleterious. In elderly, age-related macular degeneration is the leading cause of vision loss. Previous animal studies have shown that exogenously administered thyroid hormone is deleterious for cone receptors in mice. In ***Chapter 5.4*** we report a higher risk of age-related macular degeneration with higher FT4 values, which is in line with the findings of animal models. In ***Chapter 5.5*** we discuss thyroid function not as a risk factor, but as an outcome. We suggest that genetic determinants, thyroid peroxidase antibodies and age are the main determinants for thyroid function, both FT4 and TSH. Unlike previous studies, we suggest that TSH is stable and FT4 increases with aging. We propose several mechanisms that could have led to discrepancies with previous literature, including the average age of the elderly population studied and historic iodine status.

Finally, in **Chapter 6** we combine results of this thesis in a comprehensive overview and suggest future research efforts that could be a continuation of the current results.

NEDERLANDSE SAMENVATTING

In **Hoofdstuk 1** van dit proefschrift geven wij een algemene introductie en achtergrond informatie over schildklierfunctie en –dysfunctie (***Hoofdstuk 1.1***).

In ***Hoofdstuk 1.2*** bespreken we het fenomeen hypothyreoïdie, waarbij we ingaan op de huidige definitie van schildklier(dys-)functie in het algemeen en hypothyreoïdie in het bijzonder. In **Hoofdstuk 2** tonen we de associatie tussen schildklierfunctie en hart- en vaatziekten. In ***Hoofdstuk 2.1*** laten we zien dat, zelfs binnen de referentiewaarden, hogere vrij T4 serumwaardes geassocieerd zijn met een hoger tienjaarsrisico op plotse hartdood. In ***Hoofdstuk 2.2*** concluderen we dat vrij T4, en niet thyroid-stimulating hormone (TSH), een risicofactor vormt voor atriumfibrilleren. In ***Hoofdstuk 2.3*** rapporteren we een U-vormige associatie tussen vrij T4 en QT variabiliteit, een marker van cardiale repolarisatie. Deze U-vormige associatie verklaart mogelijk de gevonden associatie tussen schildklierfunctie enerzijds en cardiovasculaire ziekten (CVD) en plotse hartdood anderzijds. In ***Hoofdstuk 2.4*** onderzoeken we klinische en subklinische atherosclerotische CVD (ASCVD). We beschrijven een relatie tussen hogere vrij T4 serumwaardes en een hoger risico op ASCVD. Deze relatie geldt name voor mensen met bestaande ASCVD. We tonen ook dat hogere vrij T4 serumwaardes geassocieerd zijn met een hogere coronary artery calcificatie score, een marker voor subklinische atherosclerose. In ***Hoofdstuk 2.5*** stellen wij voor om de referentiewaarden voor schildklierfunctie te definiëren aan de hand van een ‘optimal health range’, door ook het risico op ziekten mee te nemen in de definitie. Wij laten in dit hoofdstuk ook zien dat vrij T4 de belangrijkste voorspeller is van het tienjaarsrisico op CVD mortaliteit. Tevens laten wij zien dat deze ‘optimal health ranges’ hoogstwaarschijnlijk afhankelijk is van geslacht en leeftijd.

In **Hoofdstuk 3** onderzoeken we de relatie tussen schildklierfunctie en de hersenen. In ***Hoofdstuk 3.1*** laten we zien dat er een gebrek aan literatuur is over het verband tussen schildklierdysfunctie en het risico op beroerte, een van de belangrijkste oorzaken van mortaliteit en morbiditeit wereldwijd. ***Hoofdstuk 3.2*** toont de individuele participanten data meta-analyse, uitgevoerd om dit hiaat op te vullen. Deze meta-analyse laat zien dat subklinische hypothyreoïdie met name bij jongere mensen geassocieerd is met een verhoogd risico op beroerte. Als we de

analyse beperken tot referentiewaardes van schildklierfunctie (***Hoofdstuk 3.3)***, vinden we dat hoog-normale schildklierfunctie een risicofactor is voor beroertes in alle leeftijdsgroepen. Dementie is een vaak voorkomende aandoening onder ouderen en is zeer belastend voor de directe omgeving en de maatschappij. Hoewel hypothyreoïdie gepaard kan gaan met geheugenstoornissen, zijn onderzoeken naar de associatie tussen schildklierdysfunctie en het risico op dementie schaars. In ***Hoofdstuk 3.4*** laten we zien dat hoge en hoog-normale schildklierfunctie (met name een laag TSH) risicofactoren zijn voor dementie. Opmerkelijk genoeg vonden wij geen associatie tussen schildklierfunctie en subklinische vasculaire brein markers. Dit maakt het minder waarschijnlijk dat de associatie tussen schildklierfunctie en dementie wordt gemedieerd door vasculaire risicofactoren. In ***Hoofdstuk 3.5*** onderzoeken we de relatie tussen schildklierfunctie, hersenvolume en markers van microstructurele organisatie van het brein (op MRI). Wij vinden een leeftijdsafhankelijke relatie tussen schildklierfunctie enerzijds, en hersenvolume en –organisatie anderzijds. Met name bij ouderen lijkt een hoge schildklierfunctie samen te hangen met een kleiner hersenvolume en markers passend bij een slechtere microstructuur van de hersenen.

In **Hoofdstuk 4** onderzoeken we het mogelijke verband tussen schildklierfunctie en het risico op diabetes en non-alcoholic fatty liver disease (NAFLD). In ***Hoofdstuk 4.1*** laten we zien dat lage en laag-normale schildklierwaardes risicofactoren zijn voor diabetes, met name bij mensen met pre-diabetes. Deze bevinding kan een eerste stap zijn om, op basis van schildklierfunctie, subgroepen met een verhoogd diabetesrisico aan te wijzen zijn binnen de normale populatie. In ***Hoofdstuk 4.2*** laten wij in een longitudinale studie zien dat lage en laag-normale schildklierwaardes geassocieerd zijn met een hoger risico op NAFLD en zelfs een hogere kans op fibrose bij mensen met NAFLD.

Hoofdstuk 5 beschrijft studies naar aandoeningen en ziekten die zich manifesteren op oudere leeftijd en een grote impact hebben in een ouder wordende populatie. Kanker heeft veel impact op de ‘global burden of disease’, met name in ontwikkelde landen, en het risico op maligniteiten neemt toe met de leeftijd. In ***Hoofdstuk 5.1*** laten wij zien dat hogere vrij T4 waardes geassocieerd zijn met een hoger risico op solide tumoren, met name borst- en longkanker. Het is

belangrijk om deze bevindingen te repliceren en, minstens zo belangrijk, om de onderliggende mechanismen te ontrafelen. In ***Hoofdstuk 5.2*** beschrijven wij de cross-sectionele en longitudinale onderzoeken naar de associatie tussen schildklier- en nierfunctie. Onze cross-sectionele bevindingen zijn in overeenstemming met resultaten van voorgaande (met name cross-sectionele) studies, die een relatie laten zien tussen lage schildklierfunctie en slechtere nierfunctie. In onze longitudinale analyses, die minder sensitief zijn voor 'reverse causation' of omgekeerde causaliteit, laten wij echter zien dat juist een hoge schildklierfunctie geassocieerd is met een hogere kans op chronische nierziekten. Naast voorgaande studies onderzochten wij het verband tussen schildklierfunctie en looppatroon. Een afwijkend looppatroon wordt gezien als een belangrijke algemene gezondheidsindicator. In ***Hoofdstuk 5.3*** beschrijven wij een niet-lineare associatie tussen TSH en de 'global gait score', een score die verschillende domeinen van het looppatroon incorporeert. Dit betekent dat zowel hoge als lage TSH waardes zouden kunnen samenhangen met een afwijkend looppatroon. Eerdere studies hebben aangetoond dat het toedienen van schildklierhormoon bij muizen schadelijke gevolgen kan hebben voor de kegeltjes in het netvlies. In ***Hoofstuk 5.4*** zien wij dat bij mensen een hogere schildklierfunctie geassocieerd is met macula degeneratie, wat de eerder genoemde dierstudies bevestigt. In ***Hoofdstuk 5.5*** onderzoeken wij welke factoren bepalend zijn voor de schildklierfunctie. Genetische factoren, schildklier peroxidase antistoffen en leeftijd lijken de belangrijkste determinanten voor TSH en vrij T4 te zijn. In tegenstelling tot eerdere onderzoeken, vinden wij dat vrij T4, in tegenstelling tot TSH, stijgt met de leeftijd. Wij zetten een aantal mechanismen uiteen die deze discrepantie met de eerdere literatuur zouden kunnen verklaren, zoals de gemiddelde leeftijd van de bestudeerde populatie en de historische jodium status van de bevolking.

Ten slotte combineren wij in **Hoofdstuk 6** de resultaten van dit proefschrift in een uitgebreid overzicht en doen wij op basis van onze resultaten suggesties voor toekomstig onderzoek.

CHAPTER 8

APPENDICES

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E. Chester Ridgway Trainee Grant Program, Clinical Track, Orlando	2015	2
Advanced Medical Writing and Editing Course, Philip Greenland, Rotterdam	2014	2
The Course on R (4 days), Postgraduate School MolMed, Rotterdam	2014	2
The SNP Course Xth edition Postgraduate School MolMed, Rotterdam	2013	2
SPSS Course (2 days), Postgraduate School MolMed, Rotterdam	2013	2

CONFERENCES AND INVITED LECTURES

European Thyroid Association Meeting, Copenhagen, *oral presentation*	2016	0.7
ENDO 2016, Boston, *press-released oral presentation*	2016	0.7
Dutch Internist Days, Maastricht, the Netherlands, m*eet-the-expert session*	2016	0.7
Thyroid Research Meeting, Groningen, Netherlands, *oral presentation*	2016	0.7
Science Days, Antwerp, Belgium, *oral presentation*	2016	0.7
International Thyroid Conference, Orlando, *highlighted oral presentations*	2015	0.7
MolMed Meeting, Rotterdam, The Netherlands, *poster presentation*	2015	0.7
KNAW, Thyroid Day, Amsterdam, The Netherlands, *invited speaker*	2015	0.7
Science Days, Antwerp, Belgium, *award-nominated poster presentation*	2015	0.7
CHARGE Consortium meeting, Washington, *poster presentation*	2014	0.7
European Thyroid Association Meeting, Spain, *oral presentation*	2014	0.7
Dutch Endocrine Meeting, The Netherlands, *oral presentation*	2014	0.7
Dutch Endocrine Meeting, The Netherlands, o*ral presentation:*	2014	0.7
Science Days, Antwerp, Belgium*, oral presentation*	2014	0.7

	Period	ECTS
TEACHING ACTIVITIES		
Curriculum Development Fellow for blended teaching program, Harvard T.H. Chan School of Public Health, Boston, Massachusetts, USA	2016-'17	20
Study Design in Clinical Epidemiology (EPI210), Teaching Assistant, Harvard T.H. Chan School of Public Health, Boston, Massachusetts, USA	2016-'17	7
Lectures on clinical reasoning to 4th year medical students, Erasmus MC	2015	1
Lectures on thyroid function to 1st year medical students, Erasmus MC	2014-'16	1
Lectures on thyroid function to TU Delft clinical technology students	2016	1
(CO-)SUPERVISION		
Arjola Bano, PhD student	2014-'17	3
Samer Khan, MSc student	2015-'17	2
Judith Heutz, MSc student	2016-'17	1
Elena Pavleska, MScs student	2015	1
AWARDS AND GRANTS		
Fulbright Center Research Grant	2016	
Dr. Catharina van Tussenbroek Stichting Research Grant	2016	
Endocrine Society Outstanding Abstract Award	2016	
Endocrine Society Travel Award	2016	
TRUSTFONDS Erasmus Travel Grant	2016	
E. Chester Ridgway Trainee Grant Program	2015	
TRUSTFONDS Erasmus Travel Grant	2015	
NIHES Research Award prize winner	2015	
OTHER		
Reviewing activities for several peer-reviewed journals including *International Journal of Epidemiology, Clinical Endocrinology*, *Endocrine*, *European Journal of Epidemiology*, *Nature Scientific Reports, Metabolomics, Annals of Oncology*.	2013-'17	4
Rotterdam Study general tasks and coding	2013-'16	1

LIST OF PUBLICATIONS

1. Khan SR, Bano A, Wakkee M, Korevaar T, Franco O, Nijsten T, Peeters RP, **Chaker L**. The Association of Autoimmune Thyroid Disease (AITD) with Psoriatic Disease: a prospective cohort study, systematic review and meta-analysis. Eur J Endocrinol. 2017 Jul 26. pii: EJE-17-0397. doi: 10.1530/EJE-17-0397.

2. Beukhof CM, van Doorn L, Visser TJ, Bins S, Visser WE, van Heerebeek R, van Kemenade FJ, de Rijke YB, de Herder WW, **Chaker L**, Mathijssen RH, Peeters RP. Sorafenib-induced changes in thyroid hormone levels in patients treated for hepatocellular carcinoma. *J Clin Endocrinol Metab*. 2017 May 26. doi: 10.1210/jc.2016-4025.

3. **Chaker L**, Korevaar TIM, Rizopoulos D, Collet TH, Völzke H, Hofman A, Rodondi N, Cappola AR, Peeters RP, Franco OH. Defining optimal health range for thyroid function based on the risk of cardiovascular disease. *J Clin Endocrinol Metab.* 2017 May 17. doi: 10.1210/jc.2017-00410.

4. Medici M, **Chaker L**, Peeters RP. A Step Forward in Understanding the Relevance of Genetic Variation in Type 2 Deiodinase. *J Clin Endocrinol Metab.* 2017 May 1;102(5):1775-1778. doi: 10.1210/jc.2017-00585.

5. **Chaker L**, Cappola AR, Rodondi N, Peeters RP; Thyroid Studies Collaboration. Response to the Letter by Choy. *J Clin Endocrinol Metab.* 2017 Apr 1;102(4):1409-1410. doi: 10.1210/jc.2016-3502.

6. Amin N, Jovanova O, Adams HH, Dehghan A, Kavousi M, Vernooij MW, Peeters RP, de Vrij FM, van der Lee SJ, van Rooij JG, van Leeuwen EM, **Chaker L**, Demirkan A, Hofman A, Brouwer RW, Kraaij R, Willems van Dijk K, Hankemeier T, van Ijcken WF, Uitterlinden AG, Niessen WJ, Franco OH, Kushner SA, Ikram MA, Tiemeier H, van Duijn CM. Exome-sequencing in a large population-based study reveals a rare Asn396Ser variant in the LIPG gene associated with depressive symptoms. *Mol Psychiatry.* 2016 Aug 9. doi: 10.1038/mp.2016.141.

7. **Chaker L**, Bianco AC, Jonklaas J, Peeters RP. Hypothyroidism. *Lancet.* 2017 Mar 20. pii: S0140-6736(17)30703-1. doi: 10.1016/S0140-6736(17)30703-1.

8. Barjaktarovic M, Korevaar TI, **Chaker L**, Jaddoe VW, de Rijke YB, Visser TJ, Steegers EA, Peeters RP. The association of maternal thyroid function with placental hemodynamics. *Hum Reprod.* 2017 Mar 1;32(3):653-661. doi: 10.1093/humrep/dew357.

9. Korevaar T, de Rijke YB, **Chaker L**, Medici M, Jaddoe VW, Steegers EA, Visser TJ, Peeters RP.Stimulation of Thyroid Function by Human Chorionic Gonadotropin During Pregnancy: A Risk Factor for Thyroid Disease and a Mechanism for Known Risk Factors. *Thyroid.* 2017 Mar;27(3):440-450. doi: 10.1089/thy.2016.0527.

10. **Chaker L**, van den Berg M, Stricker BH, Peeters RP. Response by Chaker et al to Letter Regarding Article, “Thyroid Function and Sudden Cardiac Death: A Prospective Population-Based Cohort Study". Circulation. 2017 Feb 21;135(8):e90-e91.

11. Korevaar TIM, Steegers EA, Pop VJ, Broeren MA, **Chaker L**, de Rijke YB, Jaddoe VW, Medici M, Visser TJ, Tiemeier H, Peeters RP. Thyroid autoimmunity impairs the thyroidal response to hCG: two population-based prospective cohort studies. J Clin Endocrinol Metab. 2017 Jan 1;102(1):69-77. doi: 10.1210/jc.2016-2942.

12. **Chaker L**, Peeters RP. What is a normal thyroid function? *Ned Tijdschr Geneeskd*. 2017;161(0):D926.

13. Bano A, **Chaker L,** Darweesh SK, Korevaar TI, Mattace-Raso FU, Dehghan A, Franco OH, van der Geest JN, Ikram MA, Peeters RP Gait patterns associated with thyroid function: The Rotterdam Study. *Sci Rep.* 2016 Dec 14;6:38912.

14. Korevaar TI, Nieboer D, Bisschop PH, Goddijn M, Medici M, **Chaker L**, de Rijke YB, Jaddoe VW, Visser TJ, Steyerberg EW, Tiemeier H, Vrijkotte TG, Peeters RP. Risk factors and a clinical prediction model for low maternal thyroid function during early pregnancy: two population-based prospective cohort studies. *Clin Endocrinol (Oxf).* 2016 Dec;85(6):902-909.

15. **Chaker L**, Sedaghat S, Hoorn EJ, Elzen WP, Gussekloo J, Hofman A, Ikram MA, Franco OH, Dehghan A, Peeters RP. The association of thyroid function and the risk of kidney function decline: a population-based cohort study. *Eur J Endocrinol.* 2016 Dec;175(6):653-660.

16. Rieben C, Segna D, da Costa BR, Collet TH, **Chaker L**, Aubert CE, Baumgartner C, Almeida OP, Hogervorst E, Trompet S, Masaki K, Mooijaart SP, Gussekloo J, Peeters RP, Bauer DC, Aujesky D, Rodondi N. Subclinical Thyroid Dysfunction and the Risk of Cognitive Decline: a Meta-Analysis of Prospective Cohort Studies. *J Clin Endocrinol Metab.* 2016 Dec;101(12):4945-4954.

17. Korevaar TI, Steegers EA, **Chaker L,** Medici M, Jaddoe VW, Visser TJ, de Rijke YB, Peeters RP The Risk of Preeclampsia According to High Thyroid Function in Pregnancy Differs by hCG Concentration. *J Clin Endocrinol Metab.* 2016 Dec;101(12):5037-5043.

18. Khan SR, **Chaker L,** Ruiter R, Aerts JG, Hofman A, Dehghan A, Franco OH, Stricker BH, Peeters RP. Thyroid Function and Cancer Risk: The Rotterdam Study. *J Clin Endocrinol Metab.* 2016 Dec;101(12):5030-5036.

19. Korevaar TI, **Chaker L**, Medici M, de Rijke YB, Jaddoe VW, Steegers EA, Tiemeier H, Visser TJ, Peeters RP Maternal total T4 during the first half of pregnancy: physiologic aspects and the risk of adverse outcomes in comparison with free T4. Clin Endocrinol (Oxf). 2016 Nov;85(5):757-763.

20. **Chaker L**, Baumgartner C, den Elzen WP,… Peeters RP; Thyroid Studies Collaboration. Thyroid Function Within the Reference Range and the Risk of Stroke: An Individual Participant Data Analysis. J Clin Endocrinol Metab. 2016 Nov;101(11):4270-4282.

21. **Chaker L**, Wolters FJ, Bos D, Korevaar TI, Hofman A, van der Lugt A, Koudstaal PJ, Franco OH, Dehghan A, Vernooij MW, Peeters RP, Ikram MA. Thyroid function and the risk of dementia: The Rotterdam Study. Neurology. 2016 Oct 18;87(16):1688-1695.

22. **Chaker L**, Ligthart S, Korevaar TI, Hofman A, Franco OH, Peeters RP, Dehghan A. Thyroid function and risk of type 2 diabetes: a population-based prospective cohort study. BMC Med. 2016 Sep 30;14(1):150.

23. **Chaker L**, van den Berg ME, Niemeijer MN, Franco OH, Dehghan A, Hofman A, Rijnbeek PR, Deckers JW, Eijgelsheim M, Stricker BH, Peeters RP Thyroid Function and Sudden Cardiac Death: A Prospective Population-Based Cohort Study. Circulation. 2016 Sep 6;134(10):713-22.

24. **Chaker L**, Korevaar TI, Medici M, Uitterlinden AG, Hofman A, Dehghan A, Franco OH, Peeters RP. Thyroid Function Characteristics and Determinants: The Rotterdam Study. Thyroid. 2016 Sep;26(9):1195-204.

25. 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 Aug;101(8):3204-1

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27. Korevaar TI, **Chaker L**, Jaddoe VW, Visser TJ, Medici M, Peeters RP. Maternal and Birth Characteristics are Determinants of Offspring Thyroid Function. J Clin Endocrinol Metab. 2016 Jan;101(1):206-13.

28. Korevaar TI, Muetzel R, Medici M, **Chaker L,** Jaddoe VW, de Rijke YB, Steegers EA, Visser TJ, White T, Tiemeier H, Peeters RP. Association of maternal thyroid function during early pregnancy with offspring IQ and brain morphology in childhood: a population-based prospective cohort study. Lancet Diabetes Endocrinol. 2016 Jan;4(1):35-43.

29. **Chaker L,** Heeringa J, Dehghan A, Medici M, Visser WE, Baumgartner C, Hofman A, Rodondi N, Peeters RP, Franco OH. Normal Thyroid Function and the Risk of Atrial Fibrillation: the Rotterdam Study. J Clin Endocrinol Metab. 2015 Oct;100(10):3718-24.

30. **Chaker L**, Baumgartner C, den Elzen WP,…, Peeters RP. Subclinical Hypothyroidism and the Risk of Stroke Events and Fatal Stroke: An Individual Participant Data Analysis. J Clin Endocrinol Metab. 2015 Jun;100(6):2181-91.

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diseases on macro-economic productivity: a systematic review. Eur J Epidemiol. 2015 May;30(5):357-95.

32. Schultheiss UT, Teumer A, Medici M, Li Y, Daya N, **Chaker L**, Homuth G, Uitterlinden AG, Nauck M, Hofman A, Selvin E, Völzke H, Peeters RP, Köttgen A. A genetic risk score for thyroid peroxidase antibodies associates with clinical thyroid disease in community-based populations. J Clin Endocrinol Metab. 2015 May;100(5):E799-807.

33. **Chaker L**, Buitendijk GH, Dehghan A, Medici M, Hofman A, Vingerling JR, Franco OH, Klaver CC, Peeters RP. Thyroid function and age-related macular degeneration: a prospective population-based cohort study - the Rotterdam Study. BMC Med. 2015 Apr 23;13:94.

34. Muka T, Imo D, Jaspers L, Colpani V, **Chaker L**, van der Lee SJ, Mendis S, Chowdhury R, Bramer WM, Falla A, Pazoki R, Franco OH. The global impact of non-communicable diseases on healthcare spending and national income: a systematic review. Eur J Epidemiol. 2015 Apr;30(4):251-77.

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36. **Chaker L**, Baumgartner C, Ikram MA, Dehghan A, Medici M, Visser WE, Hofman A, Rodondi N, Peeters RP, Franco OH. Subclinical thyroid dysfunction and the risk of stroke: a systematic review and meta-analysis. Eur J Epidemiol. 2014 Nov;29(11):791-800.

37. Medici M, Porcu E, Pistis G, Teumer A, Brown SJ, Jensen RA, Rawal R, Roef GL, Plantinga TS, Vermeulen SH, Lahti J, Simmonds MJ, Husemoen LL, Freathy RM, Shields BM, Pietzner D, Nagy R, Broer L, **Chaker L**,…, Peeters RP. Identification of novel genetic Loci associated with thyroid peroxidase antibodies and clinical thyroid disease. PLoS Genet. 2014 Feb 27;10(2):e1004123.

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ABOUT THE AUTHOR

Layal Chaker was born on August 20, 1983 in al-Lādhiqīyah (Latakia), Syria. She moved to the Netherlands in 1990, completed high school at the Christelijk Gymnasium Sorghvliet in The Hague in 2001 and started medical school at the Erasmus University Medical Center (EMC) in Rotterdam in that same year. During her years in medical school, she worked for the International Federation of Medical Students Associations and held various positions of which two years in the international board as Director on Human Rights and Peace issues. She completed medical school *cum laude* in 2008 and started working at the internal medicine department of the Reinier de Graaf Gasthuis in Delft.

In 2013, after 3 years of residency in internal medicine, she started her PhD at the Academic Center for Thyroid Diseases (Schildkliercentrum) under the supervision of Prof. Robin P. Peeters and the Department of Epidemiology under the supervision of Prof. Oscar H. Franco and Dr. Abbas Dehghan at the EMC. At the same time, she start her master in clinical epidemiology at the Netherlands Institute of Health Sciences (NIHES). She graduated in 2015 and her research project was awarded as the NIHES best master research thesis. Furthermore, she was privileged to give the Student Address during that years' graduation ceremony.

In 2016, she was awarded a Fulbright Research Scholarship to study mediation analysis at the department of Epidemiology Harvard. T.H. Chan School of Public Health in Boston. During this year, she also worked as a Curriculum Fellow for a blended Master of Public Health in Epidemiology (MPH-EPI) at the same department. As a Curriculum Fellow, she helped with the running of existing courses, creating material for new courses and lecturing on different epidemiological topics for the MPH-EPI program.