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Thyroid Function within the Reference Range and the Risk of Stroke: An Individual Participant Data Analysis

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Abstract

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

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

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

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

Introduction

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

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

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

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

Material and methods

Data Sources and Study Selection

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

Data Extraction

We requested individual participant characteristics related to prior cardiovascular risk factors and disease, including systolic blood pressure, serum total cholesterol, history of diabetes, smoking, previous cardiovascular disease and previous stroke. We also collected available information on demographic information (age, sex, race), anthropometric measurements (height and weight), medication use (thyroid hormone replacement, lipid-lowering and anti-hypertensive therapy) and the outcome. Individual participant information from all cohorts were collected and analyzed in one center (Rotterdam, The Netherlands). The primary outcome measures were all stroke (combined fatal and non-fatal) and fatal stroke. Stroke was defined according to World Health Organization (WHO) criteria as a syndrome of rapidly developing clinical signs of focal (or global) disturbance of cerebral function, with symptoms lasting 24 hours or longer or leading to death, with no apparent cause other than of vascular origin, including ischemic or hemorrhagic strokes.

Thyroid Function Testing Definition

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

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

Data synthesis and Statistical Analysis

We performed a Cox proportional hazards model in each cohort separately to assess the association of TSH or FT4 continuously with all stroke and fatal stroke (IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp). We investigated the linearity assumption using cubic restricted splines (rms package, R-project, Institute for Statistics and Mathematics, R Core Team (2013), Vienna, Austria, version 3.0.2). Due to departure from linearity for the TSH analysis in the 4D cohort (p for non-linearity = 0.03), TSH was log transformed for all continuous analyses (natural logarithm). We found no departure from non-linearity in the transformed TSH or any of the FT4 analyses and no threshold effect was therefore detected. The analyses are presented as Hazard Ratios (HR) across the reference range of TSH (0.45-4.49 mIU/L). This corresponds to the HR when comparing participants with a TSH in the upper limit of the reference range (4.49 mIU/L) to participants with a TSH in the lower limit of the reference range (0.45 mIU/L). The FT4 analyses were performed in a standardized manner (per SD) as well as per 1 ng/dL increase, for which the Whickham study (27) was excluded. We assessed the proportional hazard assumption in each cohort for each outcome, by Schoenfeld residual plots and the Schoenfeld test. All studies met the proportional hazard assumption except for the Birmingham study and PROSPER trial for the analyses with TSH, for which we performed a sensitivity analysis excluding these two cohorts. There was no interaction between FT4 and TSH levels for the all stroke events or stroke mortality analyses ($p=0.099$ and $p = 0.28$ respectively), as assessed by introducing an interaction term between FT4 (ng/dL) and TSH values.

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

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

In order to evaluate the robustness of our findings and identify possible sources of heterogeneity and populations at risk, we conducted pre-defined subgroup and sensitivity analyses. We performed stratified analyses by age, sex, history of stroke, subtype of stroke (including only classified strokes) and race, in concordance with previous reports (1,4). If the parameter estimates were infinite due to a small number of events in a stratified study-specific analysis, we used Firth's penalized maximum likelihood bias reduction method for the Cox proportional hazards model (31,32) to estimate hazard ratios (HRs) and 95% CIs. For the continuous TSH analyses, we conducted the following sensitivity analyses: 1) excluding participants who had thyroid-hormone replacement at baseline and during follow-up 2) excluding studies that included transient ischemic attack as a stroke event 3) excluding studies with self-reported stroke data 4) excluding studies that did not meet the proportional hazard assumption 5) excluding cohorts with potential co-morbidities (e.g. diabetes patients) and 6) excluding studies without formal adjudication procedures. We also conducted additional multivariable analyses including prevalent atrial fibrillation, prevalent cardiovascular disease, body mass index (BMI) or lipid-lowering, and anti-hypertensive therapy at baseline to the previous multivariable model. Furthermore, we performed the following methodological sensitivity analyses: 1) perform the meta-analysis in a two-step approach using the restricted maximum-likelihood estimator also using the metafor package and 2) calculate the risk estimates using a one-step frailty Cox proportional hazards model (coxme package, R-project, Institute for Statistics and Mathematics, R Core Team (2013), Vienna, Austria, version 3.0.2.) We assessed age- and sex-adjusted funnel plots and conducted Egger tests (33) to evaluate potential publication bias statistically. There was no specific funding for this study.

Results

We identified 17 cohorts from the United States (17,21,34), Europe (18,20,24,27,28,35-40), Australia (25), Brazil (41) and Japan (19) that assessed stroke outcomes prospectively and agreed to share IPD (**Table 1**). The included studies provided information on a total of 43,598 participants with thyroid function within the reference range and a follow-up from 1972 to 2014, a median follow-up ranging between 1.5 and 20 years and a total follow-up of 450,684 person-years. All studies, except one (34), included both female (49.6%) and male participants. All cohorts reported fatal stroke and 12 studies reported both fatal and non-fatal stroke, contributing to the all stroke analyses among 34,853 participants. During follow-up, 2271 participants had a stroke, with an incidence rate of 6.3 per 1000 person-years and 907 a fatal stroke with 2.0 per 1000 person-years. The FT4 analyses included 24,888 participants for all stroke and 32,580 for fatal stroke. Two studies (25,39) used variations of the WHO criteria to define all stroke and fatal stroke (**Supplemental Table 1**) and four studies included information on type of stroke (hemorrhagic versus ischemic) (17,21,28,40). One study (39) used questionnaires for the assessment of non-fatal stroke. Formal adjudication, defined as having clear criteria for the outcomes that were reviewed by experts for each potential case, was used for all stroke in six studies (17,21,28,36,42,43) and for fatal stroke in two additional studies (34,38).

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

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

The association between TSH and the risk of stroke

The age- and sex-adjusted pooled HR for all stroke was 0.78 (95% CI, 0.65-0.95, across the reference range of TSH mIU/L) and for fatal stroke 0.83 (95% CI, 0.62-1.09) (**Figure 1**). This corresponds to a 1.28-fold and 1.20-fold increase in all and fatal stroke risk respectively for a participant with a TSH in the lower limit of the reference range (0.45 mIU/L) compared to a participant with a TSH in the upper limit of the reference range (4.49 mIU/L).We found no heterogeneity for the analyses of all stroke or fatal stroke analyses (I^2=0%). Multivariable analyses, adjusting for sex, age, smoking, total cholesterol, systolic blood pressure and history of diabetes yielded similar results with a HR of 0.76 (95% CI, 0.63-0.91) for all stroke and 0.78 (95 % CI, 0.58-1.07) for fatal stroke (**Table 2**). Subsequent subgroup analyses did not show a differential risk when stratifying by sex, age groups, history of stroke or race (**Table 2**). The information on type of stroke was available in a subgroup of 11,192 participants in four studies (17,21,28,40). Stratifying by type of stroke showed a lower estimate in hemorrhagic fatal stroke compared to ischemic stroke (HR 0.37, 95% CI 0.12-1.12 vs HR 0.78, 95% CI 0.33-1.80), but with an insignificant p for interaction (p= 0.30). Sensitivity analyses excluding specific studies or participants using thyroid hormone replacement therapy did not meaningfully affect the risk estimates (**Supplemental Table 2**). Additional adjustment for prevalent atrial fibrillation, prevalent cardiovascular disease (defined as previous coronary heart disease or stroke), BMI or lipid-lowering and anti-hypertensive therapy did not attenuate the associations. Estimates derived by the methodological sensitivity analyses were similar to the results of the two-step random-effects model according to DerSimonian and Laird (**Supplemental Table 3**). We did not find any evidence of publication bias for the TSH analyses, either with visual assessment of age- and sex-adjusted funnel plots or with the Egger test for all stroke (p = 0.75) or fatal stroke (p = 0.29).

The association between FT4 and the risk of stroke

The age- and sex-adjusted pooled HR for the per SD increase of FT4 and stroke analyses were 1.08 (95% CI, 0.99-1.15) for all stroke and 1.10 (95% CI, 1.04-1.19) for fatal stroke (**Table 3, Figure 2**). We found substantial heterogeneity for the analyses on all stroke (I^2=55%) but no heterogeneity for fatal stroke (I^2=0%,). When analyzing the association per 1 ng/dL FT4 increase and risk of stroke, the age- and sex-adjusted pooled HRs were 1.40 (95% CI, 0.95-2.05) for all stroke and 1.44 (95% CI, 1.10-1.89) for fatal stroke (**Supplemental Table 4**). Multivariable analyses, adjusting for sex, age, smoking, total cholesterol, systolic blood pressure and history of diabetes did not change risk estimates substantially (**Table 3**). Subsequent subgroup analyses showed a differential risk for the different age categories, where the risk estimates went from protective to deleterious with increasing age (p for trend 0.024, **Table 3**). When stratifying by sex, history of stroke or race no differential effects were detected. Stratifying for type of stroke also did not show differential risk (**Table 3**), but this was only possible in one study that was included in the FT4 analyses. We did not find any evidence of publication bias for the FT4 and stroke analyses, either with visual assessment of age- and sex-adjusted funnel plots or with the Egger test for all stroke (p = 0.41) or for fatal stroke (p = 0.28).

Discussion

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

Thyroid dysfunction is defined by the biochemical reference ranges for TSH and FT4. These reference ranges, defining the normal range, depend on the assay used, the distribution of thyroid measurements in the population, or both. A thyroid function within the “normal range” would imply that the levels of circulating thyroid hormone are not accompanied by symptoms, an increased risk of disease or adverse events. In recent years, the applied reference ranges have been debated in the context of mainly the latter two: adverse events and diseases. Higher levels of TSH within the reference range are associated with an increase in systolic and diastolic blood pressure (44,45). Moreover, increased TSH levels within the reference range are linearly associated with an unfavorable serum lipid profile (46). On the other hand, lower TSH levels within the reference range are associated with an increased risk of heart failure, coronary heart disease and atrial fibrillation in an elderly population (7). The arbitrary nature of the cut-offs currently used is an important factor hampering decision making on screening and treatment of thyroid dysfunction (13). In the context of defining the reference range of thyroid function, our study provides additional evidence that lower levels of TSH and higher levels of FT4 within the reference range are associated with a negative clinical outcome, namely stroke, a major cause of morbidity and mortality.

In contrast to blood pressure or cholesterol, reference ranges for thyroid function are currently based on distribution in the population rather than risks of major diseases. It is more challenging to establish reference ranges for thyroid function based on risk of outcomes than for cardiovascular risk factors such as blood pressure and cholesterol, where the increase in risk mainly occurs for values higher than the upper limit. However, both low and high thyroid function is associated with clinical disease, also within the reference range. Furthermore, a previous study from the TSC provided no evidence for a higher risk of coronary heart disease within the normal reference range as currently defined (10). Also, thyroid function is not solely associated with cardiovascular disease but also a wide variety of clinical outcomes including fracture risk and possibly cognitive function decline (7,14). Therefore, future research should investigate if re-evaluation of the currently used reference ranges for thyroid function is meaningful, and if so, to what extent this should be done for specific populations or subgroups (e.g. elderly).

Several pathways could explain the relation between thyroid function and stroke. Thyroid hormone has direct effects on the cardiovascular system and is known to decrease systemic vascular resistance (47), increase left ventricular contractile function and alter systolic and diastolic cardiac function (48). Differences in thyroid hormone function are associated with the risk of several cardiovascular risk factors including hypertension, (49) dyslipidemia (50) and atherosclerosis (51). These changes have also been reported in subjects with subclinical thyroid dysfunction (42) and some also with differences of thyroid function within the reference range (44-46). The fact that adjustment for these cardiovascular risk factors in our multivariable analyses did not substantially alter risk estimates, suggests an effect on the risk of stroke, which is independent of classical risk factors such as hypertension.

Another explanation might be that the lack of effect of multivariable adjustment is due to residual confounding or unmeasured mediators. For example, in the current analysis, additional adjustment for atrial fibrillation, a plausible biological mediator for the association between thyroid function and the risk of stroke (52), did not alter risk estimates substantially. However, detecting an effect may have been hampered by the lack of information on prevalent atrial fibrillation in nine studies and insufficient incidence information. There was no sufficient information available on anti-coagulant medication use of participants, which did not allow for further exploration of possible mediating and confounding effects. Various abnormalities in the hemostatic system have been reported in overt (53) and subclinical thyroid dysfunction (54). Hypercoagulability is seen in hyperthyroidism while hypothyroidism has been associated with mainly hypocoagulability (55,56). Alterations in coagulability and the fibrinolytic system have been linked to a higher risk of cardiovascular disease (57). Whether hemostasis is also affected within the reference range of thyroid function is not known but might be one of the pathways that play a role in the increased risk of stroke associated with differences in thyroid function within the reference range. Changes in coagulation patterns due to thyroid hormone could imply that thyroid function tending towards hyperthyroidism might increase the risk of ischemic stroke mainly. We only had a small subgroup of studies including information on type of stroke (hemorrhagic vs ischemic), limiting our analysis on type of stroke. The exact mechanism explaining the association between differences in thyroid function within the reference range and the risk of stroke therefore remains to be determined.

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

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

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

Despite the large number of participants, we had limited numbers of events in those with a history of stroke and only four studies included data on type of stroke. Information needed for certain stratification and sensitivity analyses e.g. by race or prevalent atrial fibrillation was not available for some cohorts. Also, there was no information available on anti-coagulant use or anticoagulant factor levels, hampering analyses concerning possible underlying pathways. Furthermore, TSH and FT4 measurements were performed only at baseline and data on thyroid medication use during follow-up were not complete, which could change risk over time, in almost all cohorts and therefore it was not possible to take changes of thyroid function over time into account. Residual confounding cannot be excluded, as is the case in all observational studies.

Conclusions

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

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Legend of Figures

<u>Figure 1.</u> **The association between TSH and Risk of All Stroke and Fatal Stroke***

* Hazard ratios (HRs) and their 95% confidence intervals (CIs) are represented by squares and are across the range of TSH (0.45 and 4.49 mIU/L). Sizes of data markers are proportional to the inverse of the variance of the hazard ratios.

Data for all stroke were available in 12 studies. Three hundred ninety-three participants were excluded from the analysis of all stroke due to missing follow-up data. Data for fatal stroke were available in 17 studies. Two hundred sixty-five participants were excluded from the analysis of fatal stroke, due to missing cause of death.

Abbreviations: TSH = thyroid-stimulating hormone.

<u>Figure 2.</u> **The association between standardized FT4 and Risk of All Stroke and Fatal Stroke***

* Hazard ratios (HRs) and their 95% confidence intervals (CIs) are represented by squares and are per one increase of one standard deviation of FT4. Sizes of data markers are proportional to the inverse of the variance of the hazard ratios.

Data for all stroke were available in 9 studies. Three hundred eighty-seven participants were excluded from the analysis of all stroke due to missing follow-up data. Data for fatal stroke were available in 13 studies. Twenty-seven participants were excluded from the analysis of fatal stroke, due to missing cause of death.

Abbreviations: FT4 = free thyroxine

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

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

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

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

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

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

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

* Participants younger than 18 years of age were not included

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

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

§ 1 pmol/L is 0.0777 ng/dL

ll Excluded patients with acute coronary syndrome or severe illness

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

Table 2. Stratified Analyses for the Associations between TSH and the Risk of All Stroke and Fatal Stroke

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

Race‡‡	White	1430/19,037	**0.76 (0.60, 0.95)**	**0.73 (0.58, 0.91)**	0%	520/23,213	0.71 (0.48, 1.02)	0.67 (0.47 1.00)	0%
	Asian	NA	NA	NA		63/3230	0.48 (0.06, 11.22)	0.62 (0.10, 3.97)	41%
	Black	150/1090	0.85 (0.26, 2.78)	0.91 (0.41, 1.99)	47%	59/1055	0.95 (0.30, 3.12)	0.89 (0.26, 2.91)	0%
	p for interaction		0.88	0.60			0.83	0.91	

Abbreviations: CI, confidence interval; HR, hazard ratio; TSH, thyroid-stimulating hormone. The HR's are across the reference range of TSH mIU/L (0.45-4.49)

*Data were available from 12 studies, 393 participants were excluded due to missing stroke event data.

† 265 participants were excluded due to missing data on cause of death.

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

§ These analyses were not adjusted for sex.

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

¶ Information on history of stroke was not available for the Pisa cohort, Birmingham Study and Busselton Health Study. Data concerning history of stroke were missing for 64 participants in total.

** Information on type of stroke was available for the Cardiovascular Health Study, Health ABC Study, PROSPER and the Rotterdam Study.

‡‡Information on race was not available for the 4D study, Birmingham study. Busselton Health Study and EPIC-Norfolk Study. Excluded 96 participants from MrOs Study due to no events in subgroup.

Table 3. Stratified Analyses for the Associations between standardized FT4 and the Risk of All Stroke and Fatal Stroke*

		All Stroke†				**Fatal Stroke‡**			
		No. events/ Total participants	**Age and Sex adjusted HR (95% CI)**	**Multivariable § HR (95% CI)**	**I^2**	**No. events/ Total participants**	**Age and Sex adjusted HR (95% CI)**	**Multivariable § HR (95% CI)**	**I^2**
Total Population FT4 per SD		1307/24,888	1.08 (0.99, 1.17)	1.06 (0.99, 1.15)	55%	598/32,580	**1.10 (1.04, 1,19)**	**1.09 (1.02, 1.18)**	0%
Sex ‖	Men	639/11,848	1.02 (0.94, 1.11)	1.00 (0.92, 1.08)	0%	284/16,651	1.10 (0.99, 1.24)	1.08 (0.96, 1.21)	0%
	Women	668/13,040	1.10 (0.99, 1.22)	**1.10 (1.01, 1.20)**	52%	314/15,929	**1.12 (1.03, 1.23)**	**1.12 (1.01, 1.24)**	0%
	p for interaction		0.27	0.12			0.79	0.65	
Age¶	18 – 49y	59/8289	0.81 (0.61, 1.07)	0.75 (0.55, 1.03)	0%	12/9507	1.50 (0.62, 3.67)	0.93 (0.32, 2.71)	36%
	50 – 64y	342/9019	1.03 (0.93, 1.29)	1.03 (0.84, 1.27)	66%	99/11,929	1.09 (0.88, 1.35)	1.06 (0.84, 1.32)	0%
	65 -79y	759/6803	**1.12 (1.05, 1.19)**	**1.10 (1.04, 1.17)**	0%	376/9897	**1.11 (1.01, 1.22)**	1.09 (0.99, 1.21)	0%
	≥80	147/777	1.15 (0.98, 1.35)	1.15 (0.96, 1.38)	0%	111/1247	1.12 (0.94, 1.33)	1.09 (0.89, 1.33)	0%
	p for trend		0.024	0.015			0.54	0.76	
Stroke history**	No	1013/22,446	1.06 (0.95, 1.18)	1.05 (0.95, 1.15)	58%	472/27,256	**1.10 (1.02, 1.19)**	**1.09 (1.00, 1.18)**	0%
	Yes	104/483	1.11 (0.95, 1.29)	1.12 (0.95, 1.32)	0%	60/668	1.07 (0.78, 1.45)	1.15 (0.77, 1.73)	26%
	p for interaction		0.64	0.51			0.87	0.80	
Stroke type‡‡	Hemorrhagic	17/1577	1.37 (0.82-2.29)	1.15 (0.64-2.07)	NA	10/1577	1.15 (0.63-2.12)	1.01 (0.49, 2.07)	NA
	Ischemic	157/1577	**1.30 (1.14-1.47)**	**1.20 (1.06-1.37)**	NA	39/1577	1.00 (0.71-1.41)	0.90 (0.62-1.28)	NA
	p for interaction		0.84	0.88			0.70	0.77	

Race§§	White	617/10,208	1.12 (0.99, 1.26)	1.11 (0.99 1.23)	51%	319/14,528	**1.13 (1.03, 1.23)**	**1.10 (1.00 1.21)**	0%
	Asian	NA	NA	NA	NA	63/3228	1.27 (0.74, 2.18)	1.27 (0.74, 2.18)	58%
	Black	NA	NA	NA	NA	2/48	0.94 (0.23, 3.88)	1.00 (0.14, 7.09)	NA
	p for interaction		NA	NA			0.89	0.87	

Abbreviations: CI, confidence interval; HR, hazard ratio; FT4, free thyroxine. NA, not applicable. The HRs are per one increase in standard deviation of FT4.

*The Whickham Survey did not measure FT4 but total T4.

† Data were available from 12 studies, 384 participants were excluded due to missing stroke event data.

‡27 participants were excluded due to missing data on cause of death.

§ Adjusted for sex, age, systolic blood pressure, total cholesterol, smoking and prevalent diabetes at baseline.

|| These analyses were not adjusted for sex.

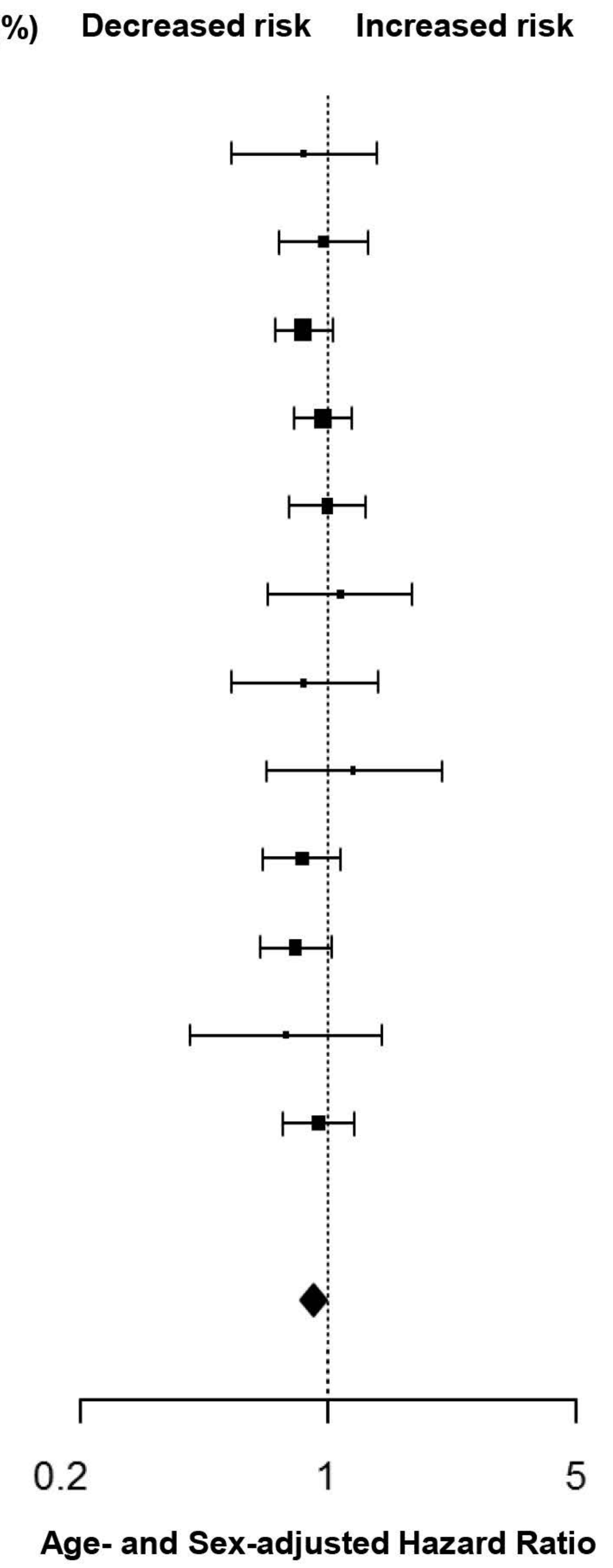
¶ These HRs were adjusted for sex and age as continuous variable to avoid residual confounding within age strata.

** Information on stroke history was not available for the Pisa cohort, Birmingham Study and Busselton Health Study. Data on stroke history were missing for 64 subjects.

‡‡ Information on type of stroke was available for the Rotterdam Study.

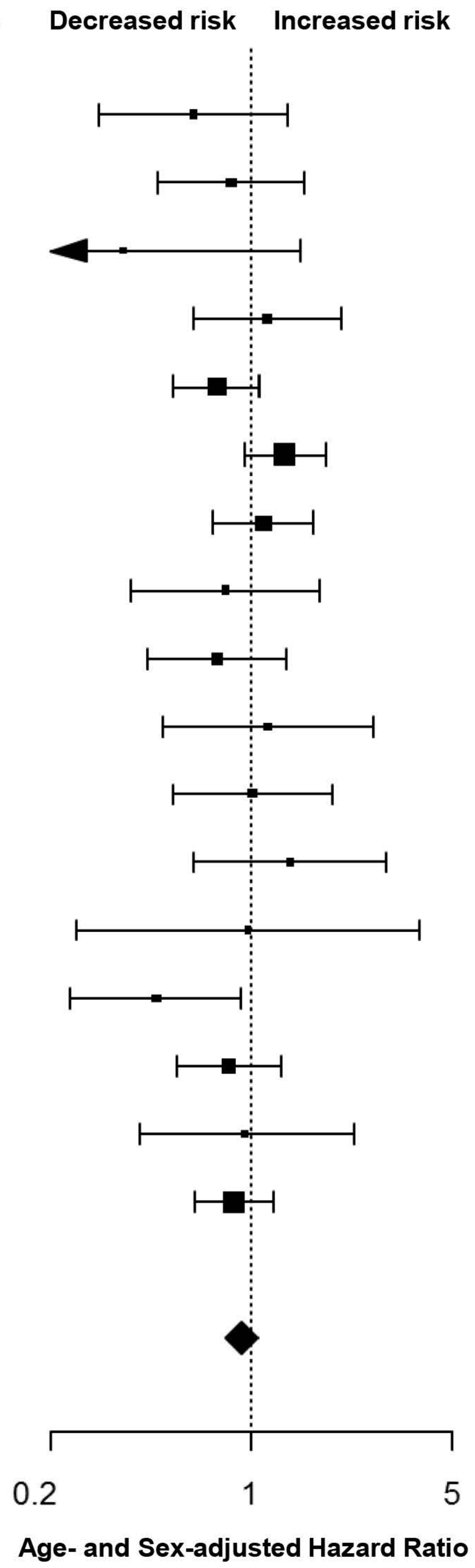
§§Information on race was not available for the 4D study, Busselton Health Study and EPIC-Norfolk Study. Excluded 96 participants from MrOs Study due to no events in subgroup.

A. All Stroke	**No. Events**	**No. Participants**	**Hazard Ratio (95% CI)**	**Weights (%)**
4D Study	66	841	0.85 [0.52, 1.38]	2.8
Busselton Health Study	186	1899	0.97 [0.73, 1.30]	7.8
Cardiovascular Health Study	425	2526	0.85 [0.85, 1.02]	18.1
EPIC-Norfolk Study	439	11986	0.96 [0.79,1.16]	17.7
Health, Aging, and Body Composition Study	249	2170	0.99 [0.77, 1.28]	10.2
InCHIANTI Study	68	963	1.08 [0.67, 1.73]	2.9
Leiden 85-plus Study	60	450	0.85 [0.52, 1.38]	2.8
PREVEND Study	52	2493	1.17 [0.66, 2.09]	2.0
Prosper Trial	234	4953	0.84 [0.65, 1.08]	9.9
Rotterdam Study	262	1543	0.81 [0.64, 1.02]	12.0
SHIP Study	67	2977	0.75 [0.40, 1.41]	1.7
Whickham Survey	163	2052	0.94 [0.83, 0.98]	12.2
Total (95% CI) I² (95% CI) = 0% (0%-18%)	2271	34853	0.90 [0.83, 0.98]	100



B. Fatal Stroke	**No. Events**	**No. Participants**	**Hazard Ratio (95% CI)**	**Weights (%)**
4D Study	27	841	0.63 [0.29, 1.33]	2.8
Birmingham Study	39	1013	0.85 [0.47, 1.53]	4.7
Brazilian Thyroid Study	7	888	0.36 [0.09, 1.48]	0.8
Busselton Health Study	43	1899	1.13 [0.43, 4.69]	4.5
Cardiovascular Health Study	124	2526	0.76 [0.53, 1.07]	13.5
EPIC-Norfolk Study	156	11986	1.31 [0.95, 1.80]	15.5
Health, Aging, and Body Composition Study	101	1934	1.10 [0.74, 1.65]	9.9
InCHIANTI Study	27	1049	0.81 [0.38, 1.74]	2.8
Leiden 85-plus Study	47	447	0.76 [0.43, 1.32]	5.2
MrOS Study	25	1410	1.17 [0.51, 2.68]	2.3
Nagasaki Adult Health Study	56	2342	1.00 [0.53, 1.91]	3.9
Pisa cohort	23	2695	1.36 [0.62, 2.95]	2.7
PREVEND Study	9	2493	0.98 [0.25, 3.90]	0.8
Prosper Trial	30	4953	0.47 [0.24, 0.92]	3.5
Rotterdam Study	81	1577	0.83 [0.55, 1.26]	9.2
SHIP Study	31	2960	0.95 [0.40,2.26]	2.2
Whickham Survey	81	2320	0.87 [0.63, 1.20]	15.7
Total (95% CI)	907	43333	0.92 [0.81, 1.04]	100

I² (95% CI) = 0% (0%-57%)



Appendix Thyroid Function within the Reference Range and the Risk of Stroke: An Individual Participant Data Analysis

Appendix Methods of electronic search strategy

Appendix Table 1 Additional Participant and Cohort Information

Appendix Table 2. Sensitivity Analyses for the association between TSH and the Risk of All Stroke Events and Fatal Stroke

Appendix Table 3. Methodological Sensitivity Analyses for the Associations between TSH and the Risk of All Stroke and Fatal Stroke

Appendix Table 4. Stratified Analyses for the Associations between FT4 in ng/dL and the Risk of All Stroke and Fatal Stroke

Example of electronic search strategy:

Medline (OvidSP):

("Thyroid Diseases"/ OR hyperthyroidism/ OR hypothyroidism/ OR "thyrotropin/bl"/ OR "thyroid function tests"/ OR (((thyroid*) ADJ3 (disease* OR abnormal* OR anomal* OR disorder* OR dysfunction* OR insufficien* OR failure* OR hyperfunction* OR subclinic* OR function*)) OR hypothyr* OR hyperthyr* OR dysthyr* OR ((thyrotropin) ADJ3 (blood OR level* OR serum)) OR ft4).ab,ti.) AND (mortality/ OR mortality.xs. OR "Cause of Death"/ OR "Survival Rate"/ OR survival/ OR exp "stroke"/ OR "Ischemic Attack, Transient"/ OR "Cerebrovascular Disorders"/ OR "brain ischemia"/ OR (mortalit* OR "death rate" OR surviv* OR ((cerebrovascular OR "cerebro vascular" OR "cerebral vascular") ADJ (accident OR disease*)) OR cva OR stroke OR "transient ischemic attack" OR "transient brain ischemia" OR "transient cerebral ischemia" OR "transient ischaemic attack" OR "transient brain ischaemia" OR "transient cerebral ischaemia" OR tia).ab,ti.) AND (exp "cohort studies"/ OR "controlled clinical trial".pt. OR "epidemiologic methods"/ OR "follow up"/ OR (cohort*).ab,ti.)

Appendix Table 1 Additional Participant and Cohort Information

Study name & Reference	TSH assay, generation*, years performed	Euthyroidism, range of TSH definition	N excluded due to TSH outside range	N included in FT4 analyses	N with data missing on any covariate†	Stroke Definition	Formal Adjudication‡
4D Study (18)	Immulite 2000; Siemens, Germany, third generation, 1998	0·45 - 4·5 ImU/L	309	797	0	Stroke was defined as neurologic deficit lasting >24 hours. Cerebral CT or MRI available in all except 16 cases	Yes
Birmingham Study (20)	Serono MAIA-clone method, second generation,1988	0·45 - 4·5 ImU/L	176	NA	NA	Cerebrovascular diseases (ICD9 430-438)	No
Brazilian Thyroid Study (41)	Immunofluorometric assay (Wallac–Delfia, PerkinElmer), third generation, 1999-2000	0·45 - 4·5 ImU/L	220	890	6	Recoded according to cause of death (ICD-9 codes 430–438)	No
Busselton Health Study (25)	Immulite 2000 chemiluminescent analyzer, third generation, 1981	0·45 - 4·5 ImU/L	202	1,900	24	First stroke event and death from stroke ICD-9 codes 430–438 (& ICD 10 I60-I69 including G45)	No
Cardiovascular Health Study (17)	LumaTaghTSH chemiluminescence (Nichols Institute, San Juan Capistrano, USA), third generation, 1991-1993	0·45 - 4·5 ImU/L	587	NA	6	First stroke event (hospitalized) and death from stroke for those with no history of stroke.	Yes
EPIC-Norfolk Study (24) (	AutoDelfia fluoroimmunoassay kits (Wallac, Finland), third generation, 1995-1998	0·45 - 4·5 ImU/L	1407	11,986	192	Cerebrovascular diseases (430-438, I60-I69) on hospital discharge, or as underlying cause of death, excl. TIA	No
Health, Aging, and Body Composition Study (21)	ACS immunoassay (Chiron Diag Corp, Emeryville, USA), third generation, 1997	0·45 - 4·5 ImU/L	628	NA	13	First stroke event and death from stroke according to adjudication	Yes
InCHIANTI Study (35)	Chemiluminescent immunoassay (Vitros Reagent, Ortho-Clinical Diagnostics, Johnson & Johnson, Italy), third generation, 1998	0·45 - 4·5 ImU/L	164	1048	26	Recoded according to cause of death (ICD-9 codes 430–438), local ascertainment for stroke events	No
Leiden 85-plus Study (36)	Elecsys 2010 system + electrochemiluminescence (Boehringer, Germany), third generation, 1997-1999	0·45 - 4·5 ImU/L	106	448	10	Adjudicated first stroke event and/or death (ICD-10 I60-I69)	Yes
MrOS Study (34)	Siemens Diagnostics, (Deerfield, IL, USA), third generation, 2000-2002	0·45 - 4·5 ImU/L	192	1410	113	Death certificates used from start of study. Adjudicated death from stroke according to ICD9 codes 430-438 from the years 2003-2005 onward.	Yes
Nagasaki Adult Health Study (19)	Eiken radioimmunoassay (Eiken Chemical Co. Ltd., Tokyo, Japan), first generation, 1984-1987	0·45 - 4·5 ImU/L	488	2340	158	Stroke specific mortality (ICD-9 430-438)	No

Appendix Table 1 Additional Participant and Cohort Information (continued)

Study name & Reference	TSH assay, generation*, years performed	Euthyroidism, range of TSH definition	N excluded due to TSH outside range	N included in FT4 analyses	N with data missing on any covariate†	Stroke Definition	Formal Adjudication‡
Pisa cohort (38)	AIA 600 system (Tosho Corp, Tokyo, Japan), third generation, 2000	0·45 - 4·5 ImU/L	446	2695	175	Recoded according to cause of death	No
PREVEND Study (37)	Microparticle enzyme immunoassay (Architect, Abbott Laboratories, Abbott Park, IL, USA), third generation	0·45 - 4·5 ImU/L	210	2493	50	Stroke event and/or death (ICD-10 I60-I69), excl. TIA	No
PROSPER Trial (28)	electrochemiluminescenceRoche Elecsys 2010 (Burgess Hill, UK), third generation, 1997-1999	0·45 - 4·5 ImU/L	843	NA	0	Adjudicated first stroke event and death from stroke	Yes
Rotterdam Study (40)	TSH Lumitest (Henning, Berlin, Germany), third generation, 1990-1992	0·45 - 4·5 ImU/L	278	1328	3	Adjudicated first stroke event and death from stroke (ICD-10 I60-I69)	Yes
SHIP Study (39)	LIA-mat, Byk Sangtec Diagnostica immunochemiluminescent procedures (Frankfurt, Germany), third generation, 1997-2001	0·45 - 4·5 ImU/L	1269	2973	12	Self-reported stroke by questionnaire	Yes
Whickham Survey (27)	TSH first generation assay, 1972-1974	0·50- 6·0 I mU/L	417	2299	36	Stroke specific mortality and incident events (ICD-9 430-438)	No

Abbreviations: ICD international classification of disease, TIA transient ischemic attack; TSH, Thyroid-Stimulating-Hormone,CT computed tomography, MRI magnetic resonance imaging; N number participants; NA not applicable.

*Third generation TSH assays have a functional sensitivity of 0·01-0·02mIU/l (the lowest TSH level at which interassay coefficient of variation is <20%)

† Covariates are sex, age, systolic blood pressure, cholesterol, smoking and prevalent diabetes at baseline.

‡ Formal adjudication is defined as having clear criteria for the outcomes that were reviewed by experts for each potential case

Appendix Table 2. Sensitivity Analyses for the association between TSH and the Risk of All Stroke Events and Fatal Stroke*

	All Stroke†			Fatal Stroke		
	No. Events/Participants	**HR (95% CI)**	**I^2,%**	**No. Events/Participants**	**HR (95% CI)**	**I^2,%**
Primary analysis with DL estimator	2271/34,853	0·78 (0·65, 0·95)	0%	907/43,333	0·78 (0·62, 1·09)	0%
Excluding those with thyroid medication use at baseline	2263/34,750	0·78 (0·65, 0·95)	0%	902/43,120	0·80 (0·60, 1·09)	0%
Excluding thyroid medication use at baseline and follow up	1477/19,965	0·80 (0·65, 1·02)	0%	562/24,987	0·71 (0·48, 1·02)	0%
Only studies with formal adjudication procedures‡	1279/12,439	0·71 (0·55, 0·91)	0%	452/16,339	0·69 (0·45, 1·07)	10%
Additional multivariate analyses§						
Multivariate + prevalent AF	1383/13,369	0·75 (0·58, 0·95)	0%	481/13,233	0·71 (0·47, 1·07)	0%
Multivariate + BMI	2271/34,853	0·76 (0·63, 0·91)	0%	868/42,320	0·80 (0·60, 1·12)	7%
Multivariate + lipid lowering and antihypertensive medication	1832/22,867	0·75 (0·60, 0·91)	0%	712/30,334	0·69 (0·50, 0·98)	0%
Multivariate + prevalent CVD	2203/33,890	0·75 (0·62, 0·91)	0%	816/39,861	0·80 (0·56, 1·09)	5%

Appendix Table 2. Sensitivity Analyses for the association between TSH and the Risk of All Stroke and Fatal Stroke[a] (Continued)

	All Stroke†			Fatal Stroke		
	No. Events/Participants	HR (95% CI)	I^2,%	No. Events/Participants	HR (95% CI)	I^2,%
Studies excluded						
Atomic Bomb Survivors; Nagasaki	NA	NA	NA	851/40,991	0·80 (0·60, 1·09)	5%
Studies where PH assumption was not met; PROPSPER trial and Birmingham Study	2037/29,900	0·80 (0·67, 0·98)	0%	838/ 37,367	0·89 (0·65, 1·19)	0%
Stroke assessed by questionnaire; SHIP Study	2204/31,876	0·80 (0·67, 0·95)	0%	876/ 40,373	0·83 (0·60, 1·12)	5%
Studies including TIAs as stroke events; Busselton Health Study	2085/32,954	0·80 (0·65, 0·95)	0%	864/41,434	0·80 (0·60, 1·09)	2%
Studies without community dwelling adults; 4D study, Pisa cohort, Prosper trial	1971/29059	0·80(0·67, 1·00)	0%	827/34,844	0·87 (0·62,1·19)	0%

Abbreviations: AF, atrial fibrillation; BMI Body-Mass Index; CVD cardiovascular disease; HR, hazard ratio; CI, Confidence Interval;T4, free thyroxine; NA, not applicable; TIA, transient ischemic attack; TSH, thyroid-stimulating hormone. The HR's are across the reference range of TSH (0·45-4·49 mIU/L)

* The HRs were adjusted for age and sex.

† Data were available from 12 studies.

‡ Formal adjudication procedure is defined as having clear criteria for the outcomes that were reviewed by experts for each potential case. This was the case for the stroke events analysis in Cardiovascular Health Study, Health ABC Study, Leiden 85-plus Study, Rotterdam Study, 4D Study and Prosper Trial and for the fatal stroke analysis additional in MrOs Study and Pisa Cohort. For CHS, 44 participants were excluded because of adjudication of stroke events was missing.

§ The Birmingham Study was excluded from the multivariable analyses because of lack of data on cardiovascular risk factors. Multivariate analyses included adjustments for sex, age, systolic blood pressure, smoking and prevalent diabetes at baseline. Information on AF at baseline was available for the 4D Study, Busselton Health Study, Cardiovascular Health Study, Health ABC Study, InCHIANTI Study, Leiden 85-plus Study, Rotterdam Study and the SHIP study. Data on lipid-lowering and hypertensive medications were not available for EPIC-Norfolk and the Nagasaki Adult Health Study. Data on history of cardiovascular disease were not available for the InChianti Study and MrOs Study. Body Mass Index defined as weight (kg) divided by height (m) squared.

Appendix Table 3. Methodological Sensitivity Analyses for the Associations between TSH and the Risk of All Stroke and Fatal Stroke

		All Stroke*				**Fatal Stroke†**			
		No. events/ Total participants	**Two-step DL** **HR (95% CI)**	**Two-step REML** **HR (95% CI)**	**One-step Cox** **HR (95% CI)**	**No. events/ Total participants**	**Two-step DL** **HR (95% CI)**	**Two-step REML** **HR (95% CI)**	**One-step Cox** **HR (95% CI)**
Total Population TSH		2271/34,853	0·78 (0·65, 0·95)	0·78 (0·65, 0·95)	0·78 (0·65, 0·95)	907/43,333	0·83 (0·62, 1·09)	0·80 (0·58, 1·12)	0·80 (0·58, 1·12)
Gender‡	Men	1091/16723	0·80 (0·62, 1·07)	0·80 (0·62, 1·07)	0·80 (0·62, 1·07)	422/21874	0·85 (0·50, 1·41)	0·85 (0·50, 1·41)	0·85 (0·53, 1·38)
	Women	1180/18130	0·78 (0·58, 1·07)	0·78 (0·58, 1·07)	0·78 (0·62, 1·00)	485/21459	0·80 (0·52, 1·25)	0·80 (0·52, 1·25)	0·80 (0·52, 1·25)
Age ǀ§	18 – 49y	60/8305	0·95 (0·31, 2·86)	0·95 (0·31, 2·86)	0·95 (0·31, 2·86)	12/9,525	0·71 (0·07, 7·47)	0·71 (0·07, 7·47)	0·78 (0·08, 7·78)
	50 – 64y	358/9145	0·75 (0·47, 1·19)	0·75 (0·47, 1·19)	0·75 (0·47, 1·19)	104/12,303	1·35 (0·55, 3·25)	1·35 (0·55, 3·25)	1·27 (0·53, 3·03)
	65 -79y	1588/15,667	0·83 (0·67, 1·05)	0·83 (0·67, 1·05)	0·83 (0·67, 1·05)	623/19,198	0·89 (0·62, 1·27)	0·87 (0·58, 1·30)	0·87 (0·58, 1·30)
	≥80	265/1736	0·69 (0·40, 1·17)	0·69 (0·40, 1·17)	0·65 (0·38, 1·12)	168/2,307	0·43 (0·22, 0·85)	0·43 (0·22, 0·85)	0·43 (0·22, 0·83)

Abbreviations: CI, confidence interval; DL DerSimonian and Laird; HR, hazard ratio; REML restricted maximum-likelihood ; The HR's are across the reference range of TSH (0·45-4·49 mIU/L)

*Data were available from 12 studies, 393 participants were excluded due to missing stroke event data.

† 265 participants were excluded due to missing data on cause of death.

‡ These analyses were adjusted for age.

§ These analyses were adjusted for sex and age as continuous variable to avoid residual confounding within age strata.

Appendix Table 4. Stratified Analyses for the Associations between FT4 in ng/dL and the Risk of All Stroke and Fatal Stroke

		All Stroke*			Fatal Stroke†		
		No. events/ Total participants	Age and sex adjusted HR (95% CI)	Multivariable‡ HR (95% CI)	No. events/ Total participants	Age and sex adjusted HR (95% CI)	Multivariable‡ HR (95% CI)
Total Population FT4		1148/22,854	1·40 (0·92, 2·13)	1·32 (0·98, 1·76)	517/30,281	1·42 (1·08, 1·87)	1·31 (1·00, 1·75)
Gender§	Men	565/10,897	1·10 (0·77, 1·57)	1·27 (0·82, 1·97)	248/15,557	1·37 (0·88, 2·13)	1·20 (0·77, 1·86)
	Women	583/11,957	1·56 (0·94, 2·59)	1·26 (0·91, 1·74)	269/14,724	1·53 (1·05, 2·23)	1·55 (0·98, 2·43)
	p for interaction		0·27	0·98		0·71	0·43
Age ‖	18 – 49y	38/7022	0·45 (0·11, 1·93)	0·63 (0·11, 3·47)	7/8190	1·19 (0·09, 15·22)	2·16 (0·08, 16·01)
	50 – 64y	284/8496	1·29 (0·40, 4·21)	1·07 (0·48, 2·41)	78/11,310	1·50 (0·68, 3·28)	1·18 (0·52, 2·70)
	65 -79y	695/6588	1·54 (1·18, 2·01)	1·50 (1·15, 1·96)	333/9578	1·30 (0·89, 1·90)	1·06 (0·92, 1·22)
	≥80	131/748	2·13 (1·03, 4·44)	1·85 (0·58, 5·86)	99/1203	1·79 (0·87, 3·70)	1·39 (0·65, 2·99)
	p for trend		0·053	0·18		0·79	0·78

Abbreviations: CI, confidence interval; HR, hazard ratio; FT4, free thyroxine (in ng/dL).

*Data were available from 11 studies, 119 participants were excluded due to missing stroke event data.

† 27 participants were excluded due to missing data on cause of death.

‡ Adjusted for sex, age, systolic blood pressure, cholesterol, smoking and prevalent diabetes at baseline.

§These analyses were not adjusted for sex.

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