Title: Defining Optimal Health Range for Thyroid Function Based on the Risk of Cardiovascular Disease.

Authors: Chaker L, Korevaar TIM, Rizopoulos D, Collet TH, Völzke H, Hofman A, Rodondi N, Cappola AR, Peeters RP, Franco OH

Journal: The Journal of clinical endocrinology and metabolism

Year: 2017 Aug 1

Issue: 102

Volume: 8

Pages: 2853-2861

DOI: 10.1210/jc.2017-00410

Defining optimal health range for thyroid function based on the risk of cardiovascular disease

L. Chaker[1,2,3], T.I.M. Korevaar[1,2,3], D. Rizopoulos[4], T-H Collet[5], H. Völzke[6], A. Hofman[3,7], N Rodondi[8,9], A.R. Cappola[10], R.P. Peeters[1,2,3], O.H. Franco[3]

[1]Rotterdam Thyroid Center, [2]Department of Internal Medicine [3]Department of Epidemiology and, [4]Department of Biostatistics, Erasmus University Medical Center, [5]Service of Endocrinology, Diabetes and Metabolism, University Hospital of Lausanne, Lausanne, Switzerland [6]Institute for Community Medicine, Clinical-Epidemiological Research/SHIP, University Medicine, Greifswald and German Centre of Cardiovascular Research, Partner Site Greifswald, Germany [7]Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, Massachusetts, USA [8]Department of General Internal Medicine, Inselspital, Bern University Hospital and [9]Institute of Primary Health Care (BIHAM), University of Bern, Switzerland. [10]Division of Endocrinology, Diabetes, and Metabolism, Department of Medicine, School of Medicine, University of Pennsylvania, Philadelphia, USA

Corresponding author

R.P. Peeters MD, PhD,

Department of Internal Medicine and Rotterdam Thyroid Center,

Erasmus University Medical Center,

Room Ee 500, PO Box 2040, 3000 CA Rotterdam, The Netherlands,

Tel: +31-10-7043363; Fax: +31-10-7035430, Email: r.peeters@erasmusmc.nl

Short Title: Defining optimal health range for thyroid function, Chaker et al.

Abstract 247, **Word Count** 3106, **Tables**: 4+ 1 supplemental, **Figures**: 1 + 1 Supplemental

Disclosure: The author reports no conflicts of interest in this work.

Abstract

Context: Reference ranges of thyroid stimulating hormone (TSH) and free thyroxine (FT4) are defined by their distribution in apparently healthy populations, (2.5th and 97.5th percentiles) irrespective of disease risk and used as cut-offs for defining and clinically managing thyroid dysfunction.

Objective: To provide a proof of concept in defining thyroid function optimal health ranges based on cardiovascular disease (CVD) mortality risk.

Design and Participants: 9,233 participants from the Rotterdam Study (mean age 65.0 years) were followed up (median 8.8 years) from baseline to date of death or end of follow-up (2012), which ever came first (689 cases of CVD mortality).

Main Outcomes: We calculated 10-year absolute risks of CVD mortality (defined according to SCORE project) using a Fine and Grey competing risk model per percentile of TSH and FT4, modelled non-linearly and sex- and age-adjusted.

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

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

Keywords: thyroid function, optimal health range, reference range, cardiovascular disease

1. Introduction

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

The reference ranges of thyroid function tests, defined by thyroid stimulating hormone (TSH) and free thyroxine (FT4), are examples of reference ranges defined by their distribution. TSh and FT4 reference ranges are currently used as cut-offs to define subclinical and overt thyroid disease, and guide treatment decisions. However, accumulating evidence suggests that subclinical thyroid dysfunction, defined by TSH outside of the reference range but FT4 within the reference range, is also associated with various clinical adverse outcomes, including coronary heart disease (CHD) and cardiovascular mortality, at the extremes.(1,2) Moreover, even differences in thyroid function within the defined reference range are associated with differing risk of cardiovascular events including atrial fibrillation, stroke, sudden cardiac death and cardiovascular mortality.(3-7) Based on the increased risk of CHD in subclinical hypothyroidism, current guidelines advocate treatment with levothyroxine above a TSH of 10 mIU/L, independent of FT4.(8) Extending this concept, the re-evaluation of thyroid function ranges could take clinical adverse events into account and thus move from reference ranges towards “optimal health ranges” for thyroid function.

This approach has been successfully applied to management of myocardial infarction, stroke and diabetes using cholesterol, blood pressure or glucose measurements.(9) For example, the defined range for total cholesterol does not rely on the distribution of total cholesterol in a specific population, but rather on the associated 10-year risk of cardiovascular mortality.(9) Pursuing the same strategy for thyroid function might not be as straightforward as for other biomarkers. The risk of adverse events is relevant for both high and low thyroid function, suggesting a non-linear association, in contrast to cholesterol for example, where the focus is on the high end of the measurement. Furthermore, thyroid dysfunction is not solely associated with cardiovascular disease (CVD), but has important implications for bone health and possibly also cognitive health. (10-13)

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

2. Subjects and Methods

A. The Rotterdam Study

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

B. Study population

We selected data from participants from the third visit of the first cohort (1997-1999, n=4797) and the first visits of the second (2000-2001, n=3011) and third cohort (2006-2008, n=3932), if TSH or FT4 measurements were performed and participants were not using thyroid function altering medication, including levothyroxine, anti-thyroid drugs, amiodarone or corticosteroids. We did not use the first visit of the first cohort as thyroid function was measured with a different assay. All participants in the present analysis provided written informed consent to participate and to obtain information from their treating physician. All study participants were followed up from the day of baseline laboratory testing to date of death or end of follow-up January 1, 2012 which ever came first.

C. Assessment of thyroid function and other baseline measurements

TSH and FT4 measurements were performed using the same methods and assay in blood samples collected between 1997 and 2008, depending on the cohort and stored at -80°C (electrochemiluminescence immunoassay for free thyroxine and thyrotropin, “ECLIA”, Roche). Body mass index was calculated as body mass (kg) divided by the square of the body height (m). Serum cholesterol was measured using standard laboratory techniques. Systolic blood pressure was calculated as the average of two consecutive measurements. Over 95% of participants were in fasting state when blood was drawn (morning) at the Rotterdam Study center visit. Information on tobacco smoking was derived from baseline questionnaires. Information on medication use was obtained from questionnaires in combination with pharmacy records. Thyroid medication at baseline and during follow-up, including thyroid hormone replacement therapy, was prescribed by participant’s own general practitioners (GP) or specialist and within the context of regular treatment and blinded to measurements of the Rotterdam Study.

D. Outcome definition

As primary outcome of interest we selected CVD since it is a leading burden of disease, morbidity and mortality.(15) Additionally, the association of subclinical and overt thyroid dysfunction with CVD mortality are well-established.(1) Secondary outcomes of interest were CHD and stroke (fatal and non-fatal). Methods for collection of data and outcome definitions have been previously described .(14,16,17) Information on the vital status of all participants was obtained on a weekly basis from the central registry of the municipality in Rotterdam and through digital linkage with records from GPs working in the study area. The cause of death was established by abstracting information from the medical records of the general practitioners or nursing home physicians and hospital discharge letters. Cardiovascular mortality was defined as according to the SCORE project definition of fatal CVD including the ICD-10 codes I10-25, I44-51, I61-73, and R96.(9,18) To test the robustness of our findings we repeated the absolute risk estimate calculations using the CVD mortality defined according to previously published definition of the Rotterdam Study, which also included non-atherosclerotic cardiovascular mortality.(16) CHD was defined as myocardial infarction, cardiac revascularization procedure or CHD mortality. Stroke was defined according to World Health Organization (WHO) criteria as a syndrome of rapidly developing clinical signs of focal (or global) disturbance of cerebral function, with symptoms lasting 24 hours or longer or leading to death, with no apparent cause other than of vascular origin, including ischemic or hemorrhagic strokes. Outcomes were adjudicated by a committee who were blinded to lab results.

Statistical analyses

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

For the CHD analyses we excluded all those with prevalent or missing information on CHD at baseline (n=685). For the stroke analyses we excluded all participants with missing information at baseline or a history of stroke (n=319). We performed a goodness-of-fit test for the Fine and Gray model for the absolute risk estimations, using the Zou Laird Fine test, and this revealed no linear, quadratic or log time varying effects of TSH or FT4 (p-value > 0.1 for all analyses). Linearity of absolute risk estimates was tested with restricted cubic splines with 3 knots at the 10^{th}, 50^{th} and 90^{th} percentile. Analyses were performed in R (survival, rms, crrSC and cmprsk packages R-project, Institute for Statistics and Mathematics, R Core Team (2013), Vienna, Austria, version 3.0.2).

3. Results

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

Absolute risk estimates cardiovascular mortality

Ten-year absolute risk estimates for CVD mortality across the range of TSH and FT4 are plotted in **Figure 1**. CVD mortality increased with higher FT4 levels (p-value 0.005) and lower TSH levels, although not statistically significantly for the latter. The best fit for both TSH and FT4 analyses was non-linear (p for non-linearity < 0.001, **Figure 1**). **Table 2** shows the different percentile cut-offs of TSH and FT4 values with the predicted absolute 10-year risk estimates, based on the non-linear association. Overall, FT4 values above the 97th percentile (absolute level of approximately 22 pmol/L or 1.7 ng/dL) were associated with a predicted 10-year risk of 9.6% (p-value = 0.005). FT4 levels above the 90th percentile corresponded to an increased risk of 7.5% and higher for CVD mortality (absolute level of approximately 19 pmol/L or 1.5 ng/dL). Sensitivity analyses additionally adjusting for cardiovascular risk factors, using the RS definition of CVD mortality or adjusting the TSH analyses for FT4 and vice versa did not change the definition of the cut-offs meaningfully (**Supplemental Table 1**). TSH levels were inversely associated with CVD mortality but not statistically significant (**Table 1**).

For men, a risk of ≥10.0% occurred at the 97th percentile of FT4 (p-value < 0.001) and a risk of ≥7.5% already occurred at the 60th percentile (**Table 3**). In women, there was no association of the thyroid function markers and risk of CVD mortality (**Table 3**). In participants younger than 65 years of age, the risk of CVD mortality increased with decreasing TSH levels (p-value = 0.009) with a risk of ≥ 2.0 % from the 30th percentile and lower (~1.40 mIU/L), while FT4 levels were not association with CVD mortality (**Table 4**). In participants older than 65 years of age (**Table 4**), the absolute risk estimates were <10.0% below the 30th percentile and ≥ 15.0% higher than the 97th percentile of FT4.

Absolute risk estimates CHD and stroke

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

4. Discussion

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

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

Overall, our study shows an absolute 10-year risk of 7.5% or higher with FT4 levels above the 90th percentile, corresponding to a cut-off level of FT4 approximately 19 pmol/L (~1.5 ng/dL). This is however, as expected, different in participants younger than 65 years of age compared to those older than 65 years. Also, there seems to be a differential association of thyroid function with absolute risk of CVD when comparing men to women. Even though this can, at least partially, be attributable to the difference in background absolute risk between the two sexes, there also seems to be a thyroid dependent differential risk when comparing men to women. These findings need to be confirmed and validated across different populations, but could suggest a sex-specific reference range is needed.

In our study, higher FT4 levels are associated with an increased risk of CVD mortality whereas TSH levels showed an expected opposite relation with CVD mortality which did not reach statistical significance. The current study is not the first to report an association of FT4 with clinical events, while the association is lower or absent with TSH.(3,6,21) Based on the log-linear relationship between TSH and FT4, TSH is perceived as the most sensitive marker in subjects with thyroid disease. The lack of association with TSH is therefore remarkable. One explanation could be that in euthyroid subjects, TSH predominantly reflects the pituitary-thyroid axis set point rather than disease risk,(28) while, independent of TSH, circulating FT4 (and subsequently FT3 acting intracellular) represents the bioavailable thyroid hormone that can be taken up by cells, thereby leading to clinical consequences of thyroid hormones peripherally.

There are several strengths to our study including the population-based design, the large size of the study population, the completeness of follow-up and the fact that outcomes were defined independently from baseline thyroid function. Nevertheless, the currently proposed optimal health ranges should be interpreted with caution. First of all, even though CVD is one of the most important clinical outcomes, the presented absolute risk estimates are solely based on cardiovascular mortality and our findings as such should be considered as a proof of concept. Furthermore, The Netherlands is classified as a low cardiovascular mortality risk country by the European Society of Cardiology and therefore estimates are not generalizable to countries with higher CVD mortality risk.(29) The Rotterdam Study consists of participants of 45 years and older and mainly Caucasians with, on average, a sufficient iodine status.(30,31) Also, only one baseline measurement of thyroid function was available and therefore changes in thyroid function could not be taken into account. The absolute levels of TSH and FT4 depend on the assay used and are therefore variable. We therefore used the percentiles of the measurements to study the associations and define the optimal health ranges, because of the strong correlation between the different assays of TSH or FT4. These results are potentially better generalizable to other populations. This is also the reason to advice that the calculation of these percentiles is country, iodine status, region and if possible even laboratory specific.

The mentioned limitations of our study also highlight the need for further research. Therefore our approach to define thyroid function adequacy focused on cardiovascular mortality need to be confirmed in similar populations but also replicated in complementary populations such as younger participants, other ethnicities and in regions with different current and historical iodine status.(32) Cardiovascular disease is an established and well-studied outcome in relation to thyroid function. However, recently, there is increasing interest in the association of thyroid function with other outcomes as well, such as cognition. Therefore, importantly, consensus is needed on which clinical outcomes are or could be relevant in defining the optimal health ranges for thyroid function, beyond cardiovascular disease. Lastly, and beyond the discussion on thyroid function optimal health ranges, consensus is also needed on which cardiovascular risk is considered too high and whether this is similar for all populations. For example, a 10-year absolute risk of 2.5% for CVD mortality for a person of 45 years of age might not be deemed equally acceptable compared to the same risk in a person of 75 years.

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

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

5. Appendix

Supplemental Table 1

Supplemental Figure 1

6. Acknowledgments

We are grateful to the study participants, the staff from the Rotterdam Study, and participating general practitioners and pharmacists.

Funding Sources

Prof. R.P. Peeters and Dr. Chaker are supported by a The Netherlands Organisation for Health Research and Development TOP grant (ZonMWTOP, nr 91212044) and an Erasmus MC Medical Research Advisory Committee. (MRACE) grant. Prof. R.P. Peeters has received lecture and consultancy fees from Genzyme, and grant support from Veracyte. Dr T-H Collet's research is supported by a grant from the Swiss National Science Foundation (PZ00P3-167826). Dr T.I.M. Korevaar, Prof. H. Völzke and Prof. R.P. Peeters are members of the EUthyroid consortium, which receives funding from the European Union's Horizon 2020 research and innovation program under grant agreement number 634453.

The Rotterdam Study is supported by the Erasmus MC and Erasmus University Rotterdam; the Netherlands Organization for Scientific Research (NWO); the Netherlands Organization for Health Research and Development (ZonMw); the Research Institute for Diseases in the Elderly (RIDE); the Netherlands Genomics Initiative (NGI); the Ministry of Education, Culture and Science; the Ministry of Health Welfare and Sports; the European Commission (DG XII); and the Municipality of Rotterdam. The funding sources had no involvement in the collection, analysis, writing, interpretation, nor in the decision to submit the paper for publication. Prof. O. H. Franco works in ErasmusAGE, a center for aging research across the life course funded by Nestle´ Nutrition (Nestec Ltd.), Metagenics Inc., and AXA. Nestle´

Nutrition (Nestec Ltd.), Metagenics Inc., and AXA had no role in design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review or approval of the manuscript.

Author contributions

Drs Chaker, Peeters and Franco had full access to all data and take responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Chaker, Korevaar, Rizopoulos, Peeters, Franco

Acquisition of data: Chaker, Korevaar, Hofman, Peeters, Franco

Analysis and interpretation of data: Chaker, Korevaar, Rizopoulos, Collet, Völzke, Hofman, Rodondi, Cappola, Peeters, Franco.

Drafting of the Manuscript: Chaker, Korevaar, Rizopoulos, Collet, Völzke, Hofman, Rodondi, Cappola, Peeters, Franco

Critical Revision of the manuscript for important intellectual content: Chaker, Rizopoulos, Korevaar, Collet, Völzke, Hofman, Rodondi, Cappola, Peeters, Franco

Statistical analysis: Chaker, Korevaar, Rizopoulos, Peeters, Franco

Obtained Funding: Hofman, Peeters, Franco

Administrative, technical, or material support: Chaker, Korevaar, Rizopoulos, Collet, Völzke, Hofman, Rodondi, Cappola, Peeters, Franco

7. References

1. Rodondi N, den Elzen WP, Bauer DC, Cappola AR, Razvi S, Walsh JP, Asvold BO, Iervasi G, Imaizumi M, Collet TH, Bremner A, Maisonneuve P, Sgarbi JA, Khaw KT, Vanderpump MP, Newman AB, Cornuz J, Franklyn JA, Westendorp RG, Vittinghoff E, Gussekloo J, Thyroid Studies C. Subclinical hypothyroidism and the risk of coronary heart disease and mortality. JAMA 2010; 304:1365-1374

2. Collet TH, Gussekloo J, Bauer DC, den Elzen WP, Cappola AR, Balmer P, Iervasi G, Asvold BO, Sgarbi JA, Volzke H, Gencer B, Maciel RM, Molinaro S, Bremner A, Luben RN, Maisonneuve P, Cornuz J, Newman AB, Khaw KT, Westendorp RG, Franklyn JA, Vittinghoff E, Walsh JP, Rodondi N, Thyroid Studies C. Subclinical hyperthyroidism and the risk of coronary heart disease and mortality. Archives of Internal Medicine 2012; 172:799-809

3. Chaker L, Heeringa J, Dehghan A, Medici M, Visser WE, Baumgartner C, Hofman A, Rodondi N, Peeters RP, Franco OH. Normal Thyroid Function and the Risk of Atrial Fibrillation: the Rotterdam Study. J Clin Endocrinol Metab 2015; 100:3718-3724

4. Chaker L, Baumgartner C, den Elzen WP, Collet TH, Ikram MA, Blum MR, Dehghan A, Drechsler C, Luben RN, Portegies ML, Iervasi G, Medici M, Stott DJ, Dullaart RP, Ford I, Bremner A, Newman AB, Wanner C, Sgarbi JA, Dorr M, Longstreth WT, Jr., Psaty BM, Ferrucci L, Maciel RM, Westendorp RG, Jukema JW, Ceresini G, Imaizumi M, Hofman A, Bakker SJ, Franklyn JA, Khaw KT, Bauer DC, Walsh JP, Razvi S, Gussekloo J, Volzke H, Franco OH, Cappola AR, Rodondi N, Peeters RP, Thyroid Studies C. Thyroid Function Within the Reference Range and the Risk of Stroke: An Individual Participant Data Analysis. J Clin Endocrinol Metab 2016; 101:4270-4282

5. Chaker L, van den Berg ME, Niemeijer MN, Franco OH, Dehghan A, Hofman A, Rijnbeek PR, Deckers JW, Eijgelsheim M, Stricker BH, Peeters RP. Thyroid Function and Sudden Cardiac Death: A Prospective Population-Based Cohort Study. Circulation 2016; 134:713-722

6. Cappola AR, Arnold AM, Wulczyn K, Carlson M, Robbins J, Psaty BM. Thyroid function in the euthyroid range and adverse outcomes in older adults. J Clin Endocrinol Metab 2015; 100:1088-1096

7. Inoue K, Tsujimoto T, Saito J, Sugiyama T. Association Between Serum Thyrotropin Levels and Mortality Among Euthyroid Adults in the United States. Thyroid 2016; 26:1457-1465

8. Pearce SH, Brabant G, Duntas LH, Monzani F, Peeters RP, Razvi S, Wemeau JL. 2013 ETA Guideline: Management of Subclinical Hypothyroidism. Eur Thyroid J 2013; 2:215-228

9. Conroy RM, Pyorala K, Fitzgerald AP, Sans S, Menotti A, De Backer G, De Bacquer D, Ducimetiere P, Jousilahti P, Keil U, Njolstad I, Oganov RG, Thomsen T, Tunstall-Pedoe H, Tverdal A, Wedel H, Whincup P, Wilhelmsen L, Graham IM, group Sp. Estimation of ten-year risk of fatal cardiovascular disease in Europe: the SCORE project. Eur Heart J 2003; 24:987-1003

10. Blum MR, Bauer DC, Collet TH, Fink HA, Cappola AR, da Costa BR, Wirth CD, Peeters RP, Asvold BO, den Elzen WP, Luben RN, Imaizumi M, Bremner AP, Gogakos A, Eastell R, Kearney PM, Strotmeyer ES, Wallace ER, Hoff M, Ceresini G, Rivadeneira F, Uitterlinden AG, Stott DJ, Westendorp RG, Khaw KT, Langhammer A, Ferrucci L, Gussekloo J, Williams GR, Walsh JP, Juni P, Aujesky D, Rodondi N, Thyroid Studies C. Subclinical thyroid dysfunction and fracture risk: a meta-analysis. JAMA 2015; 313:2055-2065

11. Pasqualetti G, Pagano G, Rengo G, Ferrara N, Monzani F. Subclinical Hypothyroidism and Cognitive Impairment: Systematic Review and Meta-Analysis. J Clin Endocrinol Metab 2015; 100:4240-4248

12. Rieben C, Segna D, da Costa BR, Collet TH, Chaker L, Aubert CE, Baumgartner C, Almeida OP, Hogervorst E, Trompet S, Masaki K, Mooijaart SP, Gussekloo J, Peeters RP, Bauer DC, Aujesky D, Rodondi N. Subclinical Thyroid Dysfunction and the Risk of Cognitive Decline: a Meta-Analysis of Prospective Cohort Studies. J Clin Endocrinol Metab 2016:jc20162129

13. Chaker L, Wolters FJ, Bos D, Korevaar TI, Hofman A, van der Lugt A, Koudstaal PJ, Franco OH, Dehghan A, Vernooij MW, Peeters RP, Ikram MA. Thyroid function and the risk of dementia: The Rotterdam Study. Neurology 2016; 87:1688-1695

14. Hofman A, Brusselle GG, Darwish Murad S, van Duijn CM, Franco OH, Goedegebure A, Ikram MA, Klaver CC, Nijsten TE, Peeters RP, Stricker BH, Tiemeier HW, Uitterlinden AG, Vernooij MW. The Rotterdam Study: 2016 objectives and design update. Eur J Epidemiol 2015; 30:661-708

15. Prince MJ, Wu F, Guo Y, Gutierrez Robledo LM, O'Donnell M, Sullivan R, Yusuf S. The burden of disease in older people and implications for health policy and practice. Lancet 2015; 385:549-562

16. Leening MJ, Kavousi M, Heeringa J, van Rooij FJ, Verkroost-van Heemst J, Deckers JW, Mattace-Raso FU, Ziere G, Hofman A, Stricker BH, Witteman JC. Methods of data collection and definitions of cardiac outcomes in the Rotterdam Study. Eur J Epidemiol 2012; 27:173-185

17. Wieberdink RG, Ikram MA, Hofman A, Koudstaal PJ, Breteler MM. Trends in stroke incidence rates and stroke risk factors in Rotterdam, the Netherlands from 1990 to 2008. Eur J Epidemiol 2012; 27:287-295

18. Mortensen MB, Afzal S, Nordestgaard BG, Falk E. The high-density lipoprotein-adjusted SCORE model worsens SCORE-based risk classification in a contemporary population of 30,824 Europeans: the Copenhagen General Population Study. Eur Heart J 2015; 36:2446-2453

19. Jonklaas J, Kahric-Janicic N, Soldin OP, Soldin SJ. Correlations of free thyroid hormones measured by tandem mass spectrometry and immunoassay with thyroid-stimulating hormone across 4 patient populations. Clin Chem 2009; 55:1380-1388

20. Sapin R, d'Herbomez M. Free thyroxine measured by equilibrium dialysis and nine immunoassays in sera with various serum thyroxine-binding capacities. Clin Chem 2003; 49:1531-1535

21. Chaker L, Buitendijk GH, Dehghan A, Medici M, Hofman A, Vingerling JR, Franco OH, Klaver CC, Peeters RP. Thyroid function and age-related macular degeneration: a prospective population-based cohort study--the Rotterdam Study. BMC Med 2015; 13:94

22. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. J Am Stat Assoc 1999; 94:496-509

23. Clark TG, Altman DG, De Stavola BL. Quantification of the completeness of follow-up. Lancet 2002; 359:1309-1310

24. Boucai L, Surks MI. Reference limits of serum TSH and free T4 are significantly influenced by race and age in an urban outpatient medical practice. Clin Endocrinol (Oxf) 2009; 70:788-793

25. Ittermann T, Khattak RM, Nauck M, Cordova CM, Volzke H. Shift of the TSH reference range with improved iodine supply in Northeast Germany. Eur J Endocrinol 2015; 172:261-267

26. Surks MI, Hollowell JG. Age-specific distribution of serum thyrotropin and antithyroid antibodies in the US population: implications for the prevalence of subclinical hypothyroidism. J Clin Endocrinol Metab 2007; 92:4575-4582

27. Jonklaas J, Bianco AC, Bauer AJ, Burman KD, Cappola AR, Celi FS, Cooper DS, Kim BW, Peeters RP, Rosenthal MS, Sawka AM, American Thyroid Association Task Force on Thyroid Hormone R. Guidelines for the treatment of hypothyroidism: prepared by the american thyroid association task force on thyroid hormone replacement. Thyroid 2014; 24:1670-1751

28. Werneck de Castro JP, Fonseca TL, Ueta CB, McAninch EA, Abdalla S, Wittmann G, Lechan RM, Gereben B, Bianco AC. Differences in hypothalamic type 2 deiodinase ubiquitination explain localized sensitivity to thyroxine. J Clin Invest 2015; 125:769-781

29. Perk J, De Backer G, Gohlke H, Graham I, Reiner Z, Verschuren M, Albus C, Benlian P, Boysen G, Cifkova R, Deaton C, Ebrahim S, Fisher M, Germano G, Hobbs R, Hoes A, Karadeniz S, Mezzani A, Prescott E, Ryden L, Scherer M, Syvanne M, Scholte op Reimer WJ, Vrints C, Wood D, Zamorano JL, Zannad F, European Association for Cardiovascular P, Rehabilitation, Guidelines ESCCfP. European Guidelines on cardiovascular disease prevention in clinical practice (version 2012). The Fifth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of nine societies and by invited experts). Eur Heart J 2012; 33:1635-1701

30. WHO: Iodine data by country.

31. World Health Organization, United Nations Children's Fund & International Council for Control of Iodine Deficiency. Disorders 2001 Assessment of iodine deficiency disorders and monitoring their elimination. WHO/NHD/01.1. 2001;

32. van de Ven AC, Netea-Maier RT, Smit JW, Kusters R, van der Stappen JW, Pronk-Admiraal CJ, Buijs MM, Schoenmakers CH, Koehorst SG, de Groot MJ, Sweep FC, Hermus AR, den Heijer M. Thyrotropin versus age relation as an indicator of historical iodine intake. Thyroid 2015; 25:629-634

Figure legend

Figure 1

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

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

Abbreviations: CVD cardiovascular disease FT4 free thyroxine, TSH thyroid-stimulating hormone.

Table 1: Baseline characteristics of included participants in the Rotterdam Study with TSH or FT4 measurements and no thyroid function altering medication

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

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

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

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

lodels are adjusted for age and sex and computed using a competing risk model. Risk legend: low risk (< 2.0% , blue), low-intermediate risk (2.0-5.0%, green), interme
.0-7.5%, yellow), high-intermediate risk (7.5-10.0%, orange), high risk (≥ 10.0%, red)
bbreviations: CVD = cardiovascular disease; FT4 = free thyroxine; N = number; TSH = thyroid stimulating hormone
6 people excluded due to missing cause of death

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

	Predicted 10-year absolute risk of event (n= 689)													
len, = 4072 cases = 357														
SH, percentile	**<2nd**	**2-5th**	**5-10th**	**10-20th**	**20-30th**	**30-40th**	**40-50th**	**50-60th**	**60-70th**	**70-80th**	**80-90th**	**90-95th**	**95-97th**	**>97th**	**P**
Absolute risk estimates	11.4%	8.6%	8.8%	8.0%	7.1%	7.0%	7.3%	6.4%	6.6%	6.4%	6.4%	7.8%	7.2%	7.1%	
N	*44*	*78*	*216*	*461*	*461*	*472*	*452*	*450*	*408*	*418*	*354*	*159*	*60*	*39*	
T4 percentiles	**<2nd**	**2-5th**	**5-10th**	**10-20th**	**20-30th**	**30-40th**	**40-50th**	**50-60th**	**60-70th**	**70-80th**	**80-90th**	**90-95th**	**95-97th**	**>97th**	**P**
Absolute risk estimates	4.4%	5.3%	6.1%	5.4%	5.5%	6.1%	6.8%	7.5%	7.6%	8.3%	8.4%	9.0%	9.0%	10.8%	<
N	*62*	*51*	*199*	*377*	*352*	*412*	*393*	*450*	*425*	*461*	*458*	*244*	*128*	*60*	
/omen, = 5155, cases = 332															
SH, percentile	**<2nd**	**2-5th**	**5-10th**	**10-20th**	**20-30th**	**30-40th**	**40-50th**	**50-60th**	**60-70th**	**70-80th**	**80-90th**	**90-95th**	**95-97th**	**>97th**	**P**
Absolute risk estimates	7.0%	8.1%	6.3%	5.9%	5.9%	4.7%	4.6%	4.7%	4.6%	4.5%	4.6%	5.0%	5.1%	5.9%	
N	*105*	*86*	*255*	*483*	*498*	*480*	*478*	*508*	*536*	*535*	*579*	*285*	*197*	*130*	
T4 percentiles	**<2nd**	**2-5th**	**5-10th**	**10-20th**	**20-30th**	**30-40th**	**40-50th**	**50-60th**	**60-70th**	**70-80th**	**80-90th**	**90-95th**	**95-97th**	**>97th**	**P**
Absolute risk estimates	4.8%	4.3%	4.2%	4.2%	4.3%	4.5%	5.0%	4.7%	5.1%	5.6%	6.7%	7.8%	8.8%	8.6%	
N	*123*	*139*	*277*	*564*	*600*	*549*	*547*	*503*	*514*	*486*	*453*	*219*	*110*	*71*	

lodels are adjusted for age and sex and computed using a competing risk model. Risk legend: low risk (< 2.0% , blue), low-intermediate risk (2.0-5.0%, green), interme
i.0-7.5%, yellow), high-intermediate risk (7.5-10.0%, orange), high risk (≥ 10.0%, red)
bbreviations: CVD = cardiovascular disease; FT4 = free thyroxine; N = number; TSH = thyroid stimulating hormone
6 people excluded due to missing cause of death

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

	Predicted 10-year absolute risk of event (n= 689)														
ge < 65 years, = 5172 cases = 82															
SH, percentile	**<2nd**	**2-5th**	**5-10th**	**10-20th**	**20-30th**	**30-40th**	**40-50th**	**50-60th**	**60-70th**	**70-80th**	**80-90th**	**90-95th**	**95-97th**	**>97th**	**P**
Absolute risk estimates	2.6%	3.0%	2.6%	2.4%	2.2%	1.9%	1.7%	1.4%	1.3%	1.2%	1.0%	0.9%	0.8%	0.9%	(
N	*56*	*59*	*234*	*490*	*523*	*557*	*532*	*573*	*564*	*580*	*554*	*233*	*134*	*83*	
T4 percentiles	**<2nd**	**2-5th**	**5-10th**	**10-20th**	**20-30th**	**30-40th**	**40-50th**	**50-60th**	**60-70th**	**70-80th**	**80-90th**	**90-95th**	**95-97th**	**>97th**	**P**
Absolute risk estimates	1.2%	1.1%	1.3%	1.3%	1.3%	1.5%	1.5%	1.8%	1.8%	1.9%	2.1%	2.2%	2.4%	2.4%	
N	*96*	*97*	*285*	*565*	*556*	*561*	*516*	*526*	*508*	*535*	*512*	*239*	*115*	*61*	
ge ≥ 65 years, = 4055, cases = 607															
SH, percentile	**<2nd**	**2-5th**	**5-10th**	**10-20th**	**20-30th**	**30-40th**	**40-50th**	**50-60th**	**60-70th**	**70-80th**	**80-90th**	**90-95th**	**95-97th**	**>97th**	**P**
Absolute risk estimates	11.8%	11.5%	12.2%	11.9%	11.5%	11.3%	11.2%	11.1%	11.1%	11.2%	10.9%	11.4%	10.5%	10.8%	
N	*93*	*105*	*237*	*454*	*436*	*395*	*398*	*385*	*380*	*373*	*379*	*211*	*123*	*86*	
T4 percentiles	**<2nd**	**2-5th**	**5-10th**	**10-20th**	**20-30th**	**30-40th**	**40-50th**	**50-60th**	**60-70th**	**70-80th**	**80-90th**	**90-95th**	**95-97th**	**>97th**	**P**
Absolute risk estimates	8.1%	7.9%	10.2%	9.3%	9.2%	10.2%	10.7%	11.1%	11.4%	13.1%	14.1%	14.7%	14.9%	15.7%	(
N	*89*	*93*	*191*	*376*	*396*	*400*	*424*	*427*	*431*	*412*	*399*	*224*	*123*	*70*	

lodels are adjusted for age and sex and computed using a competing risk model. Risk legend: low risk (< 2.0% , blue), low-intermediate risk (2.0-5.0%, green), interme
.0-7.5%, yellow), high-intermediate risk (7.5-10.0%, orange), high risk (≥ 10.0%, red)
bbreviations: CVD = cardiovascular disease; FT4 = free thyroxine; N = number; TSH = thyroid stimulating hormone
6 people excluded due to missing cause of death