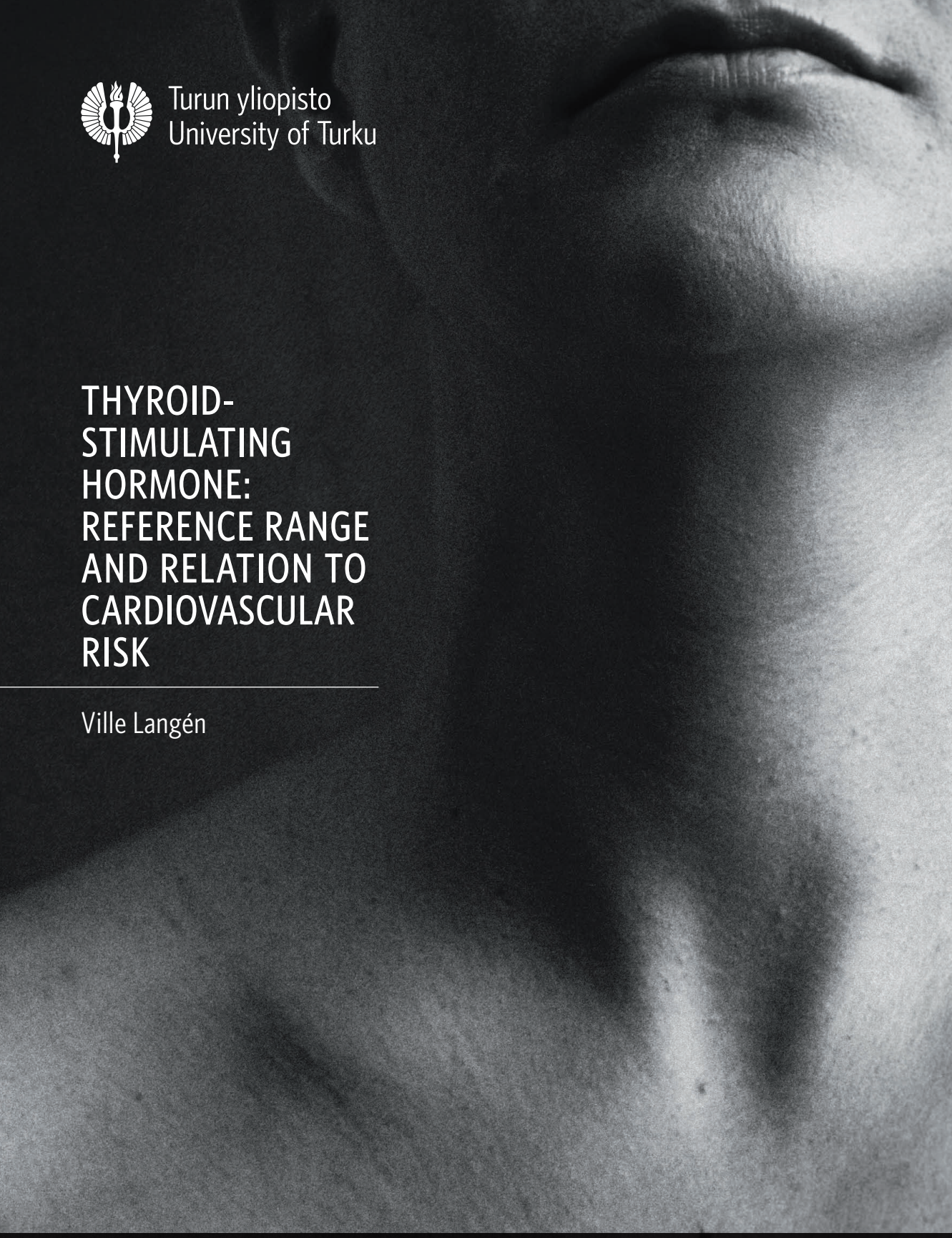

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Ville Langén

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To my family

ABSTRACT

Ville Langén
THYROID-STIMULATING HORMONE: REFERENCE RANGE AND RELATION TO CARDIOVASCULAR RISK

University of Turku, Faculty of Medicine, Department of Internal Medicine,
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Thyroid dysfunctions, especially subclinical forms, are common and could be related to an elevated cardiovascular risk. Previous data on the associations of thyroid function with cardiovascular disease entities and their risk factors are conflicting and inconclusive, particularly in the area of subclinical hypo- and hyperthyroidism and their clinical significance in different age groups. In addition, there has been a debate on where to set the upper limit of the thyroid-stimulating hormone (TSH) reference range, which is the cornerstone for defining the different thyroid dysfunctions.

The aim of this study was to establish a new reference range for TSH and to assess the associations of TSH levels with blood pressure, lipid concentrations and cardiovascular diseases and mortality in a large, randomly selected, population-based sample of Finnish adults (N=8028).

Based on our findings, we propose a new TSH reference range of 0.4–3.4 mU/L to be used for the Finnish population in laboratory units that utilise the Abbott Architect ci8200 platform.

Every 1 mU/L higher concentration of TSH associated cross-sectionally with a 0.5 mmHg higher diastolic blood pressure in men but not in women. In addition, TSH values in the highest versus the lowest tertile of the reference range associated with prevalent hypertension. However, TSH did not associate longitudinally with higher blood pressure or incident hypertension over a follow-up period of 11 years.

Every 1 mU/L higher level of TSH associated cross-sectionally with a 0.02 mmol/L higher concentration of low-density lipoprotein cholesterol (P=0.002). The results were essentially similar when total cholesterol, apolipoprotein B and triglyceride levels were the outcome variables. However, higher levels of TSH at baseline displayed no adverse associations with the lipid concentrations measured at the 11-year follow-up when individuals with a high-risk lipid profile at baseline were excluded from the analyses.

TSH had a U-shaped association with total mortality. In addition, even subclinical form of hypothyroidism associated with the risk of sudden cardiac death. However, we could not link abnormal levels of TSH to cardiovascular disease, coronary heart disease, stroke, major adverse cardiac events or atrial fibrillation and can only speculate on the underlying mechanism of the mortality outcomes.

To conclude, even subclinical hypothyroidism might be linked to an increased cardiovascular risk, but sufficiently powered randomised controlled trials are still needed to determine the cut-off levels of TSH that would indicate when different thyroid therapies should be initiated.

Keywords: thyroid-stimulating hormone, thyroid dysfunction, blood pressure, lipid concentration, cardiovascular disease, epidemiology

TIIVISTELMÄ

Ville Langén
TYREOIDEAA STIMULOIVA HORMONI: VIITEALUE JA YHTEYS SYDÄN- JA VERISUONISAIRAUKSIEN RISKIIN

Turun yliopisto, Lääketieteellinen tiedekunta, Sisätautioppi, Turun kliininen tohtoriohjelma
Turun yliopistollinen keskussairaala, Sydänkeskus
Terveyden ja hyvinvoinnin laitos, Kansanterveysratkaisut

Turun yliopiston julkaisuja
Painosalama Oy – Turku 2017

Kilpirauhasen toimintahäiriöt, etenkin niiden subkliiniset muodot, ovat yleisiä ja saattavat suurentaa sydän- ja verisuonitautiriskiä. Yhteys on kuitenkin ollut vielä epäselvä eritoten subkliinisissä toimintahäiriöissä ja ikäalaryhmissä aiemmin julkaistun kirjallisuuden perusteella. Tyreoideaa stimuloivan hormonin (TSH) viiteylärajan täsmällisestä arvosta on lisäksi käyty väittelyä tiedeyhteisön piirissä. TSH:n viitealue taas on ratkaisevassa asemassa kaikkien kilpirauhasen toimintahäiriöiden diagnostiikassa.

Väitöskirjan tarkoituksena oli määrittää uusi viitealue TSH:lle sekä arvioida TSH:n ja verenpaineen, lipidipitoisuuksien sekä sydän- ja verisuonisairauksien ja kuolleisuuden välisiä yhteyksiä suuressa väestöpohjaisessa, suomalaisia aikuisia kuvaavassa satunnaisotoksessa (N=8028).

Tulostemme perusteella ehdotamme, että määrittämämme TSH:n uusi viitealue 0.4–3.4 mU/L otetaan käyttöön laboratorioissa, joissa TSH määritetään Abbott Architect ci8200 –menetelmällä suomalaisista tutkittavista.

TSH-pitoisuuden 1 mU/L:n nousu oli poikittaisesti yhteydessä 0.5 mmHg:n verran korkeampaan diastoliseen verenpaineeseen miehillä, mutta ei naisilla. Lisäksi TSH:n viitealueen yläkolmannes oli alakolmannekseen verrattuna yhteydessä verenpainetautiin. Suurempi TSH-pitoisuus ei kuitenkaan ollut yhteydessä pitkittäisasetelmassa korkeampaan verenpaineeseen tai verenpainetaudin kehittymiseen 11 vuoden seurannassa.

TSH-pitoisuuden 1 mU/L:n nousu oli poikittaisesti yhteydessä 0.02 mmol/L:n verran korkeampaan LDL-kolesterolin pitoisuuteen (P=0.002). Vastaavan kaltaiset yhteydet havaittiin myös tilastomalleissa, joissa oli vastemuuttujana joko kokonaiskolesterolin, apolipoproteiini B:n tai triglyseridien pitoisuus. Korkeampi TSH-pitoisuus ei kuitenkaan ollut yhteydessä pitkittäisanalyyseissä 11 vuoden seurannan jälkeen mitattuihin haitallisempiin lipidipitoisuuksiin yksilöillä, joilla oli tutkimuksen alkuvaiheessa normaali lipidiprofiili.

TSH-pitoisuudella oli U-kirjaimen muotoinen yhteys kokonaiskuolleisuuteen. Lisäksi jo subkliinisen vaiheen hypotyreoosi oli yhteydessä sydänperäisiin äkkikuolemiin. TSH:lla ei ollut kuitenkaan yhteyttä sydän- ja verisuonisairauksien, sepelvaltimotaudin, aivohalvauksen, sydäntapahtumien (major adverse cardiac events, MACE) tai eteisvärinän kehittymiseen. Täten tutkimuksessa ei saatu määritettyä mekanismia kokonaiskuolleisuuden ja sydänperäisten äkkikuolemien suurentuneelle riskille kilpirauhasen vajaatoimintatiloissa.

Yhteenvetona todetaan, että jopa subkliininen hypotyreoosi saattaa olla yhteydessä suurentuneeseen sydän- ja verisuonisairauksien riskiin. Tarvitaan kuitenkin satunnaistettuja kontrolloituja tutkimuksia, jotta saadaan määritettyä kilpirauhashoitoa edellyttävät TSH:n pitoisuuksien päätäntärajat.

Avainsanat: tyreoideaa stimuloiva hormoni, kilpirauhasen toimintahäiriö, verenpaine, kolesteroli, sydän- ja verisuonisairaudet, epidemiologia

TABLE OF CONTENTS

ABBREVIATIONS

AACE	American Association of Clinical Endocrinologists
AF	atrial fibrillation
ATA	American Thyroid Association
β	beta coefficient
BMI	body mass index
BP	blood pressure
CHD	coronary heart disease
CHS	Cardiovascular Health Study
CI	confidence interval
CVD	cardiovascular disease
CYP7A1	cholesterol 7α-hydroxylase
ECG	electrocardiogram
EPIC	European Prospective Investigation of Cancer
FT4	free thyroxine
HDL	high-density lipoprotein
HR	hazard ratio
HUNT	Nord-Trøndelag Health Study
LCL	lower 95% confidence limit
LDL	low-density lipoprotein
LDLR	low-density lipoprotein receptor
LV	left ventricle
LVEF	left ventricle ejection fraction
MACE	major adverse cardiac event
N	number
NACB	National Academy of Clinical Biochemistry
NHANES	National Health and Nutrition Examination Survey
OR	odds ratio
PCSK9	proprotein convertase subtilisin/kexin type 9
SCD	sudden cardiac death

SD	standard deviation
SE	standard error
SERCA2	sarco/endoplasmic reticulum Ca2+-ATPase type 2 isoform
SHIP	Study of Health in Pomerania
SREBP-2	sterol regulatory element-binding protein-2
T3	triiodothyronine
T4	thyroxine
TgAb	thyroglobulin antibody
TPOAb	thyroid peroxidase antibody
TR	thyroid hormone receptor
TSH	thyroid-stimulating hormone
UCL	upper 95% confidence limit

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications referred to in the text by Roman numerals I-IV:

I. Langén VL, Niiranen TJ, Mäki J, Sundvall J, Jula AM. Thyroid-stimulating hormone reference range and factors affecting it in a nationwide random sample. Clin Chem Lab Med. 2014;52:1807-13.

II. Langén VL, Niiranen TJ, Puukka P, Sundvall J, Jula AM. Association between thyroid-stimulating hormone and blood pressure in adults: an 11-year longitudinal study. Clin Endocrinol (Oxf). 2016;84:741-7.

III. Langén VL, Niiranen TJ, Puukka P, Sundvall J, Jula AM. Association of thyroid-stimulating hormone with lipid concentrations: an 11-year longitudinal study. Clin Endocrinol (Oxf). 2017;86:120-127.

IV. Langén VL, Niiranen TJ, Puukka P, Lehtonen AO, Hernesniemi JA, Sundvall J, Salomaa V, Jula AM. Thyroid-stimulating hormone and risk of sudden cardiac death, total mortality and cardiovascular morbidity. Clin Endocrinol (Oxf). 2017. [Epub ahead of print]

1 INTRODUCTION

The thyroid is situated at the front of the neck and is the largest endocrine gland in the human body (1,2). The main role of the thyroid is to synthesise thyroid hormones, namely thyroxine (T4) and triiodothyronine (T3) (3). Its activity is regulated by a negative feedback system involving the hypothalamic-pituitary-thyroid axis. Thyroid-stimulating hormone (TSH) induces the synthesis of thyroid hormones, which in turn, downregulate the secretion of TSH (4). Hence, the TSH concentration in blood reflects thyroid function in an inverse manner (5); it is the most important thyroid function test in the diagnostics of all of the usual forms of thyroid dysfunction in the outpatient setting (6).

Thyroid dysfunction can be classified into hypo- and hyperthyroidism and furthermore into subclinical and overt disease. Subclinical dysfunctions are common. In a state-wide study that examined 25,862 individuals in Colorado in the United States, 9.0% had subclinical hypothyroidism and 2.1% subclinical hyperthyroidism. Overt hypo- and hyperthyroidism were rare, comprising only 0.4% and 0.1% of the study population, respectively (7).

By definition, subclinical hyperthyroidism is denoted by TSH levels below the reference range coupled with normal concentrations of thyroid hormones; subclinical hypothyroidism, in turn, is designated by TSH levels above the reference range in conjunction with normal concentrations of thyroid hormones (8). Due to these definitions, the reference range of TSH is biochemically the key determinant of the vast majority of thyroid dysfunctions.

There has been some debate on the upper limit of the TSH reference range (5,9–11), with some authors proposing that a “legitimate” upper limit might be closer to 2.5 mU/L in a complete thyroid-healthy population (5). The rationale behind this suggestion is the possibility that the presently used TSH reference ranges, with upper limits typically cited between 4.0–5.0 mU/L (11), have been established on populations with some individuals already displaying subclinical hypothyroidism (5,11). In an attempt to allay these concerns, the National Academy of Clinical Biochemistry (NACB) has suggested that prior to establishing a TSH reference range, the reference individuals should be screened rigorously for both obvious and occult thyroid disorders (5).

Thyroid hormones regulate a myriad of physiological actions in the body and are necessary for the normal functioning of almost all tissues (4). They also exert a considerable influence on the cardiovascular system (12). Since cardiovascular disease (CVD) is the leading cause of death in global terms (13), it would be

beneficial to determine if thyroid dysfunction is a modifiable risk factor of cardiovascular morbidity and mortality.

Many authors have detected an association between higher TSH levels and higher blood pressure (BP) (14–21), albeit only in a few studies conducted in a longitudinal setting (20,21). High BP, in turn, is the most important cardiovascular risk factor in global terms (22).

Furthermore, high cholesterol levels contribute substantially to the global disease burden (22,23). An association between hypothyroidism and hypercholesterolemia, in turn, was proposed already at the beginning of the 20th century (24), but population-based prospective studies on this association have been non-existent except for one study (22) prior to the work described in this thesis.

One of the key questions in thyroidology is whether even subclinical thyroid dysfunction can trigger the pathogenesis of major CVDs, such as coronary heart disease (CHD), stroke or heart failure. In fact, the epidemiological evidence of these associations has been often contradictory and largely inconclusive (25–34), especially on the question of whether the clinical significance of subclinical thyroid dysfunction differs in different age groups and the levels of TSH at which the hazard appears.

This thesis was designed to elucidate these questions in a large, population-based sample representative of Finnish adults.

2 REVIEW OF LITERATURE

2.1 Thyroid and its functions

The thyroid is a bi-lobed organ situated anterolaterally to the trachea (1). During its embryological development, the organ derives from an endodermal thickening in the primitive pharynx, developing first as a spherical form and only later becoming transformed into its characteristic lobulated shape. The primitive thyroid penetrates through the underlying tissues via a channel called the thyroglossal duct and migrates to its final pre-tracheal location (35). In the mature thyroid, the left and right lobe are normally connected by an isthmus, in such a way that the characteristic appearance of the thyroid is that of a capital letter H. With respect to its size, the thyroid is 3–4 cm long and 6–7 cm wide and in a normal adult, it weighs 15–25 g (36). An enlargement of the thyroid is called goitre (3). Sometimes goitre may be associated with hypo- or hyperthyroidism; in global terms, its major cause is iodine deficiency (37). Conversely, also excessive dietary intake of iodine has been associated with goitre according to some reports (38,39), but there is no consensus on this topic (40). It is noteworthy that ectopic thyroid tissue may occasionally be found along the thyroid's embryological migration pathway (41,42) or more rarely in more distant locations such as the mediastinum (43), the heart (44) or even in subdiaphragmatic organs (45).

Histologically, the thyroid is made up of follicles that are lined with a single layer of thyroid epithelial cells (1), whose raison d'être is the synthesis of the two thyroid hormones, T4 and T3 (3). Thyroid hormones regulate a multitude of physiological actions in the body. They have a critical role in cell and tissue differentiation, normal growth and metabolism; in fact, they are essential for the optimal functioning of almost all tissues (4). These properties are attributable mainly to the actions of T3, which is considered the biologically more active thyroid hormone (46–48). Most of the T3 is produced in the periphery rather than in the thyroid, after its conversion from T4 by deiodinases (49). The majority of the actions of T3 involve regulation of gene expression via its nuclear receptor (1). The two primary isoforms of the thyroid hormone receptor (TR), α and β, are expressed diversely in development and in adult tissues (50). The primary isoforms display further heterogeneity (4): TRα1, TRβ1 and TRβ2 are the major TR splice products in humans with ability to bind T3 (51). So far, the expression of each of the TR isoforms in different tissues has mainly been studied in mice rather than in humans (51). Along with the multiplicity of the TR isoforms, additional factors such as cross-talk with other signalling pathways and interactions with corepressors and coactivators contribute to the profound complexity of the thyroid hormone

signalling pathway (50). In addition, non-genomic actions that do not require the nuclear receptor have also been described (52). The thyroid has also parafollicular cells (C cells) that produce calcitonin (53). As opined by some authors, the precise role of calcitonin in humans has not been fully clarified (54).

The synthesis and secretion of thyroid hormones is regulated by a negative feedback system that involves the hypothalamic-pituitary-thyroid axis. Thyrotropin-releasing hormone is synthesised in the hypothalamus and stimulates the synthesis and subsequent release of TSH from the pituitary. TSH, in turn, regulates the secretion of thyroid hormones from the thyroid. Conversely, thyroid hormones downregulate the secretions of both thyrotropin-releasing hormone and TSH (4).

Thyroid disorders can be divided into hypo- and hyperthyroidism and further to overt and subclinical dysfunctions; the latter are emerging thyroid disorders, i.e. thyroid hormone levels are still normal but TSH is already abnormally high or low, denoting subclinical hypo- or hyperthyroidism, respectively (8). In central hypothyroidism, which is considered a rare disease, an otherwise normal thyroid gland is unable to secrete sufficiently thyroid hormone due to hypothalamic or pituitary dysfunction (55). Conversely, in central hyperthyroidism, which also is a rare disease, inappropriately high levels of TSH cause a hyperstimulation of the thyroid gland, leading to thyrotoxicosis (56).

TSH is considered the first-line biomarker of choice for detecting all of the more common forms of hypo- and hyperthyroidism in most outpatient settings (6). This may appear paradoxical at first glance, as TSH is merely an indirect measure of the function of the thyroid gland. It is also a potentially labile biomarker as it has a very short half-life of approximately one hour. For comparison, the half-lives of the actual thyroid hormones are markedly longer, i.e. 7 days for T4 and approximately 24 hours for T3 (5). However, the rationale of measuring TSH concentration is that it reflects inversely thyroid hormone function as it is being sensed by the pituitary gland (5) and thus its concentrations in blood can detect both overt and subclinical disorders of the thyroid function (6). It is noteworthy that in the rare case of central thyroid dysfunctions, a sole TSH reading without thyroid hormone measurement carries a risk of missed diagnosis (57).

It is recommended that if an abnormally high TSH result is measured in a previously euthyroid patient, the test should be repeated coupled by an assessment of free T4 (FT4) levels (58). In contrast, according to the clinical practice guidelines for hypothyroidism issued by the American Association of Clinical Endocrinologists (AACE) and the American Thyroid Association (ATA), T3 should not be measured to diagnose hypothyroidism (59). However, T3 measurements may be useful and already display abnormalities in the diagnostics

of milder forms of overt hyperthyroidism, where TSH is abnormally low but T4 levels are still normal. In more advanced cases of overt hyperthyroidism, both T4 and T3 concentrations are elevated and TSH is already undetectable (60).

2.2 Thyroid-stimulating hormone reference range

The definition of the TSH reference range is pivotal in the diagnostics of thyroid dysfunction because the biochemical definition of thyroid disorders, especially in subclinical cases, relies entirely on it.

The sensitivity of TSH assays has improved substantially over the last decades, but some variability persists even between the present TSH immunometric assay methods (61,62). The first generation TSH methods were based on radioimmunoassay techniques, and their functional sensitivity was approximately 1.0 mU/L. Their sensitivity was insufficient, above all, in the diagnostics of hyperthyroid conditions. The currently used third generation TSH methods are automated immunochemiluminometric assays, and their functional sensitivity is ≤0.01 mU/L (62). They are sensitive enough to detect even subclinical hyperthyroidism (63).

TSH concentrations display a two-fold diurnal variation, with the nadir occurring between 10 and 16 hours (5). According to the laboratory medicine practice guidelines issued by NACB, this variation does not compromise the interpretability of TSH results, as TSH is typically measured between 8 and 18 hours both in the clinical setting and when a TSH reference range is established (5).

In most clinics, the TSH upper reference limit varies between 4.0–5.0 mU/L (11). However, it has been recognised that with the currently used reference range, the TSH distribution is not Gaussian but rather skewed at the upper end, which is also called “tailing” toward higher levels of TSH (an example of the tailing of the TSH levels is shown in **Figure 1**) (64). In its guidelines, NACB has argued that this finding could be due to the fact that the TSH reference limits were established from populations comprising also individuals with undiagnosed subclinical hypothyroidism. To remedy this potential problem, NACB has proposed that before the TSH reference range is statistically calculated, the reference population needs to be carefully screened for emerging thyroid disorders. In the NACB guidelines, it is also postulated that this rigorous approach to screening could result in a TSH upper limit of 2.5 mU/L, which is markedly lower than the values presently used (5,9).

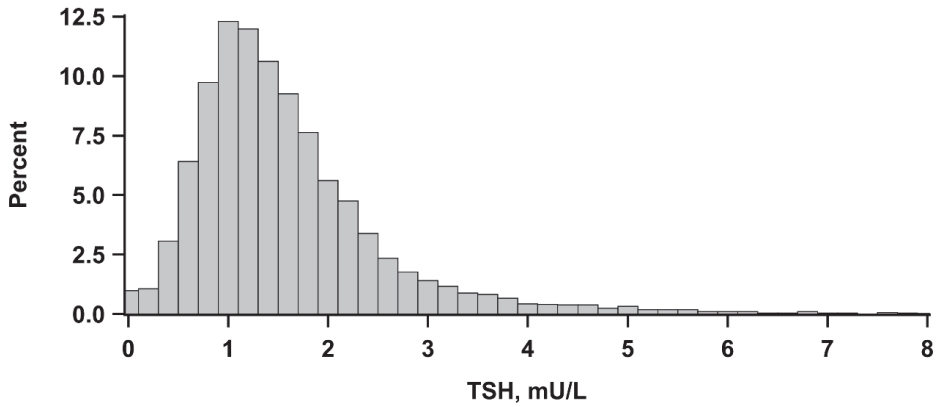


Figure 1 TSH distribution in those participants of the Health 2000 Survey with a successfully determined baseline serum TSH concentration (n=6247). "Tailing" toward higher levels of TSH (5) is clearly visible. Extreme observations are not shown in the histogram.

An inherent problem of a strict screening approach is the exclusion of a substantial number of participants in the population for which the reference range is established. The National Health and Nutrition Examination Survey (NHANES) III has demonstrated that this could be a considerable concern in older adults, as in that survey, only 10% of those aged 70–80 years could be included in a healthy reference group (65).

According to the stringent NACB criteria (**Table 1**), the TSH reference range should be established from individuals with no detectable thyroid peroxidase or thyroglobulin antibodies (TPOAb and TgAb, respectively), goitre, any medication (excluding oestrogen) or personal or family history of thyroid dysfunction. As an additional criterion, the TSH reference range should be calculated from 95% confidence interval (CI) limits of TSH values that have first been power-transformed with a logarithmic function, rather than by a nonparametric method from the 2.5th and 97.5th percentiles (5). The fact that the exclusion of TPOAb positive individuals from reference populations has lowered the TSH upper limit in some previous studies (66,67) supports this paradigm. Stringent screening is also supported by results from the Study of Health in Pomerania (SHIP), which examined an unselected population-based sample representative of German adults. Rigorous screening in the SHIP study yielded a TSH reference range of 0.25–2.12 mU/L, i.e. there was a strikingly reduced TSH upper limit compared with the one presently used in most clinics (11,68).

Table 1 Criteria on how to establish a TSH reference range, as issued by the NACB in its laboratory medicine practice guidelines (5).

- The reference population has to comprise at least 120 volunteers
- The volunteers have to be euthyroid and ambulatory, fulfilling the following criteria:
  - No goitre
  - No thyroid dysfunction in personal or family history
  - TPOAb and TgAb cannot be detectable (as measured by sensitive immunoassay)
  - With the exception of oestrogen, no medications
- TSH reference range should be established from the 95% confidence limits of the TSH values
- Before the calculation, the TSH values have to first be log-transformed

Note: it is customary to establish the TSH reference range from specimens collected between 0800 and 1800 hours.

However, when another German research group adhered scrupulously to the NACB criteria and performed thyroid ultrasound as an additional biomarker to rule out thyroid disorders, they reported the rather usual TSH reference range of 0.40–3.77 mU/L. That study sample was selected, i.e. it consisted of blood donors. Thus, it seemed that the rigorous screening in this population did not lower the TSH upper limit (69). Another German study came to the same conclusion (70). These results question not only the draconian approach to screening of a reference population (71) but also the driving force behind the tailing of TSH.

Other authors have suggested additional theories to explain the tailing phenomenon. Surks and Hollowell have shown that the skewing can be mitigated by using age-specific TSH reference limits (72). They noted that in the NHANES III cohort not only the TSH upper reference limit but also the median increased with age. Surks and Boucai have also described potentially important race-specific differences in the TSH distribution (73) that could contribute to the skewing. If age- and race-specific distributions truly are the main underlying mechanism of the tailing of TSH, lowering the TSH upper limit of the general population to 2.5 mU/L could lead to spurious overdiagnosis of hypothyroidism in older individuals (10). However, as opposed to the theory of Surks et al., the levels of TSH have not increased with age in all published studies (67,74,75). For instance, in a Finnish study conducted by Schalin-Jäntti et al., no significant age-specific differences between TSH means existed, but nonetheless the TSH distribution seemed to be skewed in the overall reference population (67). Despite the previously mentioned moderate evidence of age- (72) and race-specific (73) differences in TSH concentrations, it is not necessary, according to the NACB guidelines, to adjust for these factors when establishing a TSH reference range (5).

In their recent report, Strich et al. theorised that the tailing of TSH was a sum effect of several phenomena, rather than a single factor, such as the TSH circadian

rhythm, exercise habits, obesity, day-night rhythms, seasonal alterations in TSH and polymorphisms in the TSH molecule and other genes in the thyroid cascade. The authors also drew attention to the lack of physiological TSH reference ranges that could serve as a starting point for individually tailored assessment of thyroid function (64). It is noteworthy that in the Rotterdam Study, not only higher BMI but also alcohol use was associated with slightly higher TSH concentrations (76). Smoking, by contrast, seems to have a negative relation with TSH levels (76,77).

In addition, the syndrome of resistance to TSH warrants mention as a potential cause of an elevated TSH level. In this condition, thyroid follicle cells display reduced sensitivity to biologically active TSH (78). Theoretically, also this syndrome might contribute to the tailing of TSH distribution if the reference population comprised affected participants. However, this syndrome is considered to be rare (79).

The TSH lower limit has not attracted similar attention in the literature as the upper limit (80). In contrast to the older TSH assay methods, the present techniques are also sensitive enough to permit the diagnostics of hyperthyroidism (81). In studies with rigorous screening of the reference population, lower limits of approximately 0.4 mU/L have usually been cited (69,74,82,83).

To summarise this section, previous studies have mainly been performed in selected populations; these have yielded conflicting TSH reference limits and there is no consensus about how screening and age affect the TSH reference range.

2.3 Association of thyroid function with blood pressure and lipid profile

2.3.1 Blood pressure

Hypertension is the leading cardiovascular risk factor for global disease burden (22). In their seminal study published in the Lancet journal, Lim et al. estimated that in global terms, a total of 7.0% of healthy life years are lost due to premature death or morbidity attributable to high BP. For comparison, this surpasses even the corresponding figure of 6.3% attributed to tobacco smoking; smoking was assessed as only the second most important cardiovascular risk factor by Lim et al. (22). An individual participant data meta-analysis of one million adults published by Lewington et al. revealed that every 10 mmHg lower systolic BP or correspondingly, every 5 mmHg lower diastolic BP, would be associated with a 40% lower risk of cerebrovascular death and a 30% lower risk of death from CHD

or other vascular disease throughout middle age. This meta-analysis also indicated that the association between higher BP and higher vascular mortality risk was evident down to at least a level of 115 mmHg systolic or 75 mmHg diastolic BP (84).

As high BP is a cardinal but also a modifiable cardiovascular risk factor, it is of interest to study its predictors comprehensively. Thyroid hormones affect the cardiovascular system in many ways that could potentially influence BP (85). T3 can directly cause a relaxation of vascular smooth muscle, leading to vasodilatation, and it also has an ino- and chronotropic effect (85,86). Together, these actions increase cardiac output (85). Thyroid hormone levels are also inversely associated with vascular calcification (87,88) and smooth muscle cell apoptosis (89). Thyroid hormones are also necessary for the optimal autonomic regulation of the cardiovascular system by the parvalbuminergic neurons in the hypothalamus (90). Conversely, in case-control studies, hypothyroid states have been associated with endothelial dysfunction (91,92) and arterial stiffness (93,94). According to the literature, thyroid hormones appear to be involved in a host of properties that maintain normal endothelial function and haemodynamics and which could thus also affect BP. Furthermore, even TSH could have direct effects on the cardiovascular system. TSH receptors appear to exist not only in the thyroid but also in other tissues, e.g. the coronary artery smooth muscle cells (95) and the heart muscle (96).

Early works with highly selected study populations, such as the case-control study conducted by Saito et al. (17) and the case series reported by Streeten et al. (18), have linked overt hypothyroidism to hypertension. In addition, higher TSH levels, even levels within the TSH reference range, have been associated with BP or prevalent hypertension in several population-based cross-sectional studies (14–16).

However, causality between thyroid function and high BP cannot be inferred from these studies i.e. either they were conducted in a cross-sectional setting or they examined a selected study population. In fact, there was very meagre longitudinal population-based data on this topic prior to our investigations; as far as we are aware, there were only two previous studies published in 2013 with conflicting results (19,20).

The first of these longitudinal studies, The Norwegian Nord-Trøndelag Health Study (HUNT), indicated that in euthyroid participants, higher baseline TSH associated longitudinally with higher 11-year follow-up BP. However, the association was very modest and could only be detected in women. The HUNT study revealed also a direct relation between the change in TSH and the change in BP over the 11-year follow-up period (20).

Conversely, in the slightly later published individual participant data meta-analysis from Germany, higher baseline TSH levels were cross-sectionally associated with higher baseline BP and prevalent hypertension but not longitudinally with the five-year change in BP or incident hypertension (19).

Recently, an additional population-based study, which examined 623 euthyroid participants, was conducted in China. In the Chinese study, no association was detected between the baseline TSH levels and the 5-year follow-up BP. However, the Chinese researchers found a correlation between the change in TSH and the change in BP over the follow-up period in women, and in that respect, their findings correlate fairly well to those of the HUNT investigators (21).

2.3.2 Lipid concentrations

High cholesterol is a significant contributor to the global disease burden (22,23). It is one of the so called "conventional risk factors" and within them, it belongs to the subgroup of the major modifiable factors, together with the likes of high BP, tobacco use and obesity (97). Statin trials have demonstrated that the risk of major vascular events, which encompasses coronary death, coronary revascularisation, non-fatal myocardial infarction and stroke, can be reduced by 20% for every 1 mmol/L reduction in the low-density lipoprotein (LDL) cholesterol concentration (98). It is therefore paramount to discover all the potentially modifiable determinants of an adverse lipid profile.

Thyroid hormone can affect lipid homeostasis in a myriad of ways as has been extensively described in the literature in recent decades; especially advances have been made at the molecular biological level. An exhaustive review of these molecular level properties is beyond the scope of this epidemiological study, but some of the most essential mechanisms warrant a mention. Two early studies conducted in rats which had first been hypophysectomised and in the second study also thyroidectomised, revealed that the expression of LDL receptors (LDLR) rapidly increased with the administration of thyroid hormone (99,100). LDLR is crucial in the uptake of several atherogenic lipoproteins into cells and thus in the optimal regulation of cholesterol metabolism (101,102). The same investigators found that in rats, thyroid hormone was able to cause an even more rapid increase in the expression of cholesterol 7α-hydroxylase (CYP7A1) (99,100). Further research has revealed that this upregulation is mediated via the hepatic TRβ (103,104). CYP7A1, in turn, acts as a rate-limiting enzyme in the production of bile acids (105). By upregulating CYP7A1, thyroid hormone is able to induce the synthesis of bile acids from cholesterol (103). In addition, thyroid hormone regulates the sterol regulatory element-binding protein-2 (SREBP-2) gene (106),

and SREBP-2, in turn, upregulates the LDLR gene. The actions of thyroid hormone also correlate inversely with the levels of lipoprotein(a) (103), which is known to have atherogenic potential (107). One intriguing new finding is the link between thyroid hormone and proprotein convertase subtilisin/kexin type 9 (PCSK9) (103) that has attracted much attention lately (108–110). Recent data indicate that thyroid hormone can reduce the levels of PCSK9 in the circulation (103). PCSK9, in turn, prevents the recycling of LDLR and thus increases circulating LDL cholesterol levels (108). However, these interactions are complex; for instance, some studies have revealed that SREBP-2 increases the transcription of both LDLR (99,100) and PCSK9 (111), which are, in fact, opposing actors in the metabolism of LDL (108).

Several studies have also addressed the role of thyroid hormone action for triglyceride metabolism. In an often cited study conducted by Valdemarsson et al., lipoprotein lipase activity was decreased in hypothyroidism compared with euthyroidism (112). Lipoprotein lipase, in turn, is considered to be vital in the catabolism of triglyceride-rich lipoproteins (113). In addition, thyroid hormone action seems to down-regulate angiopoietin-like protein 3 (114), which inhibits lipoprotein lipase activity (115). Perhaps the most essential gene involved in the regulation of triglyceride metabolism is apolipoprotein AV gene whose variants may cause hypertriglyceridemia (116). The expression of this gene is upregulated, in turn, by T3 (117).

Also HDL metabolism and the reverse cholesterol transport are influenced by thyroid hormone action (118). The reverse cholesterol transport delivers cholesterol from the periphery to be metabolised in the liver. In the first step of this pathway, HDL particles receive free cholesterol from cells, such as lipid-laden macrophages in an artery wall, in a process called cholesterol efflux (119). Later along the pathway, HDL particles mature into bigger, spherical forms and can release cholesterol esters to the apolipoprotein B –containing lipoproteins, such as the LDL (120,121). The latter process is mediated by cholesteryl ester transfer protein, whose activity thyroid hormone action seems to correlate with (121,122). In the final step of this route, larger apolipoprotein B –containing lipoproteins may release triglycerides in a process that is facilitated by hepatic lipase (123,124), and the now smaller atherogenic lipoproteins can finally be taken up into hepatocytes via interaction of the LDLR (120). Hepatic lipase also mediates the conversion of larger HDL particles into smaller ones by modulating the phospholipid content of HDL (125). Thyroid hormone, in turn, seems to affect the activity of hepatic lipase (118,126). In summary, thyroid hormone modulates the distribution of HDL by influencing the activity of cholesterol ester transfer protein and hepatic lipase (118).

To summarise the previous paragraphs, molecular evidence that has accumulated during the past few decades reveals that thyroid hormone plays a vital role in lipid metabolism. In fact, the clinical association in humans was observed as early as in the 1920's, even before the present-day confirmatory molecular evidence (106). Early case series clearly demonstrated the capacity of thyroid hormone replacement therapy to reduce high cholesterol levels among hypothyroid patients (127–129). However, case series inherently carry a risk of sample bias. Subsequent cross-sectional studies provided further evidence for the association between thyroid function and lipid homeostasis. In the Colorado Thyroid Disease Prevalence Study, which comprised 25,862 participants, there was a significant trend towards higher triglyceride and total and LDL cholesterol levels across different thyroid disorder states as TSH values increased. The mean LDL cholesterol levels for the groups that according to the biochemical definition expressed either normal thyroid function, subclinical hypothyroidism or overt hypothyroidism, were 3.6 mmol/L, 3.8 mmol/L and 4.4 mmol/L, respectively (7). Since then, major additional cross-sectional studies have been conducted in different countries. These works have utilised either TSH within the reference range (14,130) or over the full range (131,132) as the exposure variable. In essence, these studies have been able to replicate the results of the Colorado Thyroid Disease Prevalence Study (14,130,131), even in adolescents (132).

All in all, the overwhelming evidence from basic science, clinical research and cross-sectional studies has enabled the scientific community to appreciate that there is a causal link between thyroid hormone and lipid homeostasis. In fact, several years ago, high cholesterol was even used as a surrogate biomarker to detect a likely case of hypothyroidism (133). To further quantify how the association between hypothyroid states and an adverse lipid profile would putatively persist over a longer period of time, prospective studies are also warranted. However, longitudinal population-based studies on this topic are extremely limited; as far as we are aware, the only study besides ours is the Norwegian HUNT study, which comprised 14,353 euthyroid adult inhabitants of Nord-Trøndelag county. The Norwegian investigators detected a modest association of the baseline TSH levels with the follow-up non-high-density lipoprotein cholesterol and triglycerides but only in men. As anticipated, the baseline TSH levels associated inversely with the follow-up high-density lipoprotein (HDL) cholesterol levels. The results revealed also a direct relationship between the change in TSH and the change in non-HDL cholesterol and triglyceride levels over an 11-year follow-up period (20).

2.4 Association of thyroid function with heart disease and stroke

2.4.1 Coronary heart disease

Already in the late 19th century, the Swiss physician Emil Theodor Kocher, who later received a Nobel Prize for his work in the field of the physiology, pathology and surgery of the thyroid (134), proposed an association between hypothyroidism and atherosclerosis (135). According to the paradigm that evolved during the following century, hypothyroidism would cause an elevated risk of CHD (135), possibly mediated via the intermediate traditional risk factors of hypercholesterolemia and hypertension (85). However, in this context, it has to be emphasised that the putative association between hypothyroidism and incident hypertension (12,135) has not been confirmed in population-based longitudinal studies (19). The results of those studies examining the associations between different states of hypothyroidism and newer cardiovascular risk factors, such as coagulation abnormalities and elevated levels of C-reactive protein or homocysteine, have been mixed (8,135). The findings of the most important epidemiological studies on the association between thyroid function and CHD will be summarised in this section.

The Whickham Survey, conducted in 1977, was the first population-based cross-sectional study to assess the relationship between thyroid status and the risk of CHD. The researchers examined a total of 2779 participants that were considered to be representative of the British adult population. CHD was assessed based on an interview, a questionnaire and an electrocardiogram (ECG). Those having a TSH concentration of 6 mU/L or higher were considered to have a minor degree of hypothyroidism. The investigators failed to detect any association between a minor degree of hypothyroidism and prevalent CHD in men. In women, a weak association between a minor degree of hypothyroidism and minor ECG changes suggestive of CHD was detected. Thyroid antibodies did not associate with prevalent CHD (136).

Twenty years later, a longitudinal follow-up study of the Whickham Survey was conducted. Those participants who had had hypothyroidism, positive antithyroid antibodies or a raised TSH level at baseline were classified as having autoimmune thyroid disease. The investigators failed to detect an association between autoimmune thyroid disease and incident CHD. In an additional nested case-control analysis, a subset of women with positive antithyroid antibodies or a raised TSH level at baseline had no greater odds of mortality or incident CHD than matched participants (137). However, the authors reanalysed their data four years later in 2010 and found that those with subclinical hypothyroidism at baseline were

at significantly greater risk of incident CHD. The reanalysis differed considerably from the original follow-up study in methodology. The original follow-up analyses had studied the effects of a composite autoimmune thyroid disease status as compared with euthyroidism. The reanalysis focused strictly on the risk of having subclinical hypothyroidism, which was defined based on TSH and T4 values rather than on broader composite criteria (138).

The Busselton Health Study conducted in Australia came to the same conclusion (139) as the reanalysis of the Whickham Survey data (138). The data from 2,108 Australian participants were analysed first in a cross-sectional setting and then longitudinally after a follow-up period of 20 years. Those with subclinical hypothyroidism at baseline had greater odds of prevalent CHD and had a higher risk of developing incident CHD and also cardiovascular death (139).

In the Norwegian HUNT study, the association between thyroid status and the risk of hospitalisation with an acute myocardial infarction was assessed in 26,707 participants over a follow-up period of 12 years. An additional outcome was death from CHD. The thyroid status variable was subdivided into 7 categories: subclinical and overt hypo- and hyperthyroidism and tertiles within the TSH reference range with the lowest tertile being used as a reference category. In women, subclinical hypo- and hyperthyroidism and also the highest tertile within the TSH reference range were associated with an increased risk of death from CHD. However, none of the thyroid status categories were associated with the risk of being hospitalised due to myocardial infarction, and thus the results were inconclusive for the risk of incident CHD (25). The results of the Nagasaki study were comparable. Subclinical hypothyroidism was cross-sectionally associated with prevalent CHD and longitudinally with the risk of death from all causes, but not with incident CHD (31). Finally, the European Prospective Investigation of Cancer (EPIC) -Norfolk study examining 11,554 participants failed to detect an association between thyroid status and incident CHD over a mean follow-up period of 10.6 years (14).

The association between hypothyroidism and CHD has also been examined exclusively in older adults in several population-based studies. The Rotterdam Study investigated the association in 1149 postmenopausal women, whose mean age was 69 years. Those with subclinical hypothyroidism had approximately doubled odds of suffering prevalent aortic atherosclerosis or had a past history of myocardial infarction compared with other participants (140). However, the cross-sectional design of the Rotterdam Study does not allow causal interpretations. In the Cardiovascular Health Study (CHS), a random sample of non-institutionalised individuals aged 65 or over were recruited from the Medicare eligibility rosters of four different counties in the United States. From that cohort, Cappola et al.

selected 3233 participants who were deemed eligible for thyroid-related analyses, with a mean follow-up time of 12.5 years. Subclinical hypothyroidism was not associated with an increased risk of incident CHD, cardiovascular death or death from all causes (26). Approximately seven years later, the authors published an additional subgroup analysis, where 679 participants had been diagnosed with persistent subclinical hypothyroidism based on serial TSH testing performed over the follow-up period. However, the results were similarly negative for the risks associated with persistent subclinical hypothyroidism (30). In the Dutch Leiden 85-Plus Study, increasing levels of TSH were, in fact, inversely associated with the risk of death from all causes in octogenarians (29). A British cohort study came to a materially same conclusion in a similarly aged cohort (33).

Perhaps the most seminal study to date on the association between subclinical hypothyroidism and CHD was published by the Thyroid Studies Collaboration in 2010. An individual participant data meta-analysis comprising 55,287 participants from the United States, Europe, Australia, Brazil and Japan was conducted. The investigators found that subclinical hypothyroidism was associated with an increased risk of CHD events and CHD mortality in participants whose TSH concentration was 10 mU/L or greater (34). These results are also reflected in the latest clinical guidelines of this topic. The clinical practice guidelines for hypothyroidism issued by ATA/AACE recommend that patients whose TSH level is 10 mU/L or more should be considered for thyroid hormone replacement therapy (59). The same recommendation, and even in situations where the patient is symptomless, is echoed in the guideline on the management of subclinical hypothyroidism issued by the European Thyroid Association (141).

Subsequently, another individual participant data meta-analysis conducted by the Thyroid Studies Collaboration revealed that even subclinical hyperthyroidism was associated with an increased risk of CHD mortality (28). This finding does not follow the logic of the classical paradigm that usually attempts to link low levels of thyroid hormone with hypercholesterolemia and an elevated risk of CHD (85). The authors speculated that the results could be due to systemic effects of thyroid hormone, such as its ability to trigger cardiac arrhythmias or cause changes in cardiac function, or alternatively, it might be a type II error, i.e. a false positive result. In fact, subclinical hyperthyroidism did not associate with incident CHD in this meta-analysis (28), in accordance with the results of the EPIC-Norfolk (14), Busselton (139) and Cardiovascular Health Studies (26).

2.4.2 Heart failure

Several review articles have emphasised the importance of adequate levels of thyroid hormone for the optimal functioning of the heart (12,85,142–144). In their comprehensive review articles, Jabbar et al. (12) and Klein et al. (85,142) have clarified the broad range of effects that thyroid hormone exerts on the heart. In brief, thyroid hormone can affect the function of the heart in three main ways: by 1) nuclear i.e. genomic and 2) non-nuclear i.e. nongenomic actions on cardiomyocytes and 3) by indirect actions on the peripheral circulation. Both TRα and TRβ are expressed in the heart, but the results of mice experiments suggest that the nuclear actions that T3 exert on the heart are predominantly mediated by TRα1 (145,146). The nongenomic actions, in turn, may initiate at the plasma membrane or in cytoplasm (147). With overall respect to the genomic and nongenomic actions on cardiomyocytes, thyroid hormone is able to increase cardiac inotropy and chronotropy (12,85), and the indirect actions affect the pre- and afterload of the heart (12). The indirect actions cause a decrease in systemic vascular resistance, which activates also the renin-angiotensin-aldosterone system, leading to an increased plasma volume (12,85). The sum effect of all these mechanisms means that thyroid hormone is able to increase cardiac output (85).

In the clinical context, the authors of several review articles have regarded low levels of thyroid hormone as a predisposing factor for heart failure, with special emphasis on diastolic dysfunction (8,12,144,148). In their review article, Jabbar et al. suggested that one important underlying mechanism for diastolic dysfunction in hypothyroidism would be the downregulation of the gene encoding the sarco/endoplasmic reticulum Ca^{2+}-ATPase type 2 isoform (SERCA2) (12). Calcium uptake in the sarcoplasmic reticulum is vital for cardiac contractile performance and is upregulated by SERCA2 and downregulated by phospholamban (149). Mouse and rat studies have revealed that thyroid hormone, in turn, upregulates SERCA2 levels and downregulates phospholamban levels (150,151).

In accordance with the review articles (8,12,144,148), several non-population-based studies, which have examined a small number of participants with either overt (152) or subclinical hypothyroidism (153–157), have detected an association between hypothyroidism and classical echocardiographic biomarkers of left ventricular diastolic dysfunction. In contrast, one case-control study that compared a small number of participants with controls could observe no association between subclinical hypothyroidism and myocardial function measured by echocardiography (94).

There are rather few epidemiological studies examining the relationship between thyroid function and echocardiographically defined myocardial function (158–161). One well-designed population-based study conducted by Iqbal et al. examined the effects of having either low or high TSH levels on echocardiographic biomarkers in 2035 participants belonging to the Tromsø study cohort. The euthyroid group had TSH values between 0.50–3.49 mU/L. In their cross-sectional analyses, the investigators found no association between TSH and left ventricle (LV) mass index. Furthermore, in a follow-up nested case-control analysis comprising a subset of 204 participants, high TSH was not associated with LV systolic or diastolic dysfunction compared with euthyroidism. In contrast, low TSH appeared to be associated with LV hyperfunction as judged by certain pulsed-wave tissue Doppler parameters (159). The results were essentially similar in the population-based SHIP study with analyses investigating 1,112 participants aged 45–81 years. Elevated TSH levels were not longitudinally associated with a change in LV mass index or incident LV hypertrophy (160). However, cross-sectional analyses involving 1510 participants revealed that hyperthyroidism associated with left ventricular mass and prevalent LV hypertrophy (161).

Three particularly important prospective outcome studies on this topic also require mention. Rodondi et al. examined 3044 participants belonging to the CHS cohort over a follow-up period of five years. Participants were subdivided into four groups according to their baseline thyroid status: 1) subclinical hyperthyroidism, 2) TSH within the reference range of 0.45–4.5 mU/L, 3) subclinical hypothyroidism without TSH level exceeding 9.9 mU/L and 4) subclinical hypothyroidism with a TSH level of 10 mU/L or higher. The study revealed that among those with a TSH level of 10 mU/L or higher, the LV mass increased during the follow-up period and also the incidence of heart failure among them was greater than in those with a TSH level within the reference range. LV ejection fraction (LVEF) measured at the 5-year follow-up did not differ among the four groups with different thyroid statuses. Interestingly, subclinical hyperthyroidism was not associated with incident heart failure (158). The detected longitudinal association between subclinical hypothyroidism and an increase in LV mass (158) is in contrast with all the other epidemiological studies mentioned in the previous paragraph (159–161).

In another population-based study conducted by Rodondi et al., 2730 participants aged 70–79 years and belonging to the Health, Aging, and Body Composition Study cohort were examined with a follow-up period of four years. The study revealed that those with subclinical hypothyroidism and a TSH level of 7.0 mU/L or higher had a greater risk of experiencing congestive heart failure events (162).

Finally, in an individual participant data meta-analysis done by the Thyroid Studies Collaboration, 25,390 participants were examined over a median follow-up time of 10.4 years. That study revealed that participants with subclinical hypothyroidism and a TSH level of 10 mU/L or higher had a moderately elevated risk of heart failure events compared with euthyroid participants. A similarly elevated risk was detected also among participants with subclinical hyperthyroidism and a TSH level of 0.10 mU/L or lower (163).

2.4.3 Sudden cardiac death

Sudden cardiac death (SCD) is arguably the most daunting manifestation of CVD. Furthermore, it is estimated that up to half of SCDs happen in individuals without any previously known heart disease (164). SCD is a preeminent issue also epidemiologically, as it accounts for one quarter of the deaths due to CVDs globally (165).

As far as we are aware, only one epidemiological study before ours has assessed the association between thyroid function and the risk of SCD in the general population. In the study of Chaker et al., data from 10,318 participants, who belonged to the Rotterdam Study cohort and were aged 45 years or over, were included in the analyses with a median follow-up of 9.1 years. Euthyroidism was defined as having a TSH level between 0.4–4.0 mU/L. The study revealed that higher levels of continuous TSH were not associated with a greater risk of SCD in all participants or in the subgroup of euthyroid participants. However, higher levels of FT4 were associated with a higher risk of SCD both in all participants and in the subgroup of euthyroid participants. According to the authors, inclusion of certain additional cardiovascular biomarkers, such as pulse rate or QT-interval duration, into the statistical models did not change the results. Hence, the investigators could not identify an exact mechanism for an elevated risk of SCD among those with higher FT4 levels. They speculated that the mechanism could be the hyperdynamic state caused by the inotropic, chronotropic and dromotropic effects of thyroid hormone in hyperthyroidism (27,166).

Thyroid dysfunction, including pronounced subclinical hyperthyroidism, has been associated with a higher risk of heart failure (163), as described in the previous section. Heart failure could, in turn, plausibly contribute to the risk of SCD, as was pointed out also by Chaker et al. (27,164). In a study that touches tangentially on this issue, Mitchell et al. examined the effects of thyroid disorders on mortality in a selected population of 2225 patients already having systolic heart failure and an LVEF at the most of 35%. This was a substudy of the participants belonging to the Sudden Cardiac Death in Heart Failure Trial cohort, which was followed for

outcomes for a median of 3.8 years. TSH values below 0.3 mU/L were designated as hyperthyroidism and values above 5.0 mU/L as hypothyroidism. The study revealed that both hyper- and hypothyroidism were associated with an increased risk of mortality compared with euthyroidism. However, mode of death did not differ among the groups, i.e. the investigators failed to detect an association between thyroid dysfunction and SCD in patients with heart failure. Interestingly, neither the baseline LVEF nor the repeat LVEF measurements during the follow-up differed among the three groups of different thyroid status (167).

Another study that recruited a special group of patients was 4D conducted in Germany. A total of 1255 diabetic haemodialysis patients were followed for a median of 3.9 years for outcomes. The study, which was conducted by Drechsler et al., was unique in that it also investigated the association of euthyroid sick syndrome with clinical outcomes. Euthyroid sick syndrome is denoted by low T3 levels coupled with normal or slightly decreased levels of TSH and T4 and is not so much considered as a thyroid disorder per se but rather as an adaptation to some other significant illness (168,169). According to Drechsler et al., euthyroid sick syndrome could be the commonest thyroid parameter abnormality in dialysis patients, with a prevalence of up to 70% (168,170). In the 4D study, a short-term analysis of the first 12 months revealed that participants with subclinical hyperthyroidism had a doubled risk and those with euthyroid sick syndrome almost a tripled risk of SCD compared with euthyroid participants (168). It is noteworthy that euthyroid sick syndrome has already been acknowledged as a possible risk factor for mortality among critically ill patients in an earlier reasonably large and well-designed prospective study of intensive care unit patients (169).

2.4.4 Atrial fibrillation

Atrial fibrillation (AF) is a major public health care concern. It is estimated that every fourth middle-aged adult in the United States and Europe will suffer AF at some time (171). In one large meta-analysis that involved 9,686,513 participants, AF was associated with a 1.46-fold risk of death from all causes and a 2.3-fold risk of stroke (172).

The first case series depicting thyroid enlargement coupled with a rapid heartbeat and palpitations date back to the end of the 18th century (173). Later, in the early 20th century, the Australian surgeon, Thomas Peel Dunhill, demonstrated that thyroidectomy could ameliorate the symptoms of AF in thyrotoxicosis (174). Subsequently, the electrophysiological background of the effects that thyroid hormone exerts on heart rhythm has been clarified; e.g. it is comprehensively described in the review article published by Kahaly and Dillmann (173). Thyroid

hormone affects the electrical impulse generation and conduction in the heart in a multitude of ways. It increases the rates of both systolic depolarisation and diastolic repolarisation and, conversely, decreases the action potential duration of the atrial myocardium and the refraction period of both the atrial myocardium and the atrioventricular node (173). Thyroid hormone also upregulates various molecules that are pivotal for intrinsic pacemaking (173). These comprise certain proteins of the I_f channel (145,175), also known as the "funny channel" (176), and the alpha 1D subunit of the L-type calcium channel (177). According to Kahaly and Dillmann, the sum effect of all these properties is chrono- and dromotropy (173). Sinus tachycardia is the commonest and most obvious effect that excess levels of thyroid hormone exert on heart rhythm (85), but atrial excitability, dromotropy and increased sympathetic tone (178) predispose also to AF (173). Additional factors that could contribute to the risk of AF in hyperthyroidism are increased LV mass (161) and perhaps atrial ectopy (173); however, the results have been mixed in studies that have assessed the association between excess levels of thyroid hormone and atrial ectopy in patients with sinus rhythm (179–182).

An association between hyperthyroidism and AF has been detected in several population-based studies. The first of these, as far as we are aware, was a report published in 1994 based on the Framingham Study cohort. In that study, 2007 participants aged 60 years or more and without prevalent AF at baseline were followed for ten years. Normal TSH was defined as being more than 0.4 mU/L but 5.0 mU/L at the highest. TSH values of 0.1 mU/L or lower were designated as low TSH and the values between the normal and low TSH categories were referred to as slightly low TSH. High TSH was defined as being more than 5.0 mU/L. In the adjusted analyses, the relative risk of AF was tripled in those with a low TSH value compared with participants with normal TSH. In contrast, slightly low or high TSH values were not significantly associated with the risk of AF (183). In another American study, which was based on the CHS cohort, 3233 participants aged 65 years or more and free from prevalent AF were followed for a mean duration of 12.5 years. In that study, the investigators detected an association between subclinical hyperthyroidism and an increased risk of incident AF, as compared with euthyroidism (26). Furthermore, an individual participant data meta-analysis by the Thyroid Studies Collaboration examining 52,675 participants revealed an association between subclinical hyperthyroidism and an increased risk of incident AF (28). Lower levels of TSH, even within the reference range, were associated with an elevated risk of incident AF in another prospective study emanating from the Rotterdam Study cohort (184).

In contrast to hyperthyroidism, hypothyroidism has not been associated with the risk of AF in population-based studies (26,185), in fact, one study even claimed that it was a protective factor against AF (186).

2.4.5 Stroke

Stroke is a disturbance of brain function due to cerebral infarction, intracerebral or subarachnoid haemorrhage or cerebral venous sinus thrombosis (187). It is a cardinal cause of premature mortality, second only to CHD in global terms (188). Similarly to the situation with CHD, there are plausible biological reasons to explain how thyroid dysfunction could elevate the risk of stroke. Many of these pathways originating from thyroid disorder are mediated via intermediate risk factors for CVD; these have been elucidated in earlier sections of this dissertation.

There are several conventional risk factors that are associated with the risks of both CHD and stroke (189), such as high BP, tobacco use, obesity, physical inactivity, an unhealthy diet, diabetes and dyslipidaemia (187,190). Of these, high BP is the most important modifiable risk factor of stroke. It is estimated that every 20 mmHg higher systolic BP or correspondingly, every 10 mmHg higher diastolic BP, is associated with a doubled risk of stroke (187). The authors of several review articles have associated low levels of thyroid hormone with higher BP values (8,12,85,144,148), but longitudinal evidence for an elevated risk of incident hypertension in hypothyroidism is still lacking (19).

In both the Global Burden of Disease, Injuries, and Risk Factor study 2013 (191) and the INTERSTROKE study (192), an unfavourable lipid profile was significantly associated with the risk of stroke. On the other hand, it has been established that pronounced hypothyroid states are associated with hypercholesterolemia (7,127–132) and atherosclerosis (34).

Approximately one quarter of strokes develop due to cardiogenic embolisation (187,193,194), which is the most common cause of stroke, especially in older adults (193). Furthermore, cardiogenic embolisation is associated with the most severe clinical forms of stroke (195,196). According to a report originating from the Framingham Study cohort, there was a doubled likelihood that a stroke due to an AF aetiology would be fatal compared to one with a non-AF aetiology (195). Hyperthyroidism, in turn, has been associated with an elevated risk of AF in several population-based studies (26,28,183,184). In cases of heart failure with severe left ventricular dysfunction, thrombi can develop even during sinus rhythm and if they become dislodged, they can trigger a stroke (197). On the other hand, both hypothyroidism (158,162,163) and hyperthyroidism (163) have been linked to an elevated risk of heart failure in population-based studies; however, several population-based studies have failed to demonstrate that thyroid dysfunction per se could cause an impairment of LVEF (158,159,167). Whatever the reason, CHD remains an important aetiology for heart failure with reduced ejection fraction

(198), and pronounced hypothyroidism, in turn, is associated with incident CHD (34).

The results in prospective outcome studies examining the associations between hypothyroidism and stroke have been conflicting. In the Nagasaki study, there was no association detected between subclinical hypothyroidism and cerebrovascular disease even in cross-sectional analyses (31). In another study that was conducted in the United States and based on the NHANES I cohort, hypothyroidism was associated with a 1.6-fold risk of stroke compared with euthyroidism, but the investigators defined thyroid status rather unconventionally i.e. solely according to FT4, with values between 3.5–10.5 mU/L denoting euthyroidism (199). In an individual participant data meta-analysis undertaken by the Thyroid Studies Collaboration involving 47,573 participants, there was a statistically nonsignificant trend with a *P*-value of 0.07 towards a higher risk of stroke with higher levels of TSH. In addition, in an age subgroup analysis examining participants aged 18–49 years, subclinical hypothyroidism was significantly associated with stroke with a 3.3-fold risk compared with euthyroidism (200). Another large meta-analysis was published by the Thyroid Studies Collaboration one year later, this time involving the individual participant data of 43,598 adults. In that study, the results seemed to point in an opposite direction compared with the results obtained one year earlier, as now higher levels of TSH within the reference range were shown to be a protective factor against stroke (201).

If one considers the intermediate contribution of AF, hyperthyroidism is biologically an even more plausible risk factor of stroke than hypothyroidism. Given the importance of both thyroid disease and stroke as public health concerns, there have been surprisingly few epidemiological studies investigating the association of hyperthyroidism with stroke. In a British study examining 7209 patients who had been treated with radioactive iodine for hyperthyroidism in Birmingham, United Kingdom, between 1950–1989, there was a 1.4-fold excess risk of death due to cerebrovascular disease (202). A Finnish study with an essentially similar design yielded an identical result (203). Another register study, which was conducted in Taiwan and with participants aged 18–44 years and a follow-up period of five years, compared 3176 participants with hyperthyroidism against 25,408 controls. In that study, hyperthyroidism was associated with a 1.44-fold risk of ischemic stroke. However, in the Taiwanese study, data for many baseline characteristics such as tobacco use, body mass index (BMI) and thyroid function test results were lacking, and thus, important residual confounding factors could have been missed, and furthermore the researchers could not double-check the diagnoses of hyperthyroidism (204).

2.5 Summary

Thyroid hormones are essential for the optimal functioning of almost all tissues (4). TSH is considered the first-line biomarker for detecting the commonest forms of thyroid dysfunctions in most outpatient settings (6). The reference range of TSH has a critical importance in the definition and diagnostics of these dysfunctions. However, TSH reference range studies have provided inconsistent results, especially concerning the TSH upper limit (68–70).

Previous major population-based studies have revealed a positive relationship between TSH and blood pressure in euthyroid participants (14–16,19). However, longitudinal evidence for this association is scarce and conflicting (19–21). Furthermore, surprisingly few population-based studies have assessed the association of frank thyroid dysfunctions, either overt or subclinical, with blood pressure (**Table 2**). The results of these studies seem to associate hypothyroidism with an elevation of at least diastolic blood pressure (14,19), although the existing scant longitudinal evidence does not confirm these cross-sectional findings (19). As far as we are aware, there are no population-based studies linking hypothyroidism with prevalent or incident hypertension (19). However, categorisation of continuous variables leads to an inevitable loss of information and statistical power (205), and in fact, Ittermann et al. were able to find an association between a power-transformed continuous TSH variable and prevalent hypertension in a cross-sectional setting (19). Additional prospective studies are needed to confirm this finding longitudinally.

Considerable molecular evidence (99,100,103,104,106) and also population-based epidemiological studies (**Table 2**) link hypothyroidism with an impaired lipid profile. However, longitudinal data on this association are limited and inconsistent between the genders (20).

Even subclinical hyperthyroidism seems to be associated with an elevated risk of AF (26,28,206). An individual-level meta-analysis has also linked subclinical hyperthyroidism with an increased risk of heart failure events (163). In addition, Chaker et al. found that higher levels of FT4 associated with a greater risk of SCD (27), but confirmatory studies are still needed.

Several studies have associated subclinical hypothyroidism with increased cardiovascular morbidity (34,138,139,200), while others have not (26,30). The conflicting results have fuelled a debate on whether even subclinical thyroid dysfunctions predispose to an excess cardiovascular risk, and whether these states already warrant thyroid therapy.

Table 2 Summary of detected associations of hypo- and hyperthyroid states with cardiovascular risk factors, as compared with euthyroidism, in previous major population-based studies.

	Thyroid status				
	Hyperthyroidism[a]		Hypothyroidism[a]		
Outcome	Association	Reference	Association	Reference	Notes
Systolic blood pressure	NS	(14,19)	+ NS	(19) (14)	Longitudinal evidence NS (19)
Diastolic blood pressure	NS	(14,19)	+	(14,19)	Longitudinal evidence NS (19)
Hypertension	NS	(19)	NS	(19)	Longitudinal evidence NS (19)
Total cholesterol	NS NS (men) - (women) -	(14,132) (207) (207) (7)	+ + (women) NS (men) NS	(7,132) (207) (207) (14)	Longitudinal evidence lacking
LDL cholesterol	NS NS (men) - (women) -	(14,132) (207) (207) (7)	+ + (women) NS (men) NS	(7,132) (207) (207) (14)	Longitudinal evidence lacking
HDL cholesterol	NS NS (men) - (women)	(7,14,132) (207) (207)	NS -	(7,14,207) (132)	Longitudinal evidence lacking
Triglycerides	- NS	(7,14,132) (207)	+ + (men) NS (women) NS	(7,132) (207) (207) (14)	Longitudinal evidence lacking

A plus sign denotes a positive relationship, a minus sign a negative relationship. NS, nonsignificant association. [a]Subclinical and overt thyroid dysfunctions pooled together.

3 AIMS OF THE STUDY

This thesis was designed to investigate whether thyroid dysfunctions, even emerging forms, would carry a risk of cardiovascular comorbidities or death. The specific aims were:

1. To define in a large population-based sample of Finnish adults a new reference range for TSH to serve as the cornerstone of the diagnostics of thyroid dysfunction.

2. To quantify the associations between thyroid function and blood pressure in both cross-sectional and prospective settings.

3. To evaluate the associations between thyroid function and lipid concentrations in both cross-sectional and prospective settings.

4. To assess the associations between thyroid function and death from any cause, sudden cardiac death and cardiovascular morbidity.

4 MATERIALS AND METHODS

4.1 Study population

This study is based on the epidemiological Health 2000 Survey and its follow-up, the Health 2011 Survey, which were conducted in Finland in 2000–2001 and 2011–2012, respectively. In the Health 2000 Survey, a stratified two stage cluster sampling approach was used nationwide and a total of 8028 persons was drawn randomly from the national population register to represent the Finnish adult population aged 30 years or more. To produce longitudinal data on the Finnish adult population, the subjects were re-invited 11 years later to a follow-up examination. A detailed report on this sampling has been published previously (208,209). The Health 2000 and 2011 Survey protocols were approved by the Epidemiology Ethics Committee for the Helsinki and Uusimaa hospital region. All the participants signed informed consent forms according to the Declaration of Helsinki.

A total of 6354 (79% of the draw) individuals agreed to participate in a health examination and in a health interview at a local facility in the baseline survey. Of these, 3857 (60.7%) individuals participated in the 11-year follow-up health examination. Of those 6247 participants for whom a baseline serum TSH was successfully determined, those with one or more of the following factors were excluded: not being ambulatory (n=8); a previous history of goitre or overt thyroid disease (n=365); use of thyroid hormone replacement medication or antithyroid agents (n=245); a TSH test drawn before 8 AM or after 6 PM (n=90) (5) and/or pregnancy or breastfeeding (n=55) (210,211). Studies I-IV were thus based on this base population of 5724 participants; subsequently, additional exclusion criteria pertinent to each study were applied (**Figure 2**).

In Study I, the TSH reference range was defined for four different subpopulations formed from the previously mentioned base population. A total of 15 participants with extreme TSH concentrations (± 5 standard deviation [SD] from mean, > 12.1 mU/L) were excluded from the base population to form a thyroid-healthy population, which consisted of 5709 participants. Subsequently, in accordance with the NACB guidelines (5), those participants who had a positive TPOAb test result (n=364) were excluded from the thyroid-healthy population. As TPOAb results were not available for all individuals who had a TSH value in the range of 0.4–2.5 mU/L, 759 randomly selected participants who had a TSH value in that range were also excluded to prevent oversampling. Consequently, the total share of excluded participants that had a TSH result within the range of 0.4–2.5 mU/L was exactly equal to the prevalence of TPOAb-positivity in that range (16.3%), as

determined from the available data. The risk factor-free subpopulation finally consisted of 4586 participants. An additional subgroup from this subpopulation was formed by excluding those participants with medication use that could potentially affect thyroid function tests. These medications have been described in a previous review article (212) and are listed in Article I, Supplemental data, Table 1 online. This subgroup totalled 3453 participants. Finally, participants on any medications (n=2737) were excluded from the risk factor-free subpopulation. In contrast to the NACB guidelines, even users of oestrogen were excluded because some oestrogen medications have been reported to affect TSH levels (213). The final reference subpopulation, therefore, consisted of 1849 participants.

In Study II, those with extreme TSH values exceeding ±5 SD from the mean or missing data for the variables used in the cross-sectional analyses (n=69) were excluded from the base population. As a result, 5655 participants were included in the cross-sectional analyses. Of these, 3453 had the available data for the planned longitudinal analyses.

In Study III, those participants either with lipid-lowering medication (n=340) or missing information (n=197) for any of the covariates used in the baseline analyses were excluded from the base population. As a result, the final baseline population of Study III consisted of 5205 participants. For those participants that were examined at follow-up, the exclusion criteria for longitudinal analyses were missing information for the variables used in analyses (n=165) or use of lipid-lowering medication (n=502) or thyroid hormone replacement medication/antithyroid agents (n=144). The resulting final follow-up population of Study III thus consisted of 2486 participants (48% of the final baseline population).

In Study IV, those participants with missing information on confounding covariates (n=55) or with a history of either a major adverse cardiac event (MACE) or CVD (n=544) were excluded from the base population. In the analyses with AF as an outcome, also those individuals with prevalent AF at baseline (n=178) were excluded. As a result, the final study population was 5211 participants, and in the analyses having AF as an outcome, the total was 5133 participants.

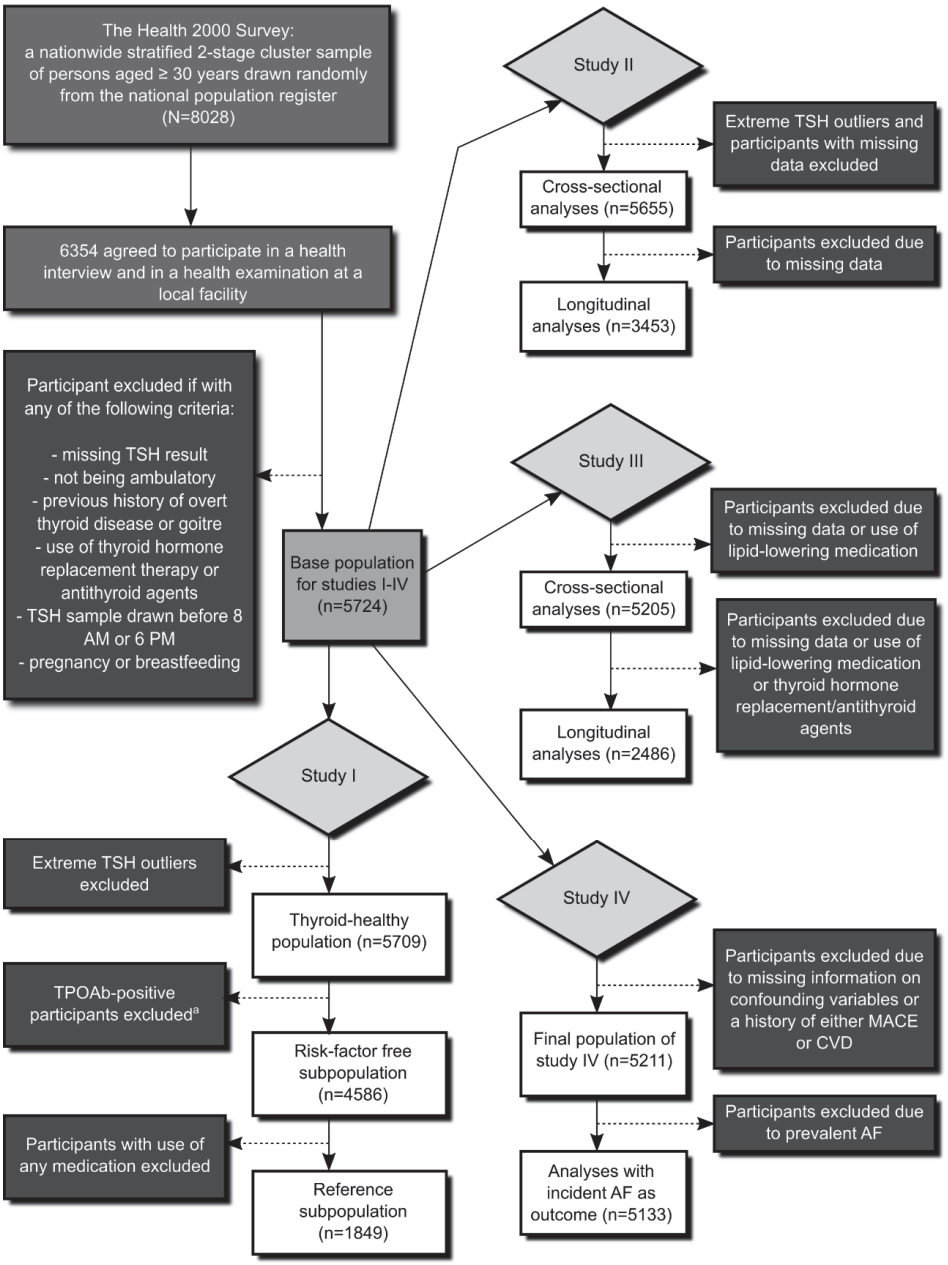


Figure 2 Flow-chart of inclusion-exclusion of participants in studies I-IV. [a]See methods.

4.2 Health interview and health examination

In the baseline survey, interviews to gather information on health, illnesses, functional capacity and medications were conducted by trained interviewers of

Statistics Finland. The subjects were examined 1–4 weeks subsequently in a single examination by centrally trained nurses and physicians at a local facility. In addition, blood samples were taken from the participants. Essentially, the same methods were used in the follow-up survey. (208,209)

4.3 Blood pressure measurement

Baseline BP was measured with a mercury manometer (Mercuro 300; Speidel & Keller, Jungingen, Germany) from the right arm if technically possible at a local facility by a nurse with the participant sitting after a 10-min rest. The follow-up measurement was similar for all except 339 (8.8%) participants in whom BP was measured during a home health examination using an oscillometric OMRON M4 device (Omron Matsusaka Co., Kyoto, Japan) from the upper arm of the non-dominant hand. The mean of two BP measurements performed at a 2-min interval was used in the analyses. (208,209) In study II, increments of 15 and 10 mmHg were added, respectively, to the measured systolic and diastolic BPs of the participants using antihypertensive medication as an estimate of the expected BP change due to BP treatment. Owing to this approach, which has been introduced in earlier well-designed works (214,215), participants with antihypertensive medication could be retained in the study sample.

4.4 Laboratory analyses

The blood samples were frozen at -20 °C immediately after separating serum and plasma and then transferred in dry ice to the laboratory once a week for biomarker analyses. The laboratory measurements for total cholesterol, HDL cholesterol, triglycerides, glucose and apolipoproteins A1 and B were performed at the Research and Development Unit in the Social Insurance Institution (Turku, Finland) using an Olympus AU400 analyser (Olympus, San Diego, CA, USA) for the baseline survey and in the biochemistry laboratory of the Genomics and Biomarkers Unit in National Institute for Health and Welfare (Helsinki, Finland) using Architect ci8200 analyser (Abbott Laboratories, Abbott Park, IL, USA) in the follow-up survey. LDL cholesterol was calculated with the Friedewald formula. FT4 and TPOAb levels were measured from those participants whose TSH level was below 0.4 mU/L or above 2.5 mU/L and also from randomly selected individuals whose TSH level was in the range of 0.4–2.5 mU/L. TSH, FT4 and TPOAb levels were analysed in the biochemistry laboratory in the National Institute for Health and Welfare (Helsinki, Finland) using a chemiluminescent microparticle immunoassay on an Abbott Architect ci8200 analyser (Abbott

Laboratories, Lake Bluff, IL, USA), from plasma samples that had been stored in -70 °C. (208,209)

4.5 Electrocardiography

At the baseline, 12-lead resting supine ECGs were obtained using a MAC 5000 recorder (Marquette Hellige, Freiburg, Germany and Milwaukee, WI, USA). The recordings were analysed using Magellan software (GE Healthcare, Milwaukee, WI, USA). Consequently, the measurements were also manually checked, and if necessary, then corrected by centrally trained nurses who were supervised by an experienced clinical physiologist. (208)

4.6 Definitions

A systolic BP ≥140 mmHg or a diastolic BP ≥90 mmHg, or self-reporting the use of medication to treat hypertension was designated as hypertension.

Diabetes mellitus was denoted by a glucose level of 7.0 mmol/L or greater or alternatively via the self-report that the participant was prescribed medications to combat diabetes.

Prevalent cancer was identified using a dichotomous questionnaire item.

The endpoints of Study IV were total mortality, SCD and incident CVD, CHD, stroke, MACE and AF. The subjects were followed up for these endpoints until 31 December 2013. Information on the endpoints was retrieved from the National Hospital Discharge and Causes of Death registers, which cover all episodes of care nationwide in secondary and tertiary care in Finland. Cardiovascular diagnoses in these registers have been outlined and validated previously (216,217). The nationwide Drug Reimbursement register that records the data on all prescription drug purchases in Finland was also utilised.

Hospitalisation with ICD-10 codes I20-I22 and/or coronary artery bypass surgery or percutaneous coronary intervention in the Hospital Discharge Register were designated as nonfatal CHD events. Codes I20–I25, I46, R96 and R98 in the Causes of Death Register as the primary underlying or immediate causes of death were designated as fatal CHD events. Nonfatal and fatal strokes were denoted by codes I60–I61 or I63–I64, excluding I63.6, in the registers. CVD event outcome was a composite endpoint comprising CHD and stroke events. MACE was a composite endpoint comprising heart failure and CVD. Heart failure was defined

as having any of the following: use of furosemide or both furosemide and potassium-sparing agents, reimbursement received from the Social Insurance Institution for medication for chronic heart failure or codes I50, I11.0, I13.0 or I13.2 in the registers. AF was denoted by the code I48 in the National Hospital Discharge or Causes of Death registers. For the outcome variables, only the first event was included in these analyses.

A report on how SCD was adjudicated in our study has been published previously (218). In brief, out-of-hospital deaths and deaths within 10 days of hospitalisation were designated as SCD. The definition of SCD included also resuscitated cardiac arrests. Deaths with a cardiac cause as the immediate or underlying cause of death in those cases without any other known cause of death other than arrhythmia were designated as probable SCDs. Deaths from a non-cardiac cause as the immediate or underlying cause of death were designated as possible SCDs, when cardiac disease was present and could reasonably have contributed to arrhythmia. In Study IV, probable and possible SCDs were pooled in the analyses.

4.7 Statistical analyses

In Study I, TSH distribution was neither normal nor perfectly log-normal in the reference subpopulation. TSH reference ranges were therefore established directly from the 2.5th and 97.5th percentiles of the TSH measurements in the thyroid-healthy population and all of its subsets, as has been done also in many other studies (67,69,82,219). In our study, the validity of this non-parametric method was controlled by defining the TSH reference range for the reference subpopulation also by using data on the 95% confidence limits; however, TSH values were first power-transformed with a suitable function to obtain a satisfactory Gaussian distribution. Kruskal-Wallis test was used to assess differences in TSH levels between the subgroups for age and gender.

In Study II, for the outlier detection mentioned earlier in the Study population section, the data for TSH were first transformed using a suitable function with the 8th-root-transformation achieving a satisfactory Gaussian distribution for TSH (skewness 0.04, kurtosis 7.14). The regression analyses were conducted using non-transformed TSH values. However, all models with TSH as a continuous variable were also reanalysed using the 8th-root-transformed TSH variable. Multiple linear regression was used for continuous outcomes and logistic regression for dichotomous outcomes. When examining curvilinear relationships between TSH and the dichotomous outcomes, five TSH categories were formed as follows: (1) TSH below the lower reference limit; (2–4) the tertiles within the reference range of 0.4–3.4 mU/L (83) and (5) TSH above the upper reference limit. TSH category

2 (TSH 0.40–1.09 mU/L) was used as the reference category. All models were adjusted for baseline age, current smoking and BMI, and also for gender in pooled analyses of women and men. In all models, the interaction terms between TSH and the other covariates were tested. The interaction term between TSH and age was statistically significant with respect to the cross-sectional outcomes of systolic BP (P=0.006), diastolic BP (P<0.001) and prevalent hypertension (P=0.01). The interaction term between TSH and gender was statistically significant for the 11-year change in systolic (P=0.003) and diastolic (P=0.01) BP and incident hypertension (P=0.03). Due to these findings, subgroup analyses for age and gender were also performed. Tukey–Kramer multiple comparisons test was used to assess differences by TSH category in BP, with an adjustment for age, smoking and BMI. To assess the representativeness of the follow-up subpopulation to the baseline population, an attrition analysis was conducted using a *t*-test for continuous and a chi-square test for categorical data.

In Study III, the cross-sectional associations between the continuous TSH variable and lipid concentrations were assessed with multivariable general linear regression models. Similar models were used to study the associations between baseline TSH and follow-up lipid concentrations. When identifying curvilinear associations between TSH and the lipid outcomes, the models were reanalysed with a categorised TSH variable. Five TSH categories were formed: (1) TSH below the lower reference limit (low TSH), (2–4) the tertiles within the reference range of 0.4–3.4 mU/L (83) and (5) TSH above the upper reference limit (high TSH). The most favourable TSH category with respect to lipid profile could not be determined by tabulation (Article III, Table 2; Article III, Supplementary Table S3 online). For that reason, the whole TSH reference range was chosen as the reference category for the models with a categorised TSH variable. All models with a continuous TSH variable were also reanalysed restricting the analyses to the reference range of TSH.

To elucidate the possible cause-effect relationship between low thyroid function and an adverse lipid profile, the longitudinal models were also reanalysed by excluding participants with high-risk baseline lipid concentrations. Thresholds for the high-risk lipid levels were obtained from the 2012 Lipid and Atherosclerosis Guidelines issued by AACE (220). The thresholds and the numbers excluded from the corresponding analyses were as follows: total cholesterol ≥ 6.2 mmol/L (n=825); HDL cholesterol < 1.3 mmol/L in women (n=430) and HDL cholesterol < 1.0 mmol/L in men (n=239); LDL cholesterol ≥ 4.1 mmol/L (n=900) and triglycerides ≥ 2.3 mmol/L (n=245). The 2012 AACE guidelines do not define thresholds for high-risk apolipoprotein A1 and B concentrations, and the models involving these outcomes were therefore not reanalysed.

All regression models of Study III were adjusted for baseline age, current smoking and BMI. Adjustment for gender was conducted in those models with pooled analyses of women and men. Due to their non-normal distribution, the data for triglycerides were $\log_{10}$-transformed. The regression analyses were conducted using non-transformed TSH values; however, all models treating TSH as a continuous variable over the full range were also reanalysed with a power-transformed TSH variable. An 8th-root function achieved an even better normal distribution for TSH than did the logarithm function (skewness -0.01, kurtosis 7.1; vs. skewness -1.3, kurtosis 11.6, respectively).

In Study IV, Cox proportional hazards regression models were used to evaluate the associations between continuous TSH and total mortality, SCD, incident CHD, stroke, CVD, MACE and AF. In addition, the linear relations of the continuous TSH variable and the outcomes were reanalysed restricting the analyses to participants with TSH levels within the reference range. To approximate normal distribution, TSH was log-transformed for all models with a continuous TSH variable. In these models, the interaction terms of TSH with gender and age were tested. The tests revealed that the interaction term for the effects of a continuous TSH variable within the reference range and gender upon incident AF was significant. That model was therefore reanalysed also separately for women and men. In order to detect curvilinear relationships, additional analyses with a categorised TSH variable were performed. The participants were divided into three categories based on their TSH levels: low TSH (<0.4 mU/L), normal TSH, which was defined as the reference range of TSH (0.4–3.4 mU/L) (83) and high TSH (>3.4 mU/L). Normal TSH was used as the reference category. All models were adjusted for baseline age, gender, smoking, diabetes, systolic BP and serum total and HDL cholesterol. The proportional hazards assumption for the Cox models was assessed using the Kolmogorov supremum test. If the test result was statistically significant, the Schoenfeld residuals were examined. No major violations of the assumption were observed. The multivariable analyses with a categorical TSH variable as the regressor and total mortality or SCD as the outcome were also reanalysed with an additional adjustment for prevalent cancer. Cross-sectional relationships between TSH and ECG conduction times were also investigated with multiple linear regression that was adjusted for the same covariates.

The relations between TSH and the risks of the main outcomes were also examined in multivariable-adjusted Cox models with a restricted cubic spline (RCS) transformation on TSH (221). The RCS transformation was conducted by placing four knots at percentiles 5, 35, 65 and 95 of the TSH distribution (222).

The associations of subclinical thyroid dysfunctions with the outcomes were also assessed, as compared with euthyroidism. TSH values within the reference range were designated as euthyroidism. Subclinical hyperthyroidism was denoted by TSH values below 0.4 mU/L coupled with FT4 values within the reference range. Subclinical hypothyroidism was designated by TSH values over 3.4 mU/L in conjunction with FT4 values within the reference range. In the FT4 assay, a manufacturer-provided reference range of 9.0–19.0 pmol/L was used.

Statistical analyses were performed using SAS software (Version 9.3 in Study I and Version 9.4 in Studies II-IV, SAS Institute, Cary, NC, USA). A *P*-value of less than 0.05 was considered statistically significant.

5 RESULTS

Baseline characteristics of the base population for Studies I-IV are shown in **Table 3** for those participants with available data on the listed variables.

Table 3 Baseline characteristics of the base population for Studies I-IV.

Characteristic	All participants	TSH category		
		Low TSH (< 0.4 mU/L)	Normal TSH (0.4–3.4 mU/L)	High TSH (>3.4 mU/L)
n	5675	129 (2.3%)[a]	5241 (92.4%)[a]	305 (5.4%)[a]
Age (years)	52.5±14.8	60.2±17.5	52±14.7	57.4±13.3
Women	2945 (51.9%)	86 (66.7%)	2677 (51.1%)	182 (59.7%)
TSH (mU/L)	1.7±2.1	0.2±0.1	1.5±0.6	6.2±7.4
Diabetes	330 (5.8%)	15 (11.6%)	293 (5.6%)	22 (7.2%)
Current smokers	1585 (27.9%)	35 (27.1%)	1494 (28.5%)	56 (18.4%)
Systolic BP (mmHg)	134.6±21.0	138.3±23.7	134.2±20.9	139.8±20.8
Diastolic BP (mmHg)	81.9±11.1	79.1±10.3	81.9±11.1	83.4±11.1
Total cholesterol (mmol/L)	5.9±1.1	5.8±1.1	5.9±1.1	6.2±1.2
HDL cholesterol (mmol/L)	1.3±0.4	1.3±0.4	1.3±0.4	1.3±0.4

Values are means ± standard deviations for continuous data and numbers and percentages for the dichotomous variables, depicting characteristics in all participants and within the TSH categories. [a]Values are numbers and row percentages.

5.1 Thyroid-stimulating hormone reference range and the factors affecting it (I)

The characteristics of the subpopulations of Study I are reported in Article I, Table 1, and the TSH medians and reference ranges are depicted correspondingly in **Figure 3**.

5.1.1 Effects of TPOAb-positivity on the TSH reference range

The exclusion of TPOAb-positive individuals from the thyroid-healthy population caused a marked decrease in the upper reference limit of TSH. The TSH upper reference limit of the thyroid-healthy population was 4.43 mU/L, while the corresponding figure for the risk factor-free subpopulation was 3.71 mU/L (**Table 4**). In contrast, the TSH lower limit remained at 0.41 mU/L even after the exclusion of TPOAb-positive participants.

5.1.2 Effects of medications on the TSH reference range

The exclusion of those individuals with a use of some medication potentially modifying thyroid function lowered the TSH upper reference limit by 0.1 mU/L. The exclusion of those individuals using any medication at all, in turn, lowered the TSH upper limit by 0.3 mU/L. These exclusions did not affect the TSH lower limit (**Figure 3**). The TSH reference range for the reference subpopulation (n=1849) without any medications was 0.43–3.37 mU/L.

5.1.3 Effects of age and gender on the TSH reference range

The TSH reference ranges were also determined for the subgroups of age and gender in the reference subpopulation. The TSH medians and reference ranges for these subgroups are given in **Table 4**. Although the small differences in TSH between the subgroups of age (P=0.002) and gender (P=0.005) did reach statistical significance, the TSH distribution curves for these subgroups could be essentially superimposed (Article I, Figure 2).

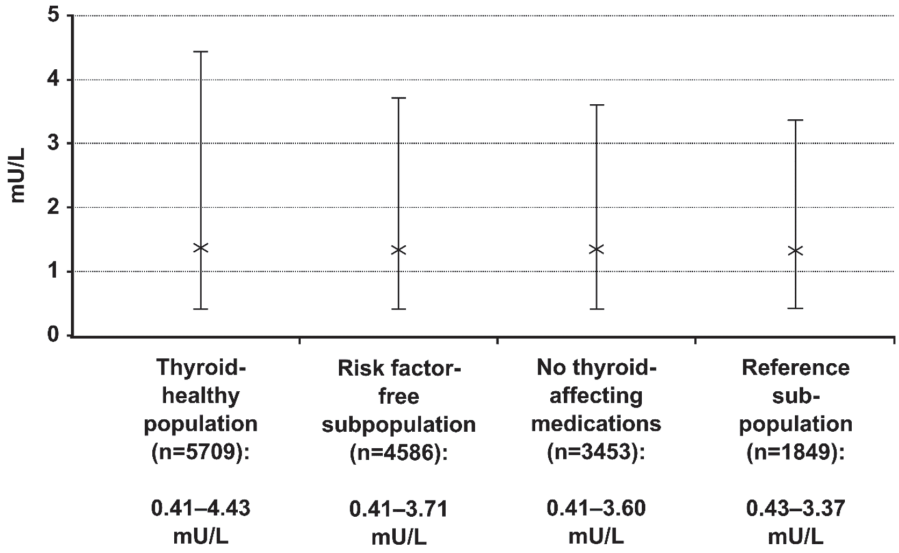


Figure 3 The four different TSH reference ranges in the present study, established from the thyroid-healthy population and its subpopulations, which were formed from the Health 2000 Survey cohort with an increasing rigour of screening for factors that could affect TSH values. The risk factor-free subpopulation was formed from the thyroid-healthy population by the exclusion of TPOAb-positive participants. The remaining two subpopulations were formed from the risk factor-free subpopulation according to the use of medications. The reference subpopulation was completely medication-free and was formed by following the NACB guidelines as closely as possible. The "x" on the range bar denotes TSH medians.

Table 4 TSH reference ranges for subgroups of age and gender in the reference subpopulation.

		TSH (mU/L)		
	n	2.5th percentile	Median	97.5th percentile
Age (years)				
30-44	953	0.42	1.29	3.07
45-59	626	0.44	1.41	4.13
≥60	270	0.38	1.33	3.41
Gender				
Women	745	0.39	1.29	3.22
Men	1104	0.50	1.36	3.55

5.2 Association of thyroid-stimulating hormone with blood pressure (II)

5.2.1 Cross-sectional analyses

BP means by TSH category after adjustment for age, smoking and BMI are described in Article II, Table 2. Mean systolic BP was lowest in TSH category 3 (TSH 1.10–1.65 mU/L) in men and in TSH category 2 (TSH 0.40–1.09 mU/L) in women. In both women and men, mean diastolic BP was lowest in TSH category 1 (TSH 0.03–0.39 mU/L). BP means were not statistically different (data not shown) between the TSH categories for either of the genders.

Regression curves for the cross-sectional association between TSH as a continuous variable and BP with an adjustment for age, gender, smoking and BMI are shown in Article II, Figure 1. Diastolic BP increased with TSH in men (beta coefficient [β]=0.50, *P*=0.01) and in the whole study population (β=0.36, *P*=0.003), but not in women (β=0.25, *P*=0.13). There was no statistically significant association between continuous TSH and systolic BP (**Figure 4**). In the age-subgroup analyses, TSH associated with systolic BP but only in men aged 65 years or less (β=0.73, *P*=0.04) and with diastolic BP but only in women (β=0.42, *P*=0.03) and men (β=0.49, *P*=0.02) aged 65 years or less (Article II, Supplementary Tables S1 and S3 online).

The odds of prevalent hypertension were greater in the highest tertile as compared with the lowest tertile of the TSH reference range (odds ratio [OR]=1.22, *P*=0.01) (**Table 5**). In the age-subgroup analyses, this association was statistically significant in participants aged 65 years or less (OR=1.24, *P*=0.02) but not in participants older than 65 years (OR=1.14, *P*=0.45). This association was not

statistically significant in the gender-specific analyses (Article II, Supplementary Tables S1 and S3 online). TSH levels over the full range were not associated with prevalent hypertension (OR=1.03, *P*=0.26) (Article II, Table 3).

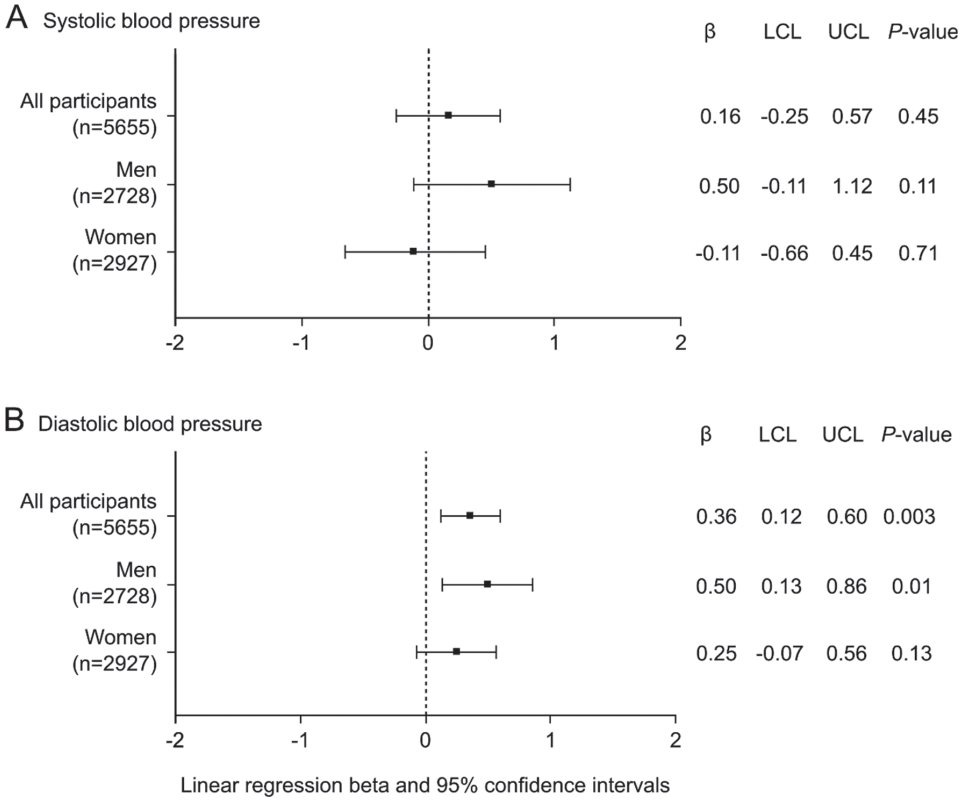


Figure 4 Association between baseline TSH (mU/L) as a continuous variable and baseline (A) systolic and (B) diastolic blood pressure (mmHg). Multiple linear regression for all outcomes, adjusted for age, smoking, BMI and also gender in models pooling women and men. LCL, lower 95% confidence limit; UCL, upper 95% confidence limit.

5.2.2 Longitudinal analyses

Regression curves depicting the association between TSH and the 11-year change in BP are shown subdivided by gender in Article II, Figure 2. The regression models showed an inverse association between continuous TSH and change in systolic BP (β=-0.92, *P*=0.03) and change in diastolic BP (β=-0.66, *P*=0.01) in men, but not in women (β=0.62, *P*=0.09 and β=0.04, *P*=0.85, respectively) (**Figure 5**). In the age subgroup analyses, TSH was inversely associated with a change in diastolic BP in men aged over 65 years (β=-1.68, *P*=0.004) but not in other men (β=-0.32, *P*=0.28). However, there was no statistically significant inverse

association between TSH and change in systolic BP, in turn, in men aged over 65 years (β=-1.46, *P*=0.21). There were no statistically significant associations between the continuous or categorised TSH variables and incident hypertension (**Table 5**; Article II, Table 4; Article II, Supplementary Tables S2 and S4 online).

5.2.3 Analyses using a power-transformed TSH variable

The 8th-root-transformed TSH variable provided similar results as the non-transformed TSH variable with one exception: in participants aged 65 years or less, the power-transformed TSH associated with systolic BP in a pooled analysis of women and men (*P*=0.03). This association was no longer significant, however, in separate analyses of women (*P*=0.22) and men (*P*=0.09) (data not shown).

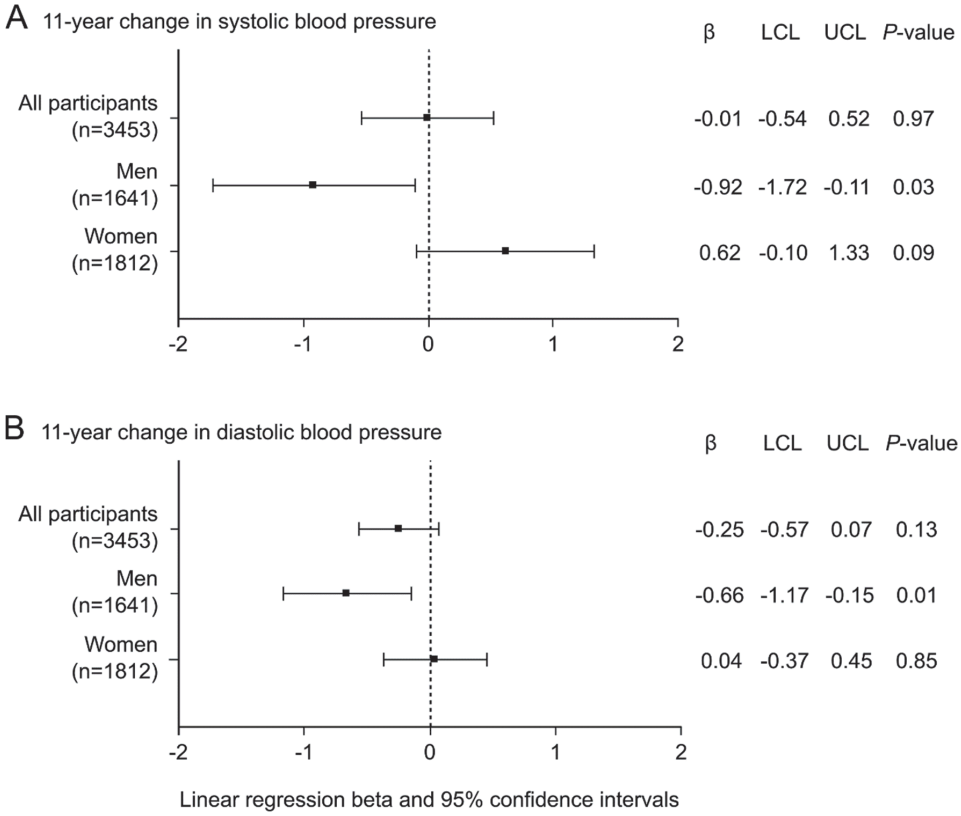


Figure 5 Association between baseline TSH (mU/L) as a continuous variable and the 11-year change in (A) systolic and (B) diastolic blood pressure (mmHg). Multiple linear regression for all outcomes, adjusted for baseline age, smoking, BMI and also gender in models pooling women and men.

Table 5 The odds ratios (with 95% confidence intervals) of prevalent and incident hypertension subdivided by categories of TSH concentration.

	TSH category				
Outcome	0.03–0.39 mU/L	0.40–1.09 mU/L	1.10–1.65 mU/L	1.66–3.40 mU/L	3.41–17.75 mU/L
Prevalent hypertension					
All (n=5655)	1.16 (0.73–1.84)	1.00	1.12 (0.96–1.31)	1.22 (1.05–1.43)*	1.14 (0.86–1.50)
Women (n=2927)	1.21 (0.65–2.23)	1.00	1.23 (0.98–1.55)	1.23 (0.98–1.55)	1.25 (0.85–1.84)
Men (n=2728)	1.03 (0.50–2.12)	1.00	1.04 (0.84–1.28)	1.19 (0.97–1.47)	1.02 (0.67–1.54)
Incident hypertension[a]					
All (n=2060)	0.87 (0.40–1.89)	1.00	0.88 (0.70–1.11)	0.93 (0.73–1.19)	1.31 (0.81–2.10)
Women (n=1164)	0.81 (0.31–2.14)	1.00	0.90 (0.65–1.24)	1.02 (0.73–1.41)	1.73 (0.92–3.25)
Men (n=896)	1.05 (0.28–3.86)	1.00	0.85 (0.60–1.19)	0.82 (0.58–1.18)	0.81 (0.38–1.75)

Logistic regression for all outcomes. Models adjusted for baseline age, smoking, BMI and also gender in models pooling women and men. * $P<0.05$. [a]Participants with prevalent hypertension at baseline were excluded from these analyses.

5.3 Association of thyroid-stimulating hormone with lipid concentrations (III)

5.3.1 Cross-sectional analyses

Regression statistics for the cross-sectional associations between baseline TSH as a continuous variable and baseline lipid concentrations are shown in **Figure 6**. The statistics of all covariates are given for the model with baseline LDL cholesterol as an outcome (Article III, Supplementary Table S5 online). In a pooled analysis of women and men combined, a 1 mU/L higher TSH level associated with a 0.02 mmol/L higher total cholesterol ($P<0.001$), a 0.01 g/L higher apolipoprotein B ($P<0.001$), a 0.02 mmol/L higher LDL cholesterol ($P=0.002$) and a 0.01 mmol/L higher log triglyceride ($P=0.004$) concentration.

The results in the gender subgroup analyses agreed well with the pooled analysis of women and men with one exception: TSH associated with log triglyceride levels in men ($P<0.001$) but not in women ($P=0.24$) (**Figure 6**).

Restricting the analyses to the TSH reference range yielded essentially similar results in the pooled analysis of women and men. In the gender subgroup analyses, a 1 mU/L higher TSH concentration within the reference range of TSH associated with a 0.04 mmol/L higher log triglyceride level also in women ($P<0.001$). However, TSH was no longer associated with total cholesterol ($P=0.07$) or LDL cholesterol ($P=0.70$) in men in the analyses that involved only the TSH reference range (Article III, Supplementary Table S4 online).

The models that had a categorised TSH variable as the regressor yielded materially similar results as those models with a continuous TSH variable. The high TSH category (denoted by TSH values above the upper reference limit) was associated with an adverse lipid profile, when compared with TSH in the reference range (Article III, Table 4; Article III, Supplementary Tables S1 and S2 online). Low TSH (denoted by TSH values below the lower reference limit), when compared with TSH in the reference range, associated with a 0.30 mmol/L lower baseline total cholesterol ($P=0.003$), a 0.05 g/L lower baseline apolipoprotein B ($P=0.045$) and a 0.26 mmol/L lower baseline LDL cholesterol ($P=0.004$) concentrations in a pooled analysis of women and men (Article III, Table 4). The analyses in the men's subgroup revealed similar findings for low TSH (Article III, Supplementary Table S2 online).

5.3.2 Longitudinal regression analyses

The regression statistics of the associations between baseline continuous TSH and follow-up lipid levels are shown in **Figure 6**. The statistics for all covariates are given for the model that had follow-up LDL cholesterol as an outcome (Article III, Supplementary Table S5 online). In pooled analyses of women and men, baseline TSH as a continuous variable did not associate significantly with follow-up lipid concentrations. However, in women, a 1 mU/L higher baseline TSH level associated with a 0.06 mmol/L higher total cholesterol concentration (P=0.03), a 0.06 mmol/L higher LDL cholesterol concentration (P=0.01) and a 0.01 g/L higher apolipoprotein B concentration (P=0.03) measured at the follow-up; in contrast, baseline TSH did not associate significantly with the corresponding outcomes in men.

Restricting the analyses to the TSH reference range did attenuate the associations in women. In men, a 1 mU/L higher baseline TSH concentration within the reference range of TSH associated with a 0.10 mmol/L lower total cholesterol concentration (P=0.04), a 0.12 mmol/L lower LDL cholesterol concentration (P=0.004) and a 0.02 g/L lower apolipoprotein B concentration (P=0.047) measured at the follow-up (Article III, Supplementary Table S4 online).

Baseline high TSH category, when compared with TSH in the reference range, associated with a 0.22 mmol/L higher follow-up LDL cholesterol concentration (P=0.03) in a pooled analysis of women and men (Article III, Table 4). This association was not observed, however, in the subgroups for women and men (Article III, Supplementary Tables S1 and S2 online). In contrast, low baseline TSH, when compared with TSH in the reference range, associated with a 0.37 mmol/L lower total cholesterol concentration (P=0.04), a 0.32 mmol/L lower LDL cholesterol concentration (P=0.04) and a 0.09 g/L lower apolipoprotein B concentration (P=0.02) at the follow-up in women (Article III, Supplementary Table S1 online). In men, similar associations could not be observed (Article III, Supplementary Table S2 online).

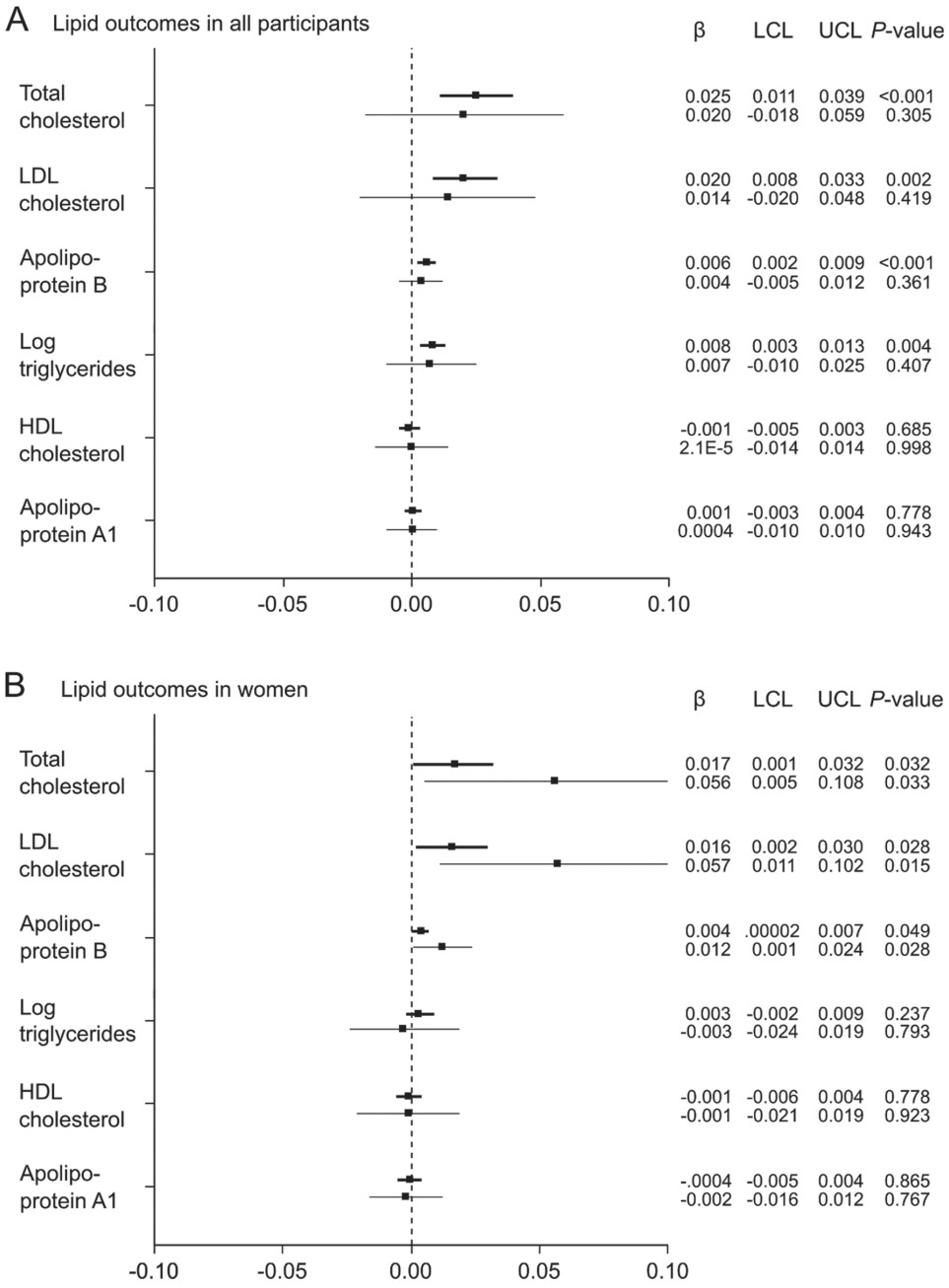
5.3.3 Effects of the exclusion of participants with high-risk baseline lipid values from the longitudinal regression analyses

After the exclusion of individuals with high-risk baseline lipid values, the continuous TSH variable over the full range and the categorised TSH variable did not associate significantly with any of the outcomes longitudinally. In those analyses restricted to the TSH reference range, baseline TSH values still associated inversely with follow-up total cholesterol concentration (β=-0.11, SE=0.05,

P=0.04) and follow-up LDL cholesterol concentration (β=-0.10, SE=0.05, *P*=0.04) in men; further, baseline TSH associated with follow-up HDL cholesterol concentration in women (β=0.04, SE=0.02, *P*=0.03) (data not shown).

5.3.4 Analyses using a power-transformed TSH variable

The analyses using an 8th-root-transformed TSH variable yielded similar results as those conducted with the non-transformed TSH variable with one exception: after the power-transformation, TSH associated cross-sectionally with log triglyceride concentration also in women (β=0.18, SE=0.08, *P*=0.02) (data not shown).



(Figure 6 continued)

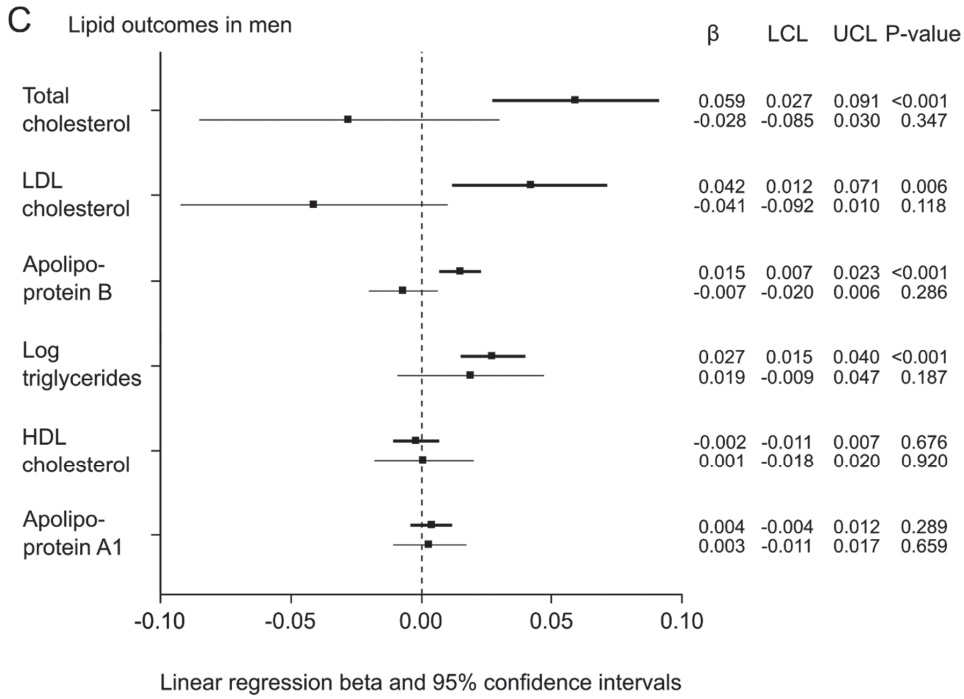


Figure 6 Associations between baseline TSH (mU/L) and baseline (thick lines) and follow-up (thin lines) lipid concentrations. Multiple linear regression for all outcomes, adjusted for baseline age, smoking, BMI and also gender in models pooling women and men. The units of concentration were mmol/L for cholesterol and triglyceride concentrations, mg/L for apolipoprotein A1 concentration and g/L for apolipoprotein B concentration. The detected longitudinal associations became statistically non-significant after the exclusion (n=245–900, depending on the lipid outcome) of participants with high-risk baseline lipid values.

5.4 Association of thyroid-stimulating hormone with the risk of sudden cardiac death, total mortality and cardiovascular morbidity (IV)

The event rates for the participants are shown by TSH categories in **Table 6**. The median follow-up time was 13.2 years.

Table 6 Event rates by TSH category in Study IV.

	TSH category		
Outcome	Low TSH (n=108)	Normal TSH (n=4834)	High TSH (n=269)
Mortality	26 (24.1%)	627 (13.0%)	57 (21.2%)
Sudden cardiac death	3 (2.8%)	72 (1.5%)	9 (3.3%)
Major coronary heart disease event	16 (14.8%)	363 (7.5%)	24 (8.9%)
Stroke	9 (8.3%)	209 (4.3%)	17 (6.3%)
Cardiovascular disease	21 (19.4%)	537 (11.1%)	38 (14.1%)
Major adverse cardiac event	26 (24.1%)	726 (15.0%)	54 (20.1%)
Atrial fibrillation[a]	15 (14.3%)	291 (6.1%)	22 (8.3%)

Values are numbers (percentages) of events within the corresponding TSH category. Low TSH: <0.4 mU/L; normal TSH: 0.4–3.4 mU/L; high TSH: >3.4 mU/L. [a]The analyses with AF as outcome had prevalent AF (n=178) as an exclusion criterion, leaving 105, 4762 and 266 participants in the low, normal and high categories of TSH, respectively.

5.4.1 Total mortality

A total of 710 (13.6%) of the 5211 participants died during the follow-up period. Continuous TSH over the full range or within the reference range was not significantly associated with overall mortality in the crude or multivariable Cox models (Article IV, Supplementary Table S1 online). However, when transformed with an RCS function, TSH displayed a U-shaped association with total mortality in a multivariable adjusted model (P=0.01) (Article IV, Figure 2). In the crude models, we observed a greater risk of death among those who belonged to either the low (hazard ratio [HR] 2.03, 95% CI 1.37–3.00, P<0.001) or the high (HR 1.70, 95% CI 1.30–2.23, P<0.001) TSH category, compared with those with normal TSH. In the multivariable models, this finding persisted for the high TSH category (HR 1.34, 95% CI 1.02–1.76, P=0.04), but it was no longer present for the low TSH category (HR 1.24, 95% CI 0.83–1.85, P=0.29) (**Figure 7**; Article IV, Table 2). Further adjustment for prevalent cancer did not essentially alter the association between high TSH category and total mortality (data not shown).

5.4.2 Sudden cardiac death

A total of 84 (1.6%) of the 5211 participants died of SCD during the follow-up period. The high TSH category associated with a higher risk of SCD both in the crude (HR 2.32, 95% CI 1.16–4.64, *P*=0.02) and the multivariable (HR 2.28, 95% CI 1.13–4.60, *P*=0.02) Cox models, compared with those with normal TSH (**Figure 7**; Article IV, Table 2). Further adjustment of the multivariable model for prevalent cancer did not essentially attenuate the relationship between high TSH category and SCD (data not shown). In contrast, the low TSH category did not associate significantly with the higher risk in the crude or multivariable models (**Figure 7**; Article IV, Table 2). Likewise, differences in continuous TSH over the full range or within the reference range were not significantly associated with the higher risk in the crude or multivariable models (Article IV, Supplementary Table S1 online). An RCS-transformed TSH variable did not associate with the risk of SCD in a multivariable-adjusted analysis (*P*=0.70) (Article IV, Figure 2). In the fully adjusted multiple linear regression models, neither high nor low TSH, as compared with normal TSH, was significantly associated with corrected QT interval duration (data not shown).

5.4.3 Incident cardiovascular disease, major coronary heart disease events, stroke and major adverse cardiac events

During the follow-up period, the rates of incident CHD, stroke, CVD and MACE were 7.7%, 4.5%, 11.4% and 15.5%, respectively, for the 5211 participants. In the crude and multivariable models, continuous TSH over the full range was not significantly associated with a greater risk of incident CHD, stroke, CVD or MACE. The results were similarly statistically nonsignificant when the analyses were restricted to individuals with TSH within the reference range (Article IV, Supplementary Table S1 online). In the crude models, the low TSH category associated with a greater risk of incident CVD (HR 1.91, 95% CI 1.23–2.95, *P*=0.004), CHD (HR 2.15, 95% CI 1.30–3.55, *P*=0.003), MACE (HR 1.76, 95% CI 1.19–2.60, *P*=0.005) and stroke (HR 2.09, 95% CI 1.07–4.08, *P*=0.03), compared with the normal TSH category. In the crude analyses, the high TSH category associated with a greater risk of MACE (HR 1.40, 95% CI 1.06–1.84, *P*=0.02) but not significantly with the risk of CHD, stroke or CVD. In the multivariable models, none of the noted statistically significant associations between the categorised TSH variables and the outcomes persisted (**Figure 7**; Article IV, Table 2). An RCS-transformed TSH variable did not associate significantly with the risks of CHD, stroke, CVD and MACE in multivariable-adjusted analyses (Article IV, Figure 2).

5.4.4 Atrial fibrillation

Of those participants without prevalent AF at baseline (n=5133), a total of 328 (6.4%) developed AF during the follow-up period. In the crude model, low TSH associated with a greater risk of AF (HR 2.45, 95% CI 1.46–4.11, $P<0.001$), compared to having a normal TSH level, but this association was no longer observed in the multivariable Cox model (HR 1.54, 95% CI 0.91–2.61, $P=0.10$) (**Figure 7**; Article IV, Table 2). Neither the high TSH category (**Figure 7**; Article IV, Table 2) nor continuous TSH over the full range/within the reference range (Article IV, Supplementary Table S1 online) was significantly associated with the risk of AF in the crude and multivariable models. Stratification by gender did not change the results statistically significantly in the multivariable model that was restricted to the TSH reference range and had incident AF as an outcome (data not shown). In a fully adjusted regression model, neither high nor low TSH values, when compared to normal TSH, was significantly associated with P-wave duration (data not shown). An RCS-transformed TSH variable did not associate with the risk of incident AF ($P=0.75$) (Article IV, Figure 2).

5.4.5 Subclinical thyroid dysfunction as a risk factor

In a fully adjusted model, subclinical hypothyroidism associated with a greater risk of SCD (HR 2.33, 95% CI 1.16–4.70, $P=0.02$) but not significantly with any other outcomes, as compared with euthyroidism (**Figure 8**; Article IV, Table 3). Further adjustment for prevalent cancer did not essentially attenuate the association with an increased risk of SCD (data not shown). Subclinical hyperthyroidism, in turn, did not associate significantly with any of the outcomes (**Figure 8**; Article IV, Table 3).

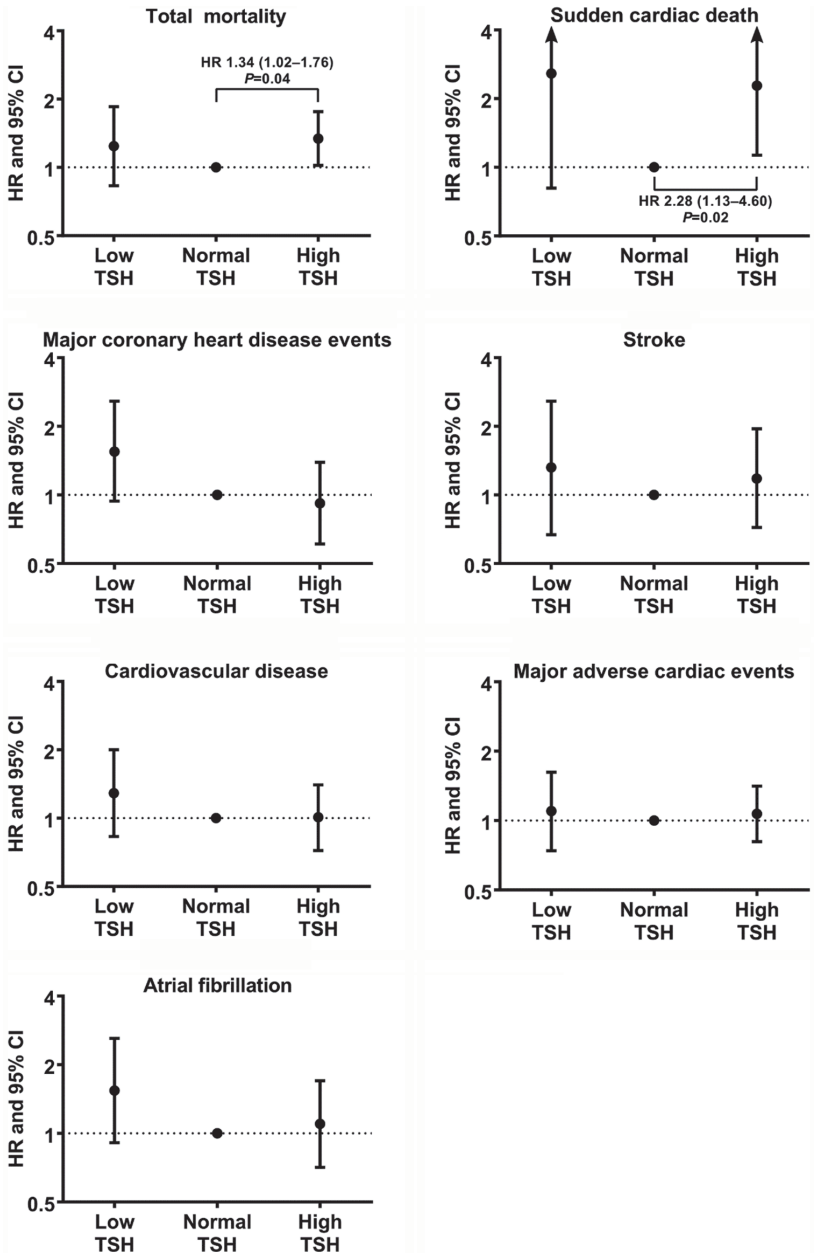


Figure 7 Adjusted hazard ratios (with 95% confidence intervals) of total mortality, sudden cardiac death, major coronary heart disease events, stroke, cardiovascular disease, major adverse cardiovascular events and atrial fibrillation by TSH category. Low TSH: < 0.4 mU/L (n=108); normal TSH: 0.4–3.4 mU/L (n=4834); high TSH: >3.4 mU/L (n=269). All depicted models were adjusted for baseline age, gender, smoking, diabetes, systolic BP and serum total and HDL cholesterol. The analysis that had AF as the outcome had prevalent AF (n=178) as an exclusion criterion and consisted of 105, 4762 and 266 participants in the low, normal and high categories of TSH, respectively.

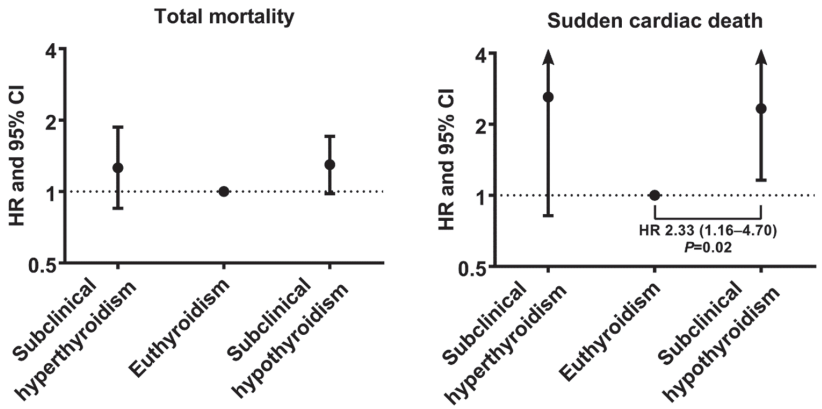


Figure 8 Adjusted hazard ratios (with 95% confidence intervals) of total mortality and sudden cardiac death by thyroid status. Subclinical hyperthyroidism: TSH<0.4 mU/L and FT4 9.0–19.0 pmol/L (n=103); euthyroidism: TSH 0.4–3.4 mU/L (n=4834); subclinical hypothyroidism: TSH>3.4 mU/L and FT4 9.0–19.0 pmol/L (n=260). Both depicted models were adjusted for baseline age, gender, smoking, diabetes, systolic BP and serum total and HDL cholesterol.

6 DISCUSSION

6.1 Thyroid-stimulating hormone reference range and factors affecting it (I)

In Study I, we determined a new reference range of 0.4–3.4 mU/L for TSH on the Abbott Architect ci8200 integrated system in a population-based sample of adults that we had first screened rigorously for thyroid diseases.

Among the other large population-based studies with probability-based samplings, the HUNT study established an upper TSH limit of 3.6 mU/L (219), which is rather similar with ours. The upper limit has been somewhat higher in studies conducted in the Netherlands (4.7 mU/L in the Nijmegen Biomedical Study) (75), the United States (4.1 mU/L in the NHANES III) (82) and Australia (4.0 mU/L in the Busselton Health Study) (74). We approximated that the mean TSH lower limit of these studies was 0.4 mU/L, with very little inter-study variation of approximately 0.2 mU/L (74,75,82). Thus, compared with the TSH lower limit, the upper limit appears to have varied more noticeably between these studies and seems to be more susceptible to the influence of unscreened factors, such as the regional iodine status. It is noteworthy that the Nijmegen Biomedical Study originated from a cohort with a longstanding borderline sufficient iodine intake (75), and, as stated above, the upper limit of the Dutch study was over 1.0 mU/L higher than that of the Nordic studies (75,83,219). The iodine intake has been sufficient in Norway (223) and also up till recent years in Finland (224,225). According to urine iodine concentration measurements conducted in 2012, the population iodine status of Finland seems to have now shifted to mild deficiency (226). Another study based on a Finnish cohort, albeit with a non-random sampling method, yielded the same upper limit as our study (67). In addition to the iodine status of the study cohorts, there are also other factors that could hinder a direct comparison between the results of these studies, such as differences in sampling methods, laboratory assays and screening for thyroid disease. Although the TSH assay sensitivity has evolved substantially over the last decades (5), there is clear inter-method variability (61), and it would therefore seem justified to implement not only population- but also assay-specific TSH reference ranges (227).

An additional aim of our study was to assess the effects of TPOAb-status, medications, gender and age on the TSH reference range. It was noted that the exclusion of TPOAb-positive participants decreased the TSH upper limit substantially from 4.4 mU/L to 3.7 mU/L. A similar finding has been made in some previous publications (66,67), although in others, the difference has not been as evident (69,228,229). In another Finnish study, the effect of TPOAb-positivity on

the TSH reference range was obvious only in women, which is an interesting finding concerning the overrepresentation of women when one assesses the prevalence of many autoimmune diseases (230). Ultimately, the effect of exclusion of the TPOAb-positive participants is dependent also on the cut-off value that is used to define an abnormal TPOAb test result. In our study, TPOAb values exceeding 5.6 IU/mL were considered abnormal, which was according to the instructions issued by the manufacturer of the assay.

Jensen et al. detected no effect of medications on the TSH reference range in their study (231). The exclusion of participants with medications that could potentially affect thyroid function reduced the TSH upper limit by a mere 0.1 mU/L in our own study. However, the exclusion due to use of any medications reduced the upper limit somewhat more substantially, by 0.3 mU/L. We speculate that the exclusion based on medications not only eradicates the effects of drugs on thyroid function but also screens for significant comorbidities that could affect the thyroid function test values.

Here, the TSH reference limits differed slightly between the gender subgroups. For women, the TSH lower limit was 0.4 mU/L and the upper limit 3.2 mU/L, whereas for men, these limits were 0.1 mU/L and 0.3 mU/L higher, respectively. Gender has had a similar detectable but trivial effect on the TSH reference range in some previous studies (66,232), whereas in others, it has been even less noticeable (82,228,229,231).

We also found differences in the TSH reference limits between the subgroups divided by age. Both the lower and upper limit were at their highest among the middle-aged, whose age-specific TSH reference range was 0.4–4.1 mU/L. Compared with the middle-aged subjects, the lower limit was 0.1 mU/L lower for both the younger and older adults, and the corresponding upper limit was 1.0 mU/L lower for the younger adults and 0.7 mU/L lower for the older adults. Also the median TSH concentration was at its highest among the middle-aged. In contrast to our results, in many previous studies (66,72,82,228,233), though not in all (67,74,75), TSH levels have increased with age.

In spite of these differences in the subgroup analyses, there were no apparent differences between the TSH distribution curves of the genders or the subgroups of age. Taking into account also the clinician's perspective, we do not think that these slight differences between the subgroups in our study were sufficiently extreme to justify the strain of using age- and gender-specific reference ranges for TSH in Finland on this assay platform.

Notwithstanding the stringent screening that we undertook, noticeable tailing remained at the upper end of the TSH distribution curves of the subgroups

according to both gender and age. Our results do not appear to corroborate the theory proposed by Surks et al. (72), according to which age-specific TSH reference ranges could mitigate the tailing of TSH. During the baseline phase of our study in 2000, Finland was ethnically rather homogenous (234); thus it may be assumed that the lack of race-specific strata cannot explain the tailing of TSH in our cohort (73). Taken as a whole, we cannot exclude the possibility that residual subclinical hypothyroidism is responsible for the tailing in this stringently screened reference population of our study. With reference to this potential problem, there has been some debate on the "true" TSH upper limit, and some authors have suggested that an upper limit of 2.5 mU/L could better represent a legitimate reference population without any emerging hypothyroidism (5,9). However, we are not in agreement with that proposition; if accepted, it would introduce a clear disadvantage, i.e. the overdiagnosis and overtreatment of hypothyroidism (10,11). Subsequently, according to a literature search performed in PubMed, the debate on the TSH upper limit seems to have abated somewhat. As if in putting an end to the debate, both a German (69) and an American (235) study that both applied rigorous screening for thyroid disorders, including a thyroid ultrasound examination, established TSH upper limits of 3.8 mU/L and 4.1 mU/L, respectively, i.e. not dramatically different from the present upper limit, which is between 4.0–5.0 mU/L in most clinics (11).

6.2 Association of thyroid-stimulating hormone with blood pressure (II)

Study II revealed a cross-sectional association between continuous levels of TSH and diastolic BP, but this finding was not consistent in both genders. In men, every 1 mU/L higher TSH level was associated with a 0.5 mmHg higher diastolic BP, but the corresponding association was not statistically significant in women. An additional cross-sectional finding of Study II was that having a high normal TSH concentration, as denoted by values within the highest tertile of the TSH reference range, was associated with 1.2-fold elevated odds of having prevalent hypertension as compared with individuals who had a low normal TSH value. However, higher baseline continuous levels of TSH were not longitudinally associated with higher future BP or incident hypertension over the 11-year follow-up period. Conversely, there was an unexpected inverse association between baseline TSH levels and the 11-year change in both systolic and diastolic BP in men.

As discussed earlier in this dissertation, there are several plausible reasons to explain why there could be an association between low concentrations of thyroid hormone and elevated BP levels. According to some investigators, thyroid

hormone can induce a relaxation of vascular smooth muscle (236) and it is inversely related to smooth muscle cell apoptosis (89) and vascular calcification (87,88). In contrast, hypothyroidism has been associated with arterial stiffness (93,94) and endothelial dysfunction (91,92).

Overt hypothyroidism was linked to prevalent hypertension already in the 1980's in smaller studies that employed non-random sampling methods (17,18). Subsequently, higher values of TSH, even in euthyroid participants, have been associated with higher BP outcomes in some studies (14–16). In study II, high normal values of TSH were associated with prevalent hypertension. In addition, other large population-based studies have yielded essentially similar results in cross-sectional analyses (14–16,19). In contrast, a moderately-sized Chinese study observed no differences in BP across the tertiles of normal TSH values (237).

Interestingly, the presence of abnormally high TSH values, i.e. exceeding 3.4 mU/L, was not associated with prevalent hypertension in Study II. Ittermann et al. detected a similar finding in a meta-analysis that examined 17,023 participants in the cross-sectional and 10,048 participants in the longitudinal analyses (19). In agreement with the results of Ittermann et al. (19), we could not confirm in a longitudinal setting, the modest cross-sectional associations that we had found between higher TSH and higher BP at baseline. Our results differ somewhat from those of the Norwegian HUNT study, which investigated exclusively euthyroid participants. The Norwegian researchers detected a positive association, albeit only in women, between higher baseline TSH values and future BP values over an 11-year follow-up period. The effects were only modest in their fully adjusted model: in women, every 1 mU/L higher baseline TSH value was associated with a 0.6 mmHg higher systolic and a 0.3 mmHg higher diastolic BP at the follow-up. In Study II, the corresponding longitudinal associations did point in the same direction in women as in the HUNT study but they did not achieve statistical difference, perhaps partially owing to the fact that our female study population was five times smaller than that in the Norwegian study.

Interestingly, the HUNT investigators also detected a direct relationship between the 11-year changes in TSH levels and BP. Thus, for every 1 mU/L increase of TSH during the follow-up period, systolic BP increased by 1.8–2.2 mmHg, depending on the gender, and diastolic BP correspondingly by 1.1–1.6 mmHg. However, it is unlikely that this finding would have been due to emerging hypothyroidism because the exclusion of TPOAb-positive participants with high normal TSH and low FT4 did not alter their results (20). In good agreement with the HUNT study, TSH and BP covaried over a follow-up period of five years also in a Chinese study that examined euthyroid participants, although the finding was statistically significant only in women (21). We were unable to replicate these

analyses in our own study because TSH concentrations were not measured at follow-up.

To summarise Study II, baseline TSH levels did not associate with higher future BP readings or incident hypertension. Thus, it seems unlikely that minor hypothyroid disorders could independently initiate a pathogenesis that would ultimately lead to incident hypertension.

6.3 Association of thyroid-stimulating hormone with lipid concentrations (III)

In Study III, we detected a cross-sectional association between higher continuous levels of TSH over the full range and an adverse lipid profile. Thus, for instance, a 1 mU/L higher baseline TSH value associated with a 0.02 mmol/L higher baseline LDL cholesterol concentration. The results were essentially similar for the models with total cholesterol, apolipoprotein B and triglycerides, i.e. other biomarkers that associate with atherogenesis (220,238). These associations were similar even within the reference range of TSH. In the analyses with a categorised TSH variable, an abnormally high TSH, which was denoted by values higher than 3.4 mU/L, was associated with an unfavourable lipid profile in comparison with euthyroid participants. Conversely, an abnormally low TSH, which was denoted by values lower than 0.4 mU/L, was associated with a more favourable lipid profile. Thus, these overall findings corroborate the proposition that TSH has no cutoff threshold with respect to its relationships with these lipid biomarkers (239), at least in a cross-sectional research setting.

In women, the initial longitudinal analyses revealed an association between higher continuous baseline values of TSH and an unfavourable lipid profile at the 11-year follow-up. In addition, in the longitudinal analysis, belonging to the category of abnormally high baseline TSH values was associated with a 0.2 mmol/L higher future LDL cholesterol concentration in all participants. In an effort to clarify the possible cause-effect relationship between low thyroid function and adverse future lipid values, we proceeded to exclude those subjects with high-risk lipid values at baseline (220). Interestingly, after this exclusion, none of the mentioned longitudinal relations remained statistically significant.

A cross-sectional association between low thyroid function and an adverse lipid profile has been detected in several earlier studies. In the Colorado Thyroid Disease Prevalence Study, the mean total and LDL cholesterol levels were significantly higher in those individuals with abnormally high TSH values than in euthyroid participants (7). In agreement with our own results, the EPIC-Norfolk

(14) and HUNT (130) studies were able to detect an association between higher levels of TSH and a poorer lipid profile also in euthyroid individuals. Similar findings have been detected in analyses involving the whole range of TSH in a Spanish study (131), in a selected population of healthy Koreans (240) and in adolescents in a German study (132). Early case series that have demonstrated beneficial effects of thyroid hormone replacement therapy on lipid concentrations in hypothyroid patients also concur with these findings (127–129). As described earlier in this dissertation, there is also considerable accumulated molecular evidence linking hypothyroidism with an impaired lipid homeostasis (99,100,103,104,106).

The most important aim of Study III was to quantify how TSH levels measured at one single time point would associate with future lipid concentrations measured 11 years later. The ultimate rationale for these analyses was to investigate whether even subtle elevations in the levels of TSH could be a harbinger of an elevated risk of CVD and an indication of thyroid hormone replacement therapy. To juxtapose this consideration with clinical studies, statin trials have demonstrated that for every 1 mmol/L reduction in LDL cholesterol concentration, there is a subsequent 20% reduction in the risk of major vascular events (98). However, the associations that we found between TSH and lipid concentrations were modest, even in the cross-sectional analyses. As far as we are aware, the Norwegian HUNT study is the only population-based study that has addressed these issues previously in a longitudinal setting. In agreement with our results, the Norwegian researchers also found only modest associations between these variables. In the HUNT study, continuous baseline levels of TSH associated inversely with the follow-up levels of HDL cholesterol, and in men, also directly with the follow-up triglyceride and non-HDL cholesterol concentrations. In addition, the Norwegian investigators detected an association between the 5-year change in TSH values and 5-year change in triglyceride and non-HDL cholesterol concentrations. However, emerging hypothyroidism is an unlikely cause for these covariations, because the results remained unchanged when those with TPOAb-positivity coupled with a high normal TSH value and a low FT4 concentration were excluded (20).

When our analyses were restricted to the euthyroid participants, higher baseline TSH levels were unexpectedly associated with favourable future changes in lipid profile in men. This result resembles our parallel findings in Study II. In addition, in Study III, there was a direct association between baseline TSH levels and future HDL cholesterol concentrations in euthyroid women, which also was unexpected. We can speculate that at least two important reasons could explain these results. First, subclinical hypothyroidism may resolve in up to 52% of the cases where there is only a moderate increase of TSH level (241). We hypothesise that these transient disorders could contribute to unexpected beta coefficients observed in the

regression analyses. Second, autoimmune diseases are far more common in women than in men (230). Thus, the possible transient fluctuations in the TSH levels in our male subjects in Studies II and III could have been non-thyroid-related but could have nonetheless created an impression that there was a longitudinal association. Whatever the reason, these findings do not support the concept of providing thyroid hormone replacement therapy to individuals with high normal TSH values.

To summarise study III, we found a modest cross-sectional association between higher levels of TSH and an adverse lipid profile, but we could not confirm this relationship indisputably in the longitudinal setting. Thus, our study does not provide evidence that a minor elevation in TSH levels would translate into any substantially elevated cardiovascular risk via an alteration in the lipid profile.

6.4 Association of thyroid-stimulating hormone with the risk of sudden cardiac death, total mortality and cardiovascular morbidity (IV)

Study IV revealed that, over a follow-up period of 13 years, those with a TSH level above the reference range had a greater risk of both SCD and death from any cause than euthyroid participants. Further adjustment of the multivariable models for cancer did not alter these associations. In the additional analyses, even those with subclinical hypothyroidism appeared to have a greater risk of SCD than euthyroid participants. We also found a U-shaped association between an RCS-transformed TSH variable and the risk of total mortality. However, we could not link abnormally high levels of TSH with incident CVD, stroke, CHD, AF or MACE. We could also not detect a cross-sectional link between baseline TSH and QT interval duration.

Among other mediators for the possible association between hypothyroid states and the risk of SCD is cardiac fibrosis. Almost all of the cardiac conditions which are associated with an increased risk of SCD feature cardiac fibrosis as a common finding (242). Hypothyroidism, in turn, could be linked with increased cardiac fibrosis (243). Previously, only Chaker et al. have investigated the association between thyroid function and SCD in a population-based study setting. Chaker et al. detected no association between a categorised TSH variable and SCD, but they found that higher levels of FT4 were associated with a higher risk of SCD. Their results cannot be directly compared with ours because Chaker et al. had categorised their TSH concentrations into evenly distributed tertiles, whereas we compared abnormally low or high levels of TSH with euthyroidism. Chaker et al. also defined SCD by more stringent criteria than those used in this thesis, as the

timespan of a witnessed stable medical condition to the SCD was limited to a maximum of 24 hours in their study (27). Based on the results of these two studies, both hyper- and hypothyroidism could be associated with an increased risk of SCD, but more studies will be needed to confirm these relationships.

Our results share some similarities with those of the Nagasaki (31) and HUNT studies (25). These two studies did detect a link between high levels of TSH and a higher risk of mortality but could not determine the underlying mechanisms with certainty. Cappola et al., in turn, did not detect any association at all between high levels of TSH and mortality (26), not even in cases of persistent subclinical hypothyroidism (30). Higher TSH levels even appeared to be a protective factor against all-cause mortality in a Dutch study that examined octogenarians (29).

As we observed a U-shaped association of an RCS-transformed TSH variable and the risk of total mortality, our results suggest that not only hypo- but also hyperthyroid states could associate with worse overall survival. Our findings are in good agreement with those of several earlier studies which have linked low levels of TSH with an increased risk of death due to any (244) or a cardiovascular reason (28,202–204,245). By contrast, a German population-based study failed to detect an association between decreased levels of TSH and mortality (32).

Several previous studies have associated subclinical hypothyroidism with cardiovascular morbidity (34,138,139,200). In a series of studies examining individuals with subclinical hypothyroidism, Rodondi et al. linked TSH levels above 7.0 mU/L to a greater risk of congestive heart failure (162) and levels exceeding 10 mU/L to a greater risk of CVD events and CVD mortality (34). Cohort studies conducted in Australia (139) and United Kingdom (138) and also a meta-analysis carried out by the Thyroid Studies Collaboration (163) revealed results that were in good agreement with those of Rodondi et al. (34,162). With reference to a possible relationship between hypothyroid states and stroke, the epidemiological evidence for this association remains conflicting (31,199–201). We visually observed a U-shaped relation between an RCS-transformed TSH variable and the risk of stroke in a graph, but the finding was not statistically significant.

Subclinical hyperthyroidism, in turn, has been associated with an increased risk of nonfatal cardiovascular morbidity in a British population record-linkage study (206). The Thyroid Studies Collaboration has also detected an association between subclinical hyperthyroidism and an increased risk of heart failure events in an individual-level meta-analysis (163). In our study, low levels of TSH were not associated with any non-fatal cardiovascular outcomes. However, in a crude analysis, having an abnormally low level of TSH was strongly associated with incident AF, and thus it is possible that an over-adjustment attenuated this relationship in the multivariable model. Several previous studies have linked even

subclinical hyperthyroidism to an elevated risk of AF (26,28,206), but it has been estimated that less than one percent of new cases of AF are caused by overt hyperthyroidism (246). According to the guidelines for the management of AF issued by the European Society of Cardiology, a new finding of AF is still always an indication to perform thyroid function testing (171).

To summarise Study IV, we found a U-shaped association between TSH and total mortality. We also detected an association between even subclinical hypothyroidism and the risk of SCD. However, we could not determine an underlying mechanism for these findings. In addition, higher TSH levels did not have a linear association with mortality or cardiovascular morbidity in euthyroid participants. According to all of the current epidemiological evidence, even subclinical hyperthyroidism seems to be associated with the risk of AF. Subclinical hypothyroidism, in turn, is likely to be associated with an increased risk of atherosclerotic CVDs, but the risk seems to be evident only at higher levels of TSH, and the cut-off value when the hazard becomes significant may be as high as 10 mU/L according to Rodondi et al. (34). Sufficiently sized randomised clinical trials to resolve these issues have yet to be conducted and would be much-needed. Meanwhile, the findings of this present work and those emerging by epidemiological studies conducted by others have found no evidence for any link between slight increases in TSH levels and an elevated cardiovascular risk.

6.5 Study strengths

The most important strength of our study was the large nationwide stratified sample, which enables us to better generalise our results to the Finnish adult population. The high-quality study material which we used can be seen to represent the whole Finnish adult population exceptionally well (208). In addition, Studies II-IV had prospective designs, which serve better for the clarification of cause-effect relationships than cross-sectional approaches. There was also a good coverage of mortality and inpatient events of CVD in Study IV.

6.6 Study limitations

6.6.1 Study I

Study I had several limitations. First, we opted to rely on a TPOAb reference range that was provided by the manufacturer of the assay kit rather than establishing the

reference range for ourselves. Second, we had no data on the participants' TgAb levels or on their family history of thyroid diseases. With reference to TgAb, TPOAb levels seem more useful in detecting thyroid disorders (231,247). Third, we had partially missing data for TPOAb levels in euthyroid participants and therefore could not completely adhere to the NACB guidelines on how to establish a TSH reference range (5).

6.6.2 Studies II-IV

Studies II-IV shared some common limitations. The most important limitation was the lack of follow-up TSH levels. Thus, we could not determine the natural course of possible emerging thyroid disorders in the participants. In addition, we defined thyroid function solely according to the TSH concentration, which is not a totally reliable method in cases of central thyroid dysfunction; however, these are believed to be rare (248,249). In Study III, we excluded participants with antithyroid, lipid-lowering or thyroid hormone replacement medication. As a result, the size of the follow-up population was approximately half of the size of the baseline population, which reduced the representativeness of our results to the general population. To avoid the same problem in Study II, we added fixed increments to the BP values of participants with hypertension medication, which, in turn, introduced uncertainty to the results of that study. In Study III, LDL cholesterol was calculated with the Friedewald equation, which becomes increasingly inaccurate when the triglyceride concentration exceeds 2.3 mmol/L (220). The AACE Lipid and Atherosclerosis Guidelines recommend that the Friedewald equation should be substituted with a more precise method when the triglyceride concentration exceeds 2.8 mmol/L (220). In Study III, the triglyceride concentration was greater than 2.8 mmol/L in 5.1% and 4.3% of the measurements at baseline and follow-up, respectively. We argue that this limitation is inherent to epidemiological studies that utilise the Friedewald equation. In Study IV, the coverage of diseases was based solely on data from secondary and tertiary care, which is a limitation that concerns mainly AF. With the exception of AF, we believe that other forms of cardiovascular morbidity are more completely covered in inpatient registers. Another limitation of Study IV was the small number of SCDs, which increases the risk that the detected association between high TSH and SCD was due to a statistical quirk of chance.

7 CONCLUSIONS

Based on the results of this and the previous studies on this topic, the following conclusions can be drawn:

1. For the Finnish population, we propose a new TSH reference range of 0.4–3.4 mU/L to be used on the Abbott Architect ci8200 platform. In all of the usual forms of hypo- and hyperthyroidism, initiation of thyroid therapy should never rely solely on a biochemical deviation from this range but rather on a careful consideration of all clinical findings and the recommendations given in concurrent international guidelines on thyroid disorders.

2. Thyroid function may have a relationship with BP but the association appears to be modest, at best. Thus, it is unlikely that minor forms of hypothyroidism would independently lead to the development of hypertension.

3. It is universally accepted in the literature that thyroid function has a cause-effect relationship with lipid homeostasis, but minor forms of hypothyroidism are not associated with a substantially poorer lipid profile and are unlikely to elevate cardiovascular risk via that mechanism.

4. Accumulated epidemiological evidence links both hyper- and hypothyroidism to adverse cardiovascular events and outcomes, including an increased risk of total mortality and possibly SCD. However, the risk appears to be evident only in pronounced forms of thyroid dysfunction. Sufficiently powered randomised controlled trials are still much-needed to determine precise cut-offs for different thyroid therapies, including thyroid hormone replacement medication in subclinical hypothyroidism.

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