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## Is thyroid status a common denominator of age-related disease?

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### Citation

Vliet, N. A. van. (2023, January 25). *Is thyroid status a common denominator of age-related disease?*. Retrieved from <https://hdl.handle.net/1887/3512954>

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**Is thyroid status a  
common denominator of  
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**N. A. van Vliet**



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Nicolet Alien van Vliet

Provided by thesis specialist Ridderprint, [ridderprint.nl](http://ridderprint.nl)  
Printing: Ridderprint  
Layout and design: Birgit Vredenburg, [persoonlijkproefschrift.nl](http://persoonlijkproefschrift.nl)  
ISBN: 9789464588194

The research described in this thesis was funded by the European Commission project THYRAGE (Horizon 2020 research and innovation program, 666869).

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# **Is thyroid status a common denominator of age-related disease?**

## **Proefschrift**

ter verkrijging van  
de graad van doctor aan de Universiteit Leiden,  
op gezag van rector magnificus prof.dr.ir. H. Bijl,  
volgens besluit van het college voor promoties  
te verdedigen op woensdag 25 januari 2023  
klokke 13.45 uur

door

Nicolet Alien van Vliet  
geboren te Alphen aan den Rijn  
in 1993

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# CHAPTER 1

General introduction and outline of the thesis

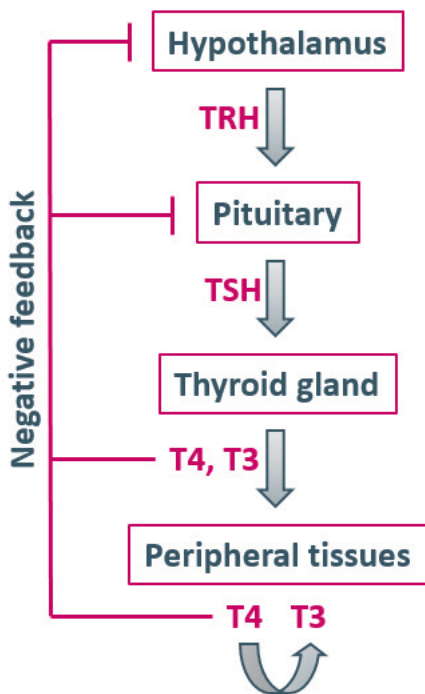
## GENERAL INTRODUCTION

Worldwide, life expectancy has increased impressively over the last centuries, mostly due to better living conditions and by improvements in health care.<sup>1</sup> One of the consequences thereof is an increasingly large proportion of older individuals in the population, which in turn gives rise to numerous societal challenges and opportunities. With the rising number of older individuals, the prevalence of age-related diseases is also on the rise. Age-related disease is an umbrella term for any illness that is more likely to strike older individuals as compared to children and young adults, and it covers a large variety of neurological, musculoskeletal, neoplastic and cardiometabolic diseases. For many conditions the reason for why aging is a major risk factor has not been fully elucidated, although the gradual accumulation of damage over time in conjunction with the body's responses to damage (as captured by the hallmarks of aging) is a popular explanatory model.<sup>2</sup> Over the past decades, it has been hypothesized that settings of endocrinological axes might influence the rate of aging.<sup>3</sup> This thesis focuses on the hypothalamic-pituitary-thyroid (HPT)-axis, which is involved in developmental and maintenance processes and in energy metabolism.

### **The hypothalamic-pituitary-thyroid-axis**

The HPT-axis entails an intricate system of feedforward and feedback signals, from central regulation in the hypothalamus and pituitary to the thyroid gland and peripheral tissues (**Figure 1**). The hypothalamus releases thyrotropin-releasing hormone (TRH) in a pulsatile fashion, which in turn stimulates the pituitary to secrete thyrotropin, also called thyroid-stimulating hormone (TSH). TSH then stimulates production and secretion of thyroid hormones by the thyroid gland. In humans, the vast majority of the thyroid hormones produced by the thyroid gland (approximately 100 microgram per day) is the inactive prohormone thyroxin (T4) and only a small proportion (estimated 30 microgram per day) is the active hormone triiodothyronine (T3).<sup>4</sup> In the blood, more than 99% of T4 and T3 are bound by various binding proteins, leaving only less than 1% unbound and free to enter cells via specialized transporter proteins.<sup>5</sup> Circulating unbound T4 (fT4) signals back to the hypothalamus and pituitary in a negative feedback loop, thus keeping circulating thyroid hormone concentrations stable by inhibiting secretion of TRH and TSH.

In human studies, thyroid status is mostly assessed by measurement of circulating levels of TSH and fT4. On top of the regulation of circulatory concentrations of thyroid hormones, peripheral tissues can locally regulate and finetune their exposure to thyroid hormone signaling, amongst other by specialized enzymes called deiodinases which can activate or deactivate thyroid hormones.<sup>6</sup>



**Figure 1.** Hypothalamic-pituitary-thyroid-axis

Feed forward and feedback signals between the hypothalamus, pituitary, thyroid gland and peripheral tissues visualized schematically.

Abbreviations: TRH; thyrotropin-releasing hormone, TSH; thyroid-stimulating hormone or thyrotropin, T4; thyroxin, T3; triiodothyronine.

### The hypothalamic-pituitary-thyroid-axis and the aging process

In various mammals an association was found between lower circulatory levels of thyroid hormones and increased longevity.<sup>7</sup> Also in exceptionally long-lived human families different tuning of the HPT-axis was observed.<sup>8,9</sup> Interestingly, a similar pattern of higher TSH was found in sporadic octogenarians who had a better functional status.<sup>10</sup> In model organisms, a role for thyroid hormones was discovered in the maintenance of muscle and brain tissue by their effect on cell fate.<sup>11,12</sup> These findings combined gave rise to the hypothesis that thyroid status might influence the apparent tradeoff between optimal development and reproductive capacity versus somatic maintenance and repair.<sup>7</sup>

### THYRAGE

This thesis is embedded in the European Commission funded Horizon 2020 project THYRAGE, which started in January 2016. The aim of the project was to investigate the effects of thyroid hormones on a wide range of age-related diseases, including osteoporosis, osteoarthritis, neurological disorders and

sarcopenia. Six partners across five European countries joined forces in a multidisciplinary team of fundamental researchers, chemists, clinicians and epidemiologists. The team at the Leiden University Medical Center led the studies involving human subjects, complementing the basic research performed in model organisms and cell cultures at the other sites in collaboration with industry-based peptide scientists. The objective for this thesis was to investigate the potential causality of associations between circulating concentrations of thyroid parameters and age-related disease or functional decline by optimally exploiting different available data.

### **Causal inference in epidemiology**

In epidemiology, the ultimate goal is to uncover why some people develop certain diseases and others do not. A first step is to investigate a well-defined group of individuals, a cohort, and simply count whether an outcome of interest occurs more frequently in individuals with certain characteristics compared to those who do not have these characteristics. By these means we can study associations. However, association does not necessarily equal causation. According to Hernán and Robins, inferences about causation are concerned with “what if questions” rather than the actual outcomes observed.<sup>13</sup> Essentially, for causality you would want to know if the outcome had been different if the exposure or characteristic of interest had been different. To answer these types of questions, one would ideally randomize individuals to either being exposed or not being exposed. By randomization, chance decides whether a person is exposed or not. Therefore, if you randomize a sufficiently large group of people, both groups will be equal in every aspect other than the exposure of interest. However, randomization is not always feasible. Observational studies can also approximate causality, though confounding and bias may influence the estimate of the effect.

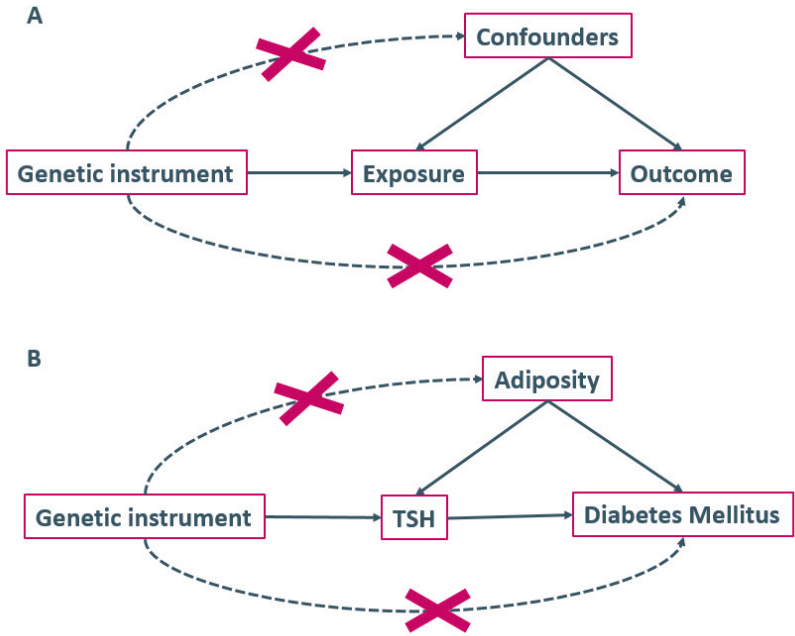
### **Multi-cohort studies**

Every epidemiological study is prone to errors; these can be divided into random and systematic errors<sup>14</sup>. The magnitude of random errors decreases with an increase in sample size. Systematic errors, also called bias, are not dependent on sample size. Bias is usually a result of study design; the most common types of bias are selection bias and information bias. Selection bias stems from non-random participation, either due to the selection criteria imposed by the researcher or due to factors influencing the choice of individuals to participate in the specific study.<sup>15</sup> Information bias is caused by systematic errors in data collection, which is most problematic when the chance of error differs between the group that develops the outcome and the group that does not.<sup>15</sup> By combining multiple cohorts, the sample size increases which directly reduces random error. In addition, sources of bias usually differ between cohorts. Therefore, if an association is consistent among multiple samples of the population, the estimate

might be closer to the true association in the source population. So, on top of increasing precision by reducing random error through increasing sample size, multi-cohort studies might also improve external validity. To conduct a multi-cohort study, either individual participant data (IPD) or summary-level data can be used. For an IPD approach, the original data for each participant is obtained and reanalyzed, allowing homogenous definitions, standardized analyses and separate analyses for subgroups; however this approach is labor intensive.<sup>16</sup> Summary-level data on the other hand means that data is only available on group level; these data are often publicly available and relatively easy to use, though no subgroups can be investigated and analytical methods are restricted. An intermediate option also explored in this thesis, is to provide a standardized protocol and automated statistical scripts to cohort-affiliated researchers and to request only the group characteristics and the results of the analyses. This hybrid method requires planning of all analyses in advance and presence of sufficient expertise at each research site to execute the protocol. However, with the increasingly restrictive privacy legislation avoidance of sharing individual data is a major advantage.

### **Mendelian randomization**

Confounding and reverse causality always threaten causal inference in observational studies. Despite advances in statistical methods, it remains essentially impossible to rule those out completely in the absence of randomized trials. Mendelian randomization was developed as an alternative method to investigate causal associations, free from most confounding and reverse causality.<sup>17</sup> Mendelian randomization utilizes Mendel's second law of independent inheritance of traits, implying that traits are randomly allocated at conception. Genetic traits can be used as instrumental variables for a given exposure, such as TSH or fT4, thereby circumventing any confounding by lifestyle or other characteristics which might confound the association between the exposure (for example TSH or fT4) and the outcome of interest (for example, a specific age-related disease).<sup>18</sup> However, for this method to be valid three assumptions should be met: 1) the instruments must be associated with the exposure, 2) the instruments must influence the outcome only through the exposure, and 3) the instruments must not associate with measured and unmeasured confounding (**Figure 2**).



**Figure 2.** Assumptions of Mendelian randomization  
Visualization of the assumed relations investigated in Mendelian randomization studies.  
**A.** Conceptual visualization,  
**B.** Example with TSH (exposure) and risk of diabetes mellitus (outcome) as explored by Bos et al.<sup>19</sup>

Oftentimes the magnitude of the association between the genetic instruments and the exposure is estimated in another study population than the population in which the association with the outcome is assessed; this strategy is called two-sample Mendelian randomization.<sup>20</sup> In a one-sample or single-sample design both estimates are derived from the same study population; this approach is vulnerable to bias towards the observational association when weaker genetic instruments are used.<sup>21</sup>

### Triangulation

Despite all efforts, each study design has its flaws. That is one of the reasons why new knowledge is built on several different pieces of evidence, usually from different branches of research. Triangulation attempts to formalize this multi-disciplinary evidence by explicitly combining different research approaches with unrelated sources of bias.<sup>22</sup> The main idea is that if different approaches point in the same direction, the findings are more likely to be true.

### **Aim of this thesis**

The aim of this thesis was to investigate the potential causal role of thyroid status in the development of age-related diseases in humans using multiple sources of available, observational data.

### **OUTLINE OF THIS THESIS**

In **Chapter 2** we put forward the viewpoint that disturbances in tissue maintenance might be at the interface between the classic hallmarks of aging and the development of age-related diseases. We present a selection of potential biomarkers of tissue maintenance that could be used to address this hypothesis. In **Chapter 3** we prospectively studied the association between different measures of thyroid status and mortality in two populations of nonagenarians. The participants of the Leiden Longevity Study were selected based on familial longevity, while the nonagenarians of the Leiden 85-plus Study were selected for survival to 90 years and living in the city of Leiden. In **Chapter 4** we investigated the association between thyroid dysfunction and cognitive function using an individual participant data analysis approach. In **Chapter 5** we performed a Mendelian randomization study and a candidate gene study on thyroid stimulating hormone and bone mineral density using summary-level data of the GEFOS (GEnetic Factors for Osteoporosis) consortium. In **Chapter 6** the relationship between thyroid function and anemia was assessed at three levels; (i) diagnosis of thyroid dysfunction, (ii) genetically determined circulating levels and (iii) genetic variants of enzymes regulating intracellular thyroid hormone availability. All analyses were performed using data from the UK Biobank; a population-based study with information on genetics, health and lifestyle of half a million inhabitants of the United Kingdom. In **Chapter 7** we investigated the interplay of thyroid status, body mass index (BMI) and diabetes mellitus with Mendelian randomization in the UK Biobank. In **Chapter 8** we explored the association between thyroid parameters and coronary artery disease with Mendelian randomization in summary-level data of the multi-ancestry CARDIoGRAMplusC4D (Coronary ARtery Disease Genome wide Replication and Meta-analysis [CARDIoGRAM] plus The Coronary Artery Disease [C4D] Genetics) consortium. In **Chapter 9** we aimed to add to the evidence on the role of thyroid status in cardiovascular disease by triangulation of the metabolomic profile associated with thyroid parameters and multi-cohort Mendelian randomization on thyroid parameters and coronary artery disease. For the triangulation, we performed multivariable regression analyses in six cohorts embedded in BBMRI-NL (Biobanking and BioMolecular resources Research Infrastructure the Netherlands), and Mendelian randomization in four cohorts with genetics data and data on the metabolomic platform used in BBMRI-NL (MAGNETIC consortium, NEO [Netherlands Epidemiology of Obesity] study,

Oxford Biobank, and PROSPER [PROspective Study of Pravastatin in the Elderly at Risk]). The Mendelian randomization study on coronary artery disease was performed with the summary-level data of three European ancestry cohorts (CARDIoGRAM [Coronary ARtery Disease Genome wide Replication and Meta-analysis] consortium, the UK Biobank and FinnGen [a concerted effort of Finnish universities, hospitals and national institute for health and welfare to combine different registries to a research source]).

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## Chapter 1

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# CHAPTER 2

Viewpoint on the role of tissue maintenance in ageing: focus on biomarkers of bone, cartilage, muscle, and brain tissue maintenance

Evie van der Spoel, Nicolien A. van Vliet, Diana van Heemst

*Ageing Res Rev 2019 Dec;56:100964.*

## **ABSTRACT**

Specific hallmarks are thought to underlie the ageing process and age-related functional decline. In this viewpoint, we put forward the hypothesis that disturbances in the process of tissue maintenance are an important common denominator that may lie in between specific hallmarks of ageing (i.e. damage and responses to damage) and their ultimate (patho)physiological consequences (i.e. functional decline and age-related disease). As a first step towards verifying or falsifying this hypothesis, it will be important to measure biomarkers of tissue maintenance in future studies in different study populations. The main aim of the current paper is to discuss potential biomarkers of tissue maintenance that could be used in such future studies. Among the many tissues that could have been chosen to explore our hypothesis, to keep the paper manageable, we chose to focus on a selected number of tissues, namely bone, cartilage, muscle, and the brain, which are important for mobility and cognition and affected in several common age-related diseases, including osteoporosis, osteoarthritis, sarcopenia, and neurodegenerative diseases. Furthermore, we discuss their advantages and limitations for use in (pre)clinical studies. The proposed biomarkers should be validated in future research, for example by measuring these in humans with different rates of ageing.

## INTRODUCTION

The ageing population is growing, with implications for nearly all sectors of society. Therefore, improving health at old age is currently one of the central aims of biomedical research. Specific hallmarks are thought to underlie the ageing process and age-related functional decline and disease. In this viewpoint, we put forward the hypothesis (**Figure 1**) that disturbances in the process of tissue maintenance are an important common denominator that may be at the interface between specific hallmarks of ageing (i.e. damage and responses to damage) and their ultimate (patho)physiological consequences (i.e. functional decline and age-related disease). Crucial for ageing research is acquiring accurate quantifiable biological markers, i.e. biomarkers, of biological age<sup>1</sup>. Biological age is different from chronological age and represents the heterogeneity in physiological health between individuals of similar calendar age; someone with a younger biological age is considered to have a physiologically younger body and hence a longer remaining life expectancy compared to someone of the same calendar age but with a higher biological age. Being able to assess biological age at the level of the individual will improve clinical decision making and the definition of endpoints for intervention studies<sup>2</sup>. Ageing can be described as time-dependent functional decline characterized by the progressive loss of physiological integrity, which results in an increased vulnerability to disease and mortality<sup>3</sup>. As a result, ageing is among others associated with loss of independence and self-reliance. A study performed by Fried *et al.* showed that maintaining independence was the most important health outcome for most older persons, compared to symptom relief, pain relief, and staying alive<sup>4</sup>. Also from a societal perspective, prolonging the independence and self-reliance of older people is of importance. Crucial for independence are, among several other factors, mobility, requiring the proper functioning of the musculoskeletal system, and cognition, requiring the proper function of the brain. As a first step towards verifying or falsifying the hypothesis that disturbances in the process of tissue maintenance are an important common denominator that may be at the interface between specific hallmarks of ageing and functional decline and age-related disease, it will be important to measure biomarkers of tissue maintenance in future studies in different study populations. The main aim of the current paper is to discuss potential biomarkers of tissue maintenance that could be used in such future studies.

Among the many tissues that could have been chosen to explore our hypothesis, to keep the paper manageable, we chose to focus on a selected number of tissues, namely bone, cartilage, muscle, and the brain, which are important for mobility and cognition and affected in several prevalent age-related diseases, including osteoporosis, osteoarthritis, sarcopenia and neurodegenerative diseases. Osteoporosis is characterized by reduced bone mass, strength,

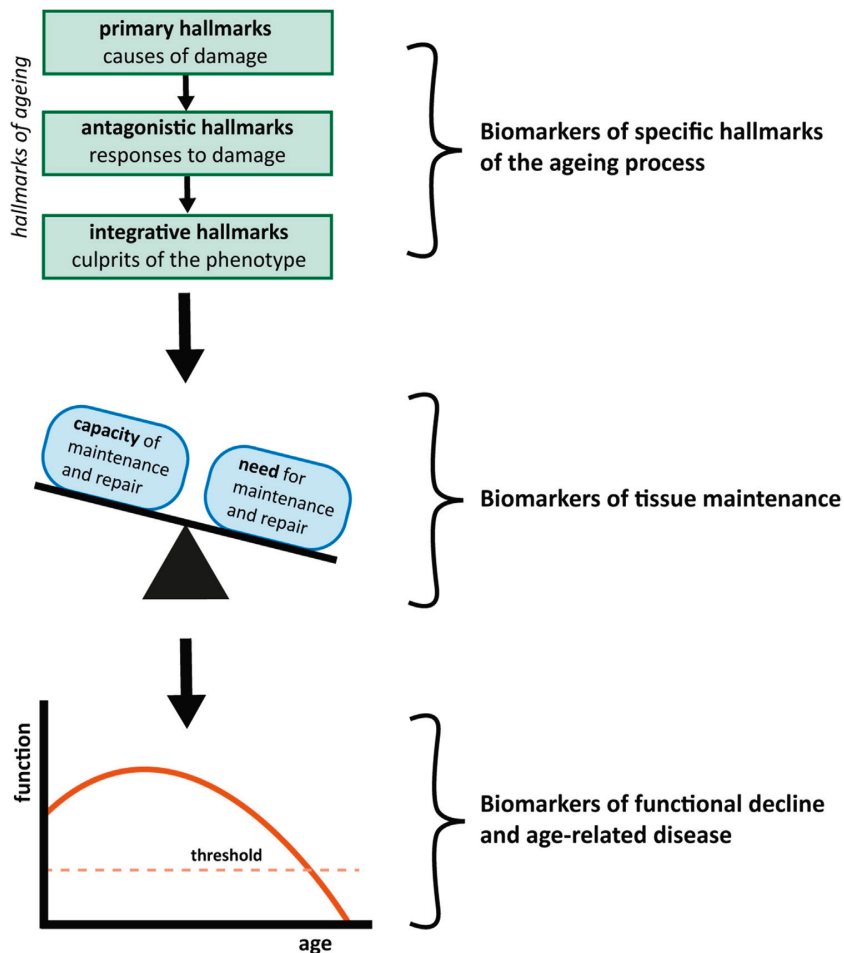
and microarchitecture, leading to increased fracture risk<sup>5</sup>. Osteoarthritis is an inflammatory process of the joints leading to damage and loss of cartilage with consequential joint pain and loss of joint mobility<sup>6</sup>, while sarcopenia is characterized by the combination of loss of skeletal muscle mass with loss of muscle strength and/or physical performance<sup>7,8</sup>. Progressive degeneration of neurons in the brain leads to neurodegenerative diseases including Parkinson's, Alzheimer's, and Huntington's disease<sup>9</sup>. Ageing is accompanied with declines in almost all our physiological systems, including the cardiovascular, respiratory, and urogenital systems, and all of these systems are crucial for maintenance of independence into old age. However, to discuss all these systems is beyond the scope of this paper. Therefore, the focus of this paper will be on the discussion of biomarkers for bone, cartilage, skeletal muscle, and brain tissue maintenance.

Several research groups were already successful in discovering biomarkers that indicate physiological and/or (sub)clinical (loss of) function of bone, cartilage, skeletal muscle, and brain, including bone mass, grip strength, gait speed and verbal fluency<sup>10-13</sup>. Preferably, biomarkers would be related to an early stage of the ageing process, where effective intervention could take place. Although some previously defined biomarkers of ageing are indeed related to earlier stages of the ageing process, we propose here to identify biomarkers specifically associated with (loss of) tissue maintenance. The specific processes underlying deficits in tissue maintenance are categorized in the conceptual framework of the hallmarks of ageing<sup>14</sup>. Factors that are associated with the different hallmarks of ageing have been investigated as possible biomarkers of ageing. Since these biomarkers have been extensively reviewed by others, these biomarkers will only briefly be discussed in this paper. The main focus of this viewpoint is on the hypothesis that disturbances in tissue maintenance are at the interface between specific hallmarks of ageing and functional decline and age-related disease.

Tissue maintenance and repair requires the replacement of lost and/or dysfunctional cells. The correct activation, amplification, and differentiation of functional adult stem cells is critically important for maintenance of the regenerative potential of tissues. Tissue regeneration continues throughout life resulting in a constant renewal of cells in the body, in order to compensate for the continuous loss of functional cells caused by exposure to internal and external stress-factors<sup>15</sup>. Tissues differ in the rates at which cells are damaged and/or lost and replaced, and as a consequence in their rate of regeneration. If tissue maintenance would be perfect, tissues could be life-long regenerated. However, reductions in numbers and flaws in the activation, amplification, and/or differentiation of functional stem cells on the one hand as well as increases in numbers and flaws in the detection, repair, and/or removal of dysfunctional cells on the other hand may lead to a reduced ability to regenerate tissues.

Therefore, we hypothesize that the capability of maintenance of tissue homeostasis decreases with age, leading to loss of function and eventually death. In contrast, longevity could be the result of a prolonged ability to maintain tissue homeostasis. Factors that reflect the activity of processes related to tissue homeostasis (i.e. the ability of repair and maintenance of tissues) might be potential biomarkers of biological age, but might also be potential early markers of age-related diseases. We propose that biomarkers of tissue maintenance comprise a novel category of biomarkers of ageing, being more tissue specific than biomarkers related to hallmarks of aging while being potentially an earlier marker of decline than the biomarkers indicating loss of function. In **Figure 1**, this central hypothesis is visually presented. However, these biomarkers should be first measured in ageing research to assess their validity before these can be used in clinical studies.

Here, we give an overview of (established) biomarkers of tissue maintenance and we will discuss their advantages and limitations for use in (pre)clinical studies. As described before, we will focus on bone, cartilage, skeletal muscle, and the brain, and on the pathophysiological processes in these tissues/organs that underlie four of the main age-related diseases; osteoporosis, osteoarthritis, sarcopenia, and neurodegenerative diseases respectively. Biomarkers described in this paper do not necessarily have to be causally involved in the process of regeneration; they could also be by-products of building up tissues or waste products of breaking down tissues. We do not aim to give a comprehensive overview of all the possible biomarkers of maintenance of these tissues, but rather a starting point when searching for potential biomarkers to measure in the context of ageing and tissue maintenance. In future studies, these biomarkers of tissue maintenance could be validated, for example by measuring them in humans with different rates of ageing.



**Figure 1.** Hypothesis: biomarkers of tissue maintenance are at the interface between the hallmarks of ageing and age-related functional decline and disease

There is a constant balance between the capacity and need for tissue maintenance and repair during life. However, it is hypothesized that the capability of maintaining tissue homeostasis decreases with age while the need for maintenance and repair increases. Consequently, due to evolved limitations in maintenance and repair, molecular damage will accumulate gradually, and interfere with the integrity of cells and tissues, thus driving functional decline and risk of age-related diseases and death. Factors that reflect the activity of processes related to tissue maintenance could therefore represent a novel category of biomarkers of ageing; at the interface between biomarkers of specific hallmarks of ageing (i.e. damage and responses to damage) and their ultimate (patho)physiological consequences (i.e. functional decline and age-related disease). However, these biomarkers should be first measured in ageing research to assess their validity before they can be used in clinic.

### **Biomarkers of biological age**

Biomarkers are defined by the World Health Organization as “any substance, its products, structure or process that can be measured in the body and that influences or predicts the incidence of outcome or disease”<sup>16</sup>. Preferably, the measurability of a biomarker is accurate and reproducible, and the biomarker has a validated association with a specific biological process or outcome, resulting in low false negatives and false positives. Intuitively, an optimal panel of biomarkers would consist of multiple biomarkers, in order to represent changes in specific organs and across different organs. Furthermore, an ideal biomarker is measured in bodily fluids that can be obtained relatively non-invasively, including blood, saliva, and urine, is assessed by a little time-consuming and low-priced assay, which is applicable for use in large-scale studies.

Biological age of an individual is challenging to measure. One of the reasons for this is that the ageing process is dependent on various external and internal factors, including genetic, environmental, and chance factors. Consequently, the human population, and especially older individuals, are a heterogeneous group. Nevertheless, several research groups were successful in discovering biomarkers that are associated with clinical features of ageing or with chronological age<sup>10-13</sup>. The ageing process becomes most noticeable when functions, including metabolic, cardiovascular, and/or cognitive function, decline under a certain threshold. Therefore, functional tests could serve as biomarkers of ageing. Tests of physical function that correlate well with biological age and/or mortality are hand grip strength, gait speed, chair stand test, and global Mini-Mental State Examination (MMSE) test<sup>10, 17-20</sup>. These markers are mostly related to a clinical feature of an older person, thus with a late phase of the ageing process. Consequently, there are fewer possibilities to intervene with the ageing process at this stage. Preferably, biomarkers of ageing are causally associated with key features of the ageing process itself.

A few years ago, Lopez-Otin *et al.* proposed to capture and categorize our currently available knowledge about the ageing process into a conceptual framework that comprises nine universal hallmarks of ageing, namely the primary hallmarks genomic instability, telomere attrition, epigenetic alterations, and loss of proteostasis, the antagonistic hallmarks deregulated nutrient sensing, mitochondrial dysfunction, and cellular senescence, and the integrative hallmarks stem cell exhaustion, and altered intercellular communication<sup>14</sup>. Theoretically, factors associated with one or more hallmarks of ageing could be potential causal biomarkers of ageing. In fact, numerous research groups have been working on the discovery of new and accurate biomarkers which are associated with the hallmarks of ageing<sup>21</sup>. The primary hallmarks of ageing reflect the accumulation of damage. Of these, diminished DNA repair

with ageing could for example be determined by measuring poly(ADP-ribose) polymerase 1 (PARP-1) expression levels, since this enzyme is one of the key players in DNA damage repair <sup>22</sup>. Also leukocyte telomere length is a potential biomarker of ageing, but the clinical evidence is yet inconclusive <sup>23</sup>. Epigenetic alterations such as DNA methylation, which involves addition or removal of methyl groups on cytosines in cytosine-phosphate-guanine (CpG) dinucleotides, can be measured using DNA methylation array technology. Several research groups are working on 'epigenetic clocks' in which these sets of CpGs are coupled with a mathematical algorithm to estimate the age of an individual. These epigenetic clocks are a promising molecular estimator of biological age <sup>24, 25</sup>. Another hallmark of ageing, loss of proteostasis, could be determined by the analysis of N-glycomic changes in glycoproteins, Advanced Glycation Endproducts (AGEs), or protein damage in blood <sup>12</sup>. Responses to damage during the ageing process are categorized as antagonistic hallmarks of ageing, including deregulated nutrient sensing. The insulin/insulin-like growth factor 1 (IGF-1) signaling pathway is important for nutrient sensing, and is one of the most evolutionarily conserved pathways across species that has been associated with ageing and longevity <sup>26</sup>. Furthermore, glucose tolerance and insulin sensitivity decline with age, which increases the risk for various age-related diseases. Insulin sensitivity and glucose tolerance are routinely assessed by measuring fasting glucose and insulin levels and by an oral glucose tolerance test <sup>27</sup>. Mitochondrial dysfunction results in the production of reactive oxygen species (ROS) which causes cellular damage <sup>28</sup>. However, the effects of ROS on ageing are probably dose dependent. Another hallmark of ageing is cellular senescence, which is defined as irreversible cell cycle arrest and which plays a role in preventing tumor formation. Although more research is required in this area, senescence seems best described by a combination of biomarkers of cellular senescence including  $\beta$ -galactosidase, elevated expression levels of p16INK4a and hypophosphorylated nuclear retinoblastoma protein, short or dysfunctional telomeres, senescence-associated heterochromatic foci (SAHF) and  $\gamma$ H2AX (DNA damage) foci, and senescent cells exhibit a senescence associated secretory phenotype (SASP) <sup>29, 30</sup>. The integrative hallmarks comprise stem cell exhaustion and altered intercellular communication. Hematopoietic stem cells (HSCs) and progenitor cells (HSPCs), which are located in the bone marrow, renew blood cells, comprising red cells, white cells, and platelets. Surface markers of HSCs and HSPCs are Lin-CD34+CD38-CD90-/+CD45RA-Flt3+CD7-CD10-, which could be potential markers to determine the level of stem cell exhaustion <sup>31</sup>. Besides increases in immunosenescence and inflammaging, features of altered intercellular communication comprise the decline in sex hormones with age, including estrogen and testosterone <sup>32, 33</sup>. All of these processes and hallmarks may ultimately result in an age-related decline in tissue maintenance. Identifying biomarkers of tissue maintenance is the central focus of the current paper.

## Bone tissue

Bone is a connective tissue in which different types of cells are separated by a matrix that is composed of collagen fibres impregnated with minerals. Bone tissue is dynamic as it is continuously resorbed, renewed, and remodeled. In healthy adults, approximately 25% of trabecular bone, the internal tissue of the skeletal bone with an open cell porous network, is remodeled annually. The outer denser layer, cortical bone, remodels less frequently<sup>32</sup>. The whole skeleton is renewed every 7-10 years. During the bone remodeling cycle, bone is resorbed at approximately the same rate as new bone is formed. Within the basic multicellular unit, bone cells control bone formation and resorption. Bone turnover starts with resorption of old or damaged bone by osteoclasts, followed by the formation of new bone by osteoblasts, which is partly regulated by osteocytes. Osteocytes are transformed osteoblasts when surrounded by osteoid<sup>34</sup>. Mechanical loading is important for maintaining bone mass and studies have shown that both bone formation and resorption markers are increased after physical activity<sup>35</sup>.

## Osteoporosis

Bone resorption starts to exceed bone formation around the age of 30 years, causing gradual bone loss with ageing. This gradual bone loss could result in osteoporosis which is characterized by reduced bone mass, strength, and microarchitecture, leading to increased fracture risk. The prevalence of osteoporosis increases with age, and especially postmenopausal women have an increased risk of osteoporosis because of the reduction in estrogen. Approximately 6% of European men and 21% of European women in the age category of 50-84 years are diagnosed with osteoporosis<sup>5</sup>. Besides age and being postmenopausal, other risk factors for low bone mineral density (BMD) are family history of osteoporosis, cigarette smoking, low physical activity, low body mass index, low calcium intake, low vitamin D level, and use of corticosteroids<sup>36</sup>. Osteoporosis is primarily diagnosed by BMD of the spine and proximal femur, which is measured by dual-energy X-ray absorptiometry (DEXA). An individual is diagnosed with osteoporosis if their BMD (i.e. T score) is at least 2.5 standard deviations below the average BMD value for young healthy white women<sup>37</sup>.<sup>38</sup> Unfortunately, osteoporosis is often only diagnosed after an incident of a fracture or when the BMD is already very low. Moreover, 37.1% of male and 26.4% of female patients with a hip fracture die within one year<sup>39</sup>. Therefore, early detection and prevention are necessary. Pharmacological interventions consist mainly of bisphosphonates, strontium ranelate, raloxifene, denosumab, and parathyroid hormone peptides. These treatments are mostly effective, but adverse effects, including nausea, headache, skin conditions, and leg edema, are common<sup>5</sup>.

### **Biomarkers of bone tissue turnover**

Bone turnover markers have been identified and reviewed by others<sup>40-42</sup>. Most of the identified bone markers are cellular components of the bone matrix, with N-terminal propeptide of type 1 procollagen (PINP) and procollagen type 1 C-terminal propeptide (PICP) as markers of bone formation. Markers of bone resorption derived from cellular components of the bone matrix are C-terminal cross-linked telopeptide of type 1 collagen (CTX-I), N-terminal cross-linked telopeptide of type 1 collagen (NTX-I), type 1 collagen alpha 1 helicoidal peptide (HELP), deoxypyridinoline (DPD), and pyridinoline (PYD). Two other specific and sensitive markers of bone formation are bone-specific alkaline phosphatase (BSAP) and serum osteocalcin. Serum tartrate-resistant acid phosphatase – isoform 5b (TRAP5b) and cathepsin K are other markers of bone resorption. Receptor activator of nuclear factor  $\kappa$ -B ligand (RANKL) and osteoprotegerin (OPG) are two proteins produced by osteoblasts. Most of these biomarkers can be measured in serum, EDTA plasma or urine. Bone turnover markers are used in clinical trials for determining the efficacy and the response to osteoporosis treatments, but for most bone markers the predictive and diagnostic value is currently limited. Vasikaran *et al.* recommended to use serum CTX-I as reference biomarker of bone resorption and serum PINP as reference biomarker of bone formation<sup>43</sup>. These two of the most widely used biomarkers of bone turnover are by-products of forming or degrading the main component of bone, type 1 collagen. An overview of the selected proposed bone markers can be found in

### **Figure 2.**

#### *CTX-I*

In the process of bone resorption, bone collagen is broken down by cleavage of the cross-linked type 1 collagen by cathepsin K, which is expressed by osteoclasts. Subsequently, the N- and C-terminal cross-linked telopeptides of type 1 collagen (NTX-I and CTX-I) are released into the blood circulation, with CTX-I as the preferred marker of bone resorption. CTX-I could be measured in serum, EDTA plasma, or urine<sup>44</sup>. CTX-I is relatively stable at room temperature, especially in EDTA<sup>45</sup>. Serum CTX-I decreased with age in both men and women. However, CTX-I levels gradually increased in men after 40-50 years of age and increased in postmenopausal women. CTX-I levels were lower in premenopausal women than in men, but levels were highest in women after menopause<sup>46-48</sup>. CTX-I shows a circadian rhythm with its nadir in the afternoon around 14:00 hours and its maximum in the night around 5:00 hours<sup>49,50</sup>. Food intake causes a reduction in CTX-I levels while fasting causes an increase in CTX-I levels, thereby strongly influencing the circadian rhythm of CTX-I<sup>51-54</sup>. In contrast, the circadian rhythm of CTX-I is not influenced by age, sex, postmenopausal status, bed rest of 5 days, by absence of a circadian rhythm of cortisol, by absence of a light-dark cycle

(blindness), or by low bone mass<sup>53,55</sup>. Generally, biomarkers of bone resorption show a stronger circadian rhythm than biomarkers of bone formation<sup>40,42</sup>.

### *PINP*

When bone is formed, osteoblasts secrete the precursor of type 1 collagen, i.e. type 1 procollagen. To form type 1 collagen, the carboxy and aminoterminal extension peptides are enzymatically cleaved off. These C- and N-terminal propeptides of type 1 procollagen (PICP and PINP) are waste products and released into the circulation. Both PICP and PINP are considered to reflect newly formed type 1 collagen. However, circulating PINP is most specific and is therefore considered as the key biomarker of bone formation. PINP can be measured in serum and EDTA plasma. PINP has a low intra individual variability, a good assay precision, and is stable at room temperature<sup>43,45,56-58</sup>. Studies on the 24-hour rhythm of PINP are conflicting. Two studies did not observe a 24-hour rhythm, while two other studies found somewhat higher levels in the night, albeit in men only in one of the two studies<sup>55-58</sup>. PINP levels were higher in men compared to premenopausal women, but levels were higher in postmenopausal women. With age, PINP levels decreased in both men and women, but increased after menopause in women<sup>47,48</sup>. PINP levels were slightly influenced by food intake, for example when measured in the fed state or during an intravenous glucose tolerance test, PINP levels were somewhat lower, but oral glucose ingestion did not have a significant effect on PINP levels<sup>59-61</sup>.

### *Osteocalcin*

Besides collagen, osteoblasts also produce a protein called osteocalcin at sites of new bone formation. Osteocalcin is mostly incorporated into the bone matrix, but a small fraction is released into the circulation<sup>62</sup>. The specific function of osteocalcin remains debatable, but it is suggested to be involved in bone mineralization. Since the process of osteoid mineralization occurs in a late stadium of the bone turnover process, osteocalcin is a late marker of osteoblast activity. Furthermore, since osteocalcin is part of the bone matrix, osteocalcin fragments are also released into the circulation during bone resorption. Therefore osteocalcin is commonly used as a biomarker of bone turnover. Osteocalcin levels decreased with age in both men and women, but increased after menopause in women and increased slightly after age 65 for men. Osteocalcin levels were higher in young men than in premenopausal women, but lower in older men compared to women after menopause<sup>46,63,64</sup>. Serum osteocalcin shows a circadian rhythm with its nadir in the afternoon and its peak at night. Age, sex, or menopausal status did not influence this circadian rhythm<sup>65-69</sup>.

### *Other (potential) biomarkers of bone tissue turnover*

The most widely used bone markers, CTX-I, PINP, and osteocalcin, represent the function of osteoblasts or osteoclasts. However, the osteocyte is the most abundant cell type of bone tissue and plays a key regulatory role in bone and mineral homeostasis<sup>70</sup>. Therefore, markers of osteocyte activity are, although less established, promising novel biomarkers of bone turnover. Osteocytes are multifunctional cells which are differentiated osteoblasts, that control calcium and phosphate levels, and detect mechanical forces<sup>70</sup>. Osteocytes were found to play a key role in the regulation of bone turnover by producing factors including sclerostin and Dickkopf-related protein 1 (DKK1). These factors are negative regulators of bone formation that inhibit osteoblast activity via blocking the Wnt signaling pathway by antagonizing the Wnt/lipoprotein receptor-related protein 5<sup>71,72</sup>. Serum sclerostin levels were higher in men than in women and levels correlated positively with age<sup>73-77</sup>. No circadian rhythm of sclerostin has been observed, although sclerostin levels varied over 24 hours in one study<sup>55,56</sup>. DKK1 levels were somewhat higher in older individuals than in younger individuals and levels were higher in female than in male geriatric patients<sup>77,78</sup>. In one study, DKK1 levels were found to have a large interindividual and intraindividual variation, so more research is needed before DKK1 can be recommended as a reliable marker for diagnostic or research purposes<sup>55</sup>. Furthermore, although osteocytes were found to play a key role in the regulation of bone turnover, it is questionable whether sclerostin and DKK1 are specific markers of bone turnover. Since osteocytes are multifunctional cells which for example also control calcium and phosphate levels, sclerostin and DKK1 might be suitable as general markers of bone activity but these markers might not be specific enough for the processes of bone resorption and formation.

### **Cartilage**

Cartilage tissue consists of chondrocytes surrounded by an extracellular matrix (ECM) which defines its properties. Three types of cartilage can be distinguished: elastic cartilage, hyaline cartilage and fibrocartilage. Articular cartilage is hyaline cartilage covering the joint surfaces. In hyaline cartilage, the ECM consists predominantly of collagen type 2, proteoglycan aggregates containing glycosaminoglycans (GAGs) and other non-collagenous proteins. Collagen stabilizes and strengthens the tissue<sup>79</sup>, while the proteoglycans bind water through their negative charge, conferring flexibility and shock-absorbing properties<sup>80</sup>, both vital to cartilage function. Turnover rate of articular cartilage ECM is generally slow but differs dramatically of each of its components; proteoglycans have an estimated half-life of 25 years<sup>81</sup>, while the estimated half-life of collagen in cartilage equals 117 years<sup>82</sup>.

## Osteoarthritis

The integrity of articular cartilage can be disrupted, due to major trauma or age-related degenerative changes. If the disruption is larger than the repair capacity, osteoarthritis may develop. Osteoarthritis is characterized by damage and eventually loss of articular cartilage, as well as remodeling of subchondral bone and aberrant bone formation at the joints margins called osteophytes<sup>83</sup>. Patients often experience joint pain and stiffness, and in severe disease limitation of movement<sup>6</sup>. The definition used in literature for presence of osteoarthritis varies, from purely radiographical criteria for which the most commonly used is the Kellgren and Lawrence score<sup>84</sup> to various questionnaire scores on subjective complaints expressed by patients. In clinical practice, the diagnosis is commonly based on the combination of features in the medical history and physical examination, sometimes supplemented with diagnostic imaging<sup>6</sup>. The reported prevalence of osteoarthritis varies between studies, although the World Health Organization estimates a prevalence of 9.6% in men and 18.0% in women worldwide<sup>85</sup>. The established risk factors for osteoarthritis include obesity, high age and female sex<sup>86</sup>, whereas the respective roles of smoking, bone density and dietary factors are still under debate<sup>86</sup>. With the increasing rates of obesity and the ageing of the population, a rise in incidence of osteoarthritis can be expected in the near future. The current treatment consists of analgesic prescription and lifestyle advise, and in late stage of disease surgical joint replacement<sup>6</sup>, which is associated with high perioperative morbidity and persistent functional impairment after 12 months<sup>87</sup>. Because of the high burden of the disease symptoms and shortcomings of current treatment modalities, identification of people at risk to target for prevention is essential. Furthermore, new treatments could possibly slow down progression in pre-symptomatic patients to postpone or even prevent symptoms and surgical treatment. To identify patients who might benefit from these treatments and to evaluate effects of treatment, we need biomarkers that give an insight in the processes taking place in articular cartilage.

## Biomarkers of cartilage turnover

Several biomarkers of articular cartilage turnover have been identified in the context of arthropathies, including osteoarthritis, rheumatoid arthritis and ankylosing spondylitis<sup>41, 88</sup>. These markers can be grouped based on the particular process they are associated with or whether they are collagen-derived or not. Most markers of cartilage turnover are based on collagen type 2 metabolism; markers for degradation include C-terminal cross-linked telopeptide of type 2 collagen (CTX-II) and type 2 collagen fragments (C2C), while the predominant markers for formation are the C- and N-terminal propeptide of type 2 procollagen (PIICP and PIINP)<sup>41, 88, 89</sup>. The main non-collagenous marker of cartilage turnover is cartilage oligomeric matrix protein (COMP), a glycoprotein constituent of articular cartilage<sup>41, 90</sup>. Additionally, some markers of aggrecan

degradation by matrix metalloproteinases (MMPs) and aggrecanases have been described such as keratin sulphate, which are mostly elevated after joint injury <sup>41</sup>. The main proposed biomarkers of cartilage turnover are listed in **Figure 2**.

### *COMP*

The ECM of articular cartilage comprises numerous proteins, all contributing to optimum functionality of the joint. One of the regulatory constituents of cartilage ECM is cartilage oligomeric matrix protein (COMP), a large pentameric glycoprotein secreted by chondrocytes <sup>91</sup>. COMP interacts with several other ECM proteins, aiding matrix assembly and stability through binding collagen fibres <sup>92</sup>. Upon degradation of cartilage, COMP is released into the synovial fluid and consequently leaks into the circulation <sup>93</sup>. Although COMP was first discovered in cartilage, it is expressed in multiple other tissues including tendons, synovium, cardiomyocytes and activated platelets <sup>91</sup>. Nevertheless, COMP is one of the most robust biomarkers for osteoarthritis <sup>94</sup>. Measurement of COMP can occur in serum or plasma <sup>95</sup> and in synovial fluid <sup>96</sup>, though collection of synovial fluid in healthy individuals proved difficult to perform <sup>97</sup>. Physical activity was found to increase circulating COMP levels acutely <sup>97-99</sup>, and a second peak occurs five hours after exercise <sup>99</sup>. However, the effect of exercise is not as pronounced in individuals who exercise habitually <sup>100, 101</sup>. On the contrary, bed rest decreases circulating levels of COMP <sup>99, 102</sup>, though serum levels normalize within one day after discontinuation of bed rest <sup>102</sup>. Because of its sensitivity to physical activity, most studies include a 24 to 36 hour limitation of exercise prior to the test day <sup>98, 99</sup> and a short resting period of 15 to 30 minutes immediately before drawing blood for COMP measurements to reduce effects of activities of daily living on circulating COMP levels <sup>98, 99, 101</sup>.

### *CTX-II*

Upon enzymatic degradation of collagen type 2, cross-linked C-telopeptide of collagen type 2 (CTX-II) is one of the collagen fragments released <sup>103</sup>. CTX-II is a very promising biomarker for cartilage degradation due to the high specificity of collagen type 2 for cartilage, and its strong correlation with joint diseases <sup>104</sup>. Measurement can be performed in plasma <sup>105</sup>, urine <sup>104</sup>, serum and synovial fluid <sup>106</sup>. Urinary excretion of CTX-II (uCTX-II) is the most common measurement, which is also very convenient considering the high stability of uCTX-II at room temperature and after multiple freeze-thaw cycles <sup>104</sup>. Generally, uCTX-II levels are lower in older than in young adults <sup>107, 108</sup>. Individuals with a higher BMI tend to have higher uCTX-II than lean individuals <sup>107, 109, 110</sup>. Levels of uCTX-II are higher in women than in men <sup>107, 110</sup>. Estrogen levels appear to influence uCTX-II, since the highest levels are observed in postmenopausal women <sup>107, 111</sup> and a decrease of uCTX-II was described with the use hormonal replacement therapy <sup>107, 112</sup> and selective estrogen receptor modulator <sup>113</sup>. Bisphosphonate use was shown to

decrease uCTX-II in postmenopausal women, with return to baseline levels after withdrawal of bisphosphonate treatment <sup>114</sup>. The presence of a circadian rhythm in uCTX-II is not established; one study did find a circadian rhythm similar to CTX-I with a nadir in the late afternoon and evening <sup>110</sup>, while no rhythmicity was observed in an earlier study <sup>104</sup>. Seasonal variation in plasma levels of CTX-II was observed, with a peak in November and nadir in May, possibly explained through seasonal variation in serum vitamin D levels which influences bone metabolism and thereby indirectly collagen type II degradation <sup>105</sup>.

### *PIINP*

Type 2 collagen is first released as a procollagen during synthesis with propeptides at the N- and C-terminus, which are removed by specific proteinases before deposition into fibrils of the ECM <sup>115</sup>. The N-terminal propeptide of type 2 procollagen (PIINP) can be measured in synovial fluid and serum, and the concentrations reflect the anabolic state of the chondrocytes <sup>116</sup>. However, type 2 procollagen is synthesized in two variants due to alternative splicing, resulting in procollagen type 2A and type 2B <sup>117</sup>. The splicing is cell type specific: procollagen 2A appears predominantly in chondroprogenitor cells, whereas type 2B is mainly produced by mature chondrocytes <sup>118</sup>. Both splice variants have distinct N-terminal propeptides; PIIANP and PIIBNP respectively. In osteoarthritis patients, PIIANP was present in deep zone cartilage <sup>119</sup> and higher circulating levels of PIIANP were associated with slower progression of disease <sup>120</sup>. However, transcription of procollagen type 2A was only seen in osteoarthritic cartilage and not in healthy cartilage tissue <sup>121</sup>. Therefore, PIIANP may not be a suitable biomarker for turnover of healthy cartilage. PIIBNP would be a more appropriate biomarker of cartilage formation in healthy populations, regarding its origin from adult cartilage tissue. An ELISA assay specific for PIIBNP has been developed <sup>122</sup>, and has recently been used to distinguish arthropathies <sup>123,124</sup> and for early drug development <sup>125,126</sup>. Even though further validation is still warranted, PIIBNP is a promising biomarker for collagen type 2 synthesis in healthy adult populations.

### **Skeletal muscle**

Skeletal muscle protein synthesis and degradation are in constant balance. Each skeletal muscle cell contains parallel arrays of myofibrils, which are composed of hundreds or thousands repeating units called sarcomeres. A sarcomere is the structural unit of skeletal muscle and consists of smaller interdigitating myofilaments; thin and thick filaments. Thin filaments are polymers of proteins with actin as the main component and molecules of tropomyosin and troponin as regulatory proteins binding to actin. Myosin-II molecules are the primary component of thick filaments. The coordinated interaction among troponin, tropomyosin, and actin allows actin-myosin interactions to be regulated by changes in calcium concentration, with an increase in calcium concentration

triggering contraction of the muscle<sup>34</sup>. An imbalance between the rate of skeletal muscle protein synthesis and breakdown results in loss of skeletal muscle mass, which occurs during ageing, disease, or inactivity<sup>127</sup>. Bedrest particularly results in increased nitrogen excretion and to a lesser extent in whole-body protein breakdown, indicating that mainly protein synthesis is inhibited during bed rest<sup>128, 129</sup>.

### **Sarcopenia**

Sarcopenia is an age-related disease that is associated with loss of skeletal muscle mass in combination with loss of muscle strength and/or physical performance<sup>7, 8</sup>. Ageing is accompanied by changes in body composition comprising an increase in fat mass and a decrease in lean body mass, in particular a decrease in skeletal muscle and bone mass. The causes of sarcopenia are multifactorial, and risk factors for sarcopenia have been reviewed by others and were found to comprise, apart from chronological age, genetic heritability, chronic disease, inflammation, insulin resistance, low physical activity, insufficient protein intake, and low estrogen, testosterone, and vitamin D levels<sup>7, 36</sup>. Sarcopenia can be diagnosed when a patient's muscle mass is less than 20<sup>th</sup> percentile of values for healthy young adults, determined by 24-hour urinary creatinine (except in patients with renal insufficiency), or more indirect by bioelectrical impedance, DEXA or imaging techniques, in combination with muscle strength (low grip strength) and poor physical functioning determined by a gait speed of 0.8 m/s or less<sup>8</sup>. Sarcopenia biomarkers have been reviewed elsewhere<sup>130</sup>. Sarcopenia is a strong predictor of late-life disability<sup>131, 132</sup>. The prevalence of sarcopenia is estimated in community-dwelling adults aged 60 years and older at 10% of men and 10% of women worldwide, rising rapidly up to 50% of men and 43.8% of women at age 80 years and older<sup>133</sup>. Early detection and prevention are necessary. Muscle resistance training combined with amino acid-containing supplements is the preferred treatment to prevent (progression of) sarcopenia in older persons<sup>134</sup>.

### **Biomarkers of muscle protein synthesis and breakdown**

Several biological markers have been shown to be associated with skeletal muscle mass, strength, and function. However, most of these biomarkers are not muscle-specific, including hemoglobin, serum albumin, urinary creatinine, and inflammatory biomarkers such as circulating C-reactive protein (CRP), interleukin 6 (IL-6), and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ )<sup>135, 136</sup>. Muscle-specific biomarkers are serum myostatin, which is a suppressor of muscle growth<sup>137</sup>, and an isoform of serum creatine kinase (CK-MM), which is a muscle enzyme that is increased as a reaction to muscle damage, for example after exercise<sup>138</sup>. An overview of the main proposed biomarkers of muscle protein synthesis and breakdown is given in **Figure 2**.

### *3-methylhistidine*

The rate of muscle protein breakdown can be determined by measuring the rate of excretion of 3-methylhistidine. 3-methylhistidine is an amino acid which is formed by post-translational methylation of histidine residues in the myofibrillar proteins actin and myosin and it is released after breakdown of both proteins<sup>139, 140</sup>. 3-methylhistidine is preferably measured in (24-hours) urine, but can also be detected in serum or plasma. Preferably, a meat-free diet is initiated around 3 days before measurements to reduce the effect of dietary 3-methylhistidine intake. However, one study showed that when using a tracer-based method this might not be necessary<sup>141</sup>. Levels of 3-methylhistidine were not responsive to exercise<sup>142, 143</sup>.

### *PIIINP*

A biomarker of skeletal muscle remodeling is plasma N-terminal propeptide of type 3 procollagen (PIIINP)<sup>136, 144</sup>. P3NP is a fragment released during final stages of collagen type 3 synthesis in soft connective tissues. During this process, N- and C-terminal portions of procollagen type 3 are removed by specific proteinases and these fragments are released in the circulation<sup>145-147</sup>. Consequently, PIIINP can be measured in serum with precision and accuracy. PIIINP levels are shown to respond to exercise, testosterone, and growth hormone (GH)<sup>148-152</sup>.

### *Infusion of isotopically labelled amino acids*

Another method to determine the rate of protein turnover in muscle is by infusion of isotopes<sup>153, 154</sup>. The general protocol for this method is to infuse isotopically labeled amino acids and subsequently measure their incorporation into muscle tissue. First, isotopically labeled amino acids such as phenylalanine and leucine are produced. Secondly, after taking a baseline blood sample and/or muscle biopsy, these amino acids are infused in the bloodstream via a catheter, in a single dose or, preferably, continuously. Subsequently, blood is withdrawn at specific time points or muscle biopsies are taken to determine the rate of muscle protein synthesis. Usually, this method is combined with a meat-free or protein controlled diet. This approach is a robust method, but a disadvantage of this method is that it is invasive, especially when muscle biopsies are taken.

## **Brain**

Brain tissue comprises of a collection of different cell types including neurons, astrocytes, and microglia. Together with the extensive vasculature, they create a dynamic and highly interdependent entity. Although each component has its own function, neurons are considered the main functional component of the brain. Therefore, the neuronal cells have been studied most extensively. In rodents, adult neurogenesis was first demonstrated in the dentate gyrus<sup>155</sup>, and later in the

subventricular zone<sup>156</sup> from which the new neurons also migrate to other areas of the brain<sup>157</sup>. However, in human brains the presence of adult neurogenesis is still under debate<sup>158, 159</sup>. If there is indeed limited or no production of new neurons in adults, then preservation of existent neurons is of crucial importance. Consequently, clearance of potentially neurotoxic substances and waste materials for optimal neuroprotection might be of greater importance to tissue homeostasis in the brain than cellular turnover.

### **Neurodegenerative disease**

The hallmark of neurodegenerative diseases is progressive loss of neurons, accompanied by gradual development of cognitive impairments and motor disorders<sup>9</sup>. According to previous research in model organisms and in post-mortem studies of human brains the neuronal loss is often accompanied by accumulation of waste products, e.g. amyloid  $\beta$ <sup>160</sup>, neurofibrillary tangles<sup>161</sup> and Lewy body inclusions<sup>162</sup>. What distinguishes the different disease entities is the localization of the most pronounced neuronal loss; e.g. for Alzheimer's disease the hippocampus and frontotemporal lobes are the most affected structures<sup>163</sup>, while in Parkinson's disease the substantia nigra and the basal ganglia are predominantly affected<sup>164</sup>. The nature of the symptoms of different neurodegenerative diseases corresponds to the functional loss of anatomical region affected, however severity of anatomical disturbances do not correspond directly with severity of symptoms<sup>165, 166</sup>. Since the course of neurodegenerative diseases is generally gradual and slow, a long period of subclinical disease such as mild cognitive impairment can be observed but is also frequently missed due to the insidious onset<sup>9</sup>. Therefore, most degenerative diseases are diagnosed when severe damage has already occurred, because only by then clearly distinguishable functional decline has developed. The disease etiology has not been fully elucidated; apart from genetic predisposition, advanced age is still the major risk factor for neurodegenerative diseases<sup>167</sup>. Other risk factors include sedentary lifestyle, obesity, tobacco smoking and hypertension<sup>168</sup>. Currently no disease modifying therapies exist for neurodegenerative diseases, despite decades of pharmaceutical research<sup>169</sup>. This could be due to the advanced stage of disease and extensive brain damage at time of diagnosis, hence the repair capacities might be exhausted. Therefore, earlier detection of processes of neurodegeneration might identify a window of opportunity for disease modifying therapies.

### **Biomarkers of brain tissue maintenance**

Research into early markers of neurodegenerative diseases has been conducted extensively, especially for Alzheimer's dementia<sup>170</sup>. However, human brain tissue is inaccessible for histological examination during life. Furthermore, measurements in serum or plasma do not always reflect the cerebral milieu

due to the blood brain barrier. Cerebrospinal fluid (CSF) is closer to the brain environment, though the acquisition via lumbar puncture is rather invasive. Therefore, most biomarkers for neuronal turnover are derived from translational research in model organisms, or are merely predictive markers mostly developed to predict incident dementia in individuals with or without mild cognitive impairment. Currently, the most promising markers for adult neurogenesis and neurodegeneration from translational research are circulating regulatory molecules such as microRNAs (miRNAs)<sup>171</sup>, natriuretic peptides (NPs)<sup>172</sup>, brain-derived neurotrophic factor (BDNF)<sup>173</sup>, and glial cell line-derived neurotrophic factor (GDNF)<sup>174, 175</sup>. The predictive markers can be grouped to three categories; they are related to accumulation of misfolded proteins, progressive brain atrophy or energy metabolism. Accumulation of amyloid  $\beta_{42}$  ( $A\beta_{42}$ ) and tau can be observed before brain atrophy occurs<sup>176</sup>, and can be considered an early marker of disturbed tissue homeostasis. After prolonged net neurodegeneration, brain atrophy can be visualized using different imaging techniques<sup>177</sup>. For energy metabolism several metabolomic biomarkers have been associated with dementia risk, of which especially compounds from lipid metabolism appear to correlate with risk of dementia<sup>178</sup>.

### *Brain atrophy*

Radiological techniques can give unique insight in the anatomical and physiological features of the brain *in vivo*. Structural imaging, using computed tomography (CT) or preferably magnetic resonance imaging (MRI), can reveal several anatomical changes reflecting tissue damage and loss. Atrophy of the medial temporal lobe and hippocampus can be observed in Alzheimer-related pathology<sup>179, 180</sup>, while in early stage Parkinson's disease atrophy of the substantia nigra and basal ganglia are the primary abnormalities<sup>164</sup>. More general anatomical abnormalities include signs of cerebral small vessel disease and loss of cortical thickness<sup>177, 181</sup>. Although the resolution of MRI scans has improved dramatically over the past decades from 0.5 Tesla to 7 Tesla<sup>182</sup>, volumetric changes are only perceivable after a prolonged period of net tissue loss. Therefore, it is a relatively late marker of brain tissue damage. A more subtle instrument could be fluorodeoxyglucose (<sup>18</sup>F-FDG) positron emission tomography (PET), which measures glucose consumption at the synaptic level as a reflection of neuronal activity<sup>183</sup>. However, quite some limitations currently remain for using <sup>18</sup>F-FDG-PET in research setting, including lack of quantitative analysis methods and distortion of the results when cortical atrophy is present<sup>184, 185</sup>. Generally, imaging techniques as biomarkers have several limitations. Most measures of atrophy correlate well with clinical outcomes on a group level, but on an individual level these correlations are weak<sup>166</sup>. Furthermore, CT and PET-scanning involve radiation exposure for the study participant, the required scanners are not yet

widely available and the economic costs for imaging technologies are relatively high.

### *A $\beta_{42}$ and tau*

As neuroprotection might be more important than cellular turnover in maintenance of the brain, accumulation of potentially harmful waste products could also be used as a biomarker for tissue homeostasis. Accordingly, accumulation of misfolded proteins such as fibrillar A $\beta$  and neurofibrillary tangles are among the earliest abnormalities in neurodegenerative processes<sup>176</sup>. It is hypothesized that these accumulated proteins are neurotoxic, especially oligomerization appears to contribute to neurotoxic effects of A $\beta_{42}$  and tau<sup>160, 161</sup>.

Since histological examination for abnormalities is only possible post-mortem, alternative measurements were sought. Amyloid  $\beta$  can be measured in CSF and in plasma and by using targeted PET imaging<sup>186, 187</sup>. Counterintuitively, higher plaque burden of amyloid deposition on autopsy was associated with lower concentrations of A $\beta_{42}$  in the CSF<sup>188, 189</sup>. This could be explained as a sign of reduced clearance of amyloid  $\beta$  from the brain. Although A $\beta_{42}$  can also be measured in plasma, these levels do not reflect cortical burden of amyloid plaques<sup>190</sup>. Cerebral burden of amyloid  $\beta$  can also be measured using PET. The first method developed is Pittsburg Compound-B (PiB) PET<sup>187</sup>. PiB PET imaging is consistent with post-mortem amyloid  $\beta$  plaque burden<sup>191, 192</sup>. Since PiB is technically difficult to work with due to its short half-life of only 20 minutes, alternative ligands have been developed with similar imaging properties<sup>193, 194</sup>. For all measurements related to amyloid  $\beta$ , it is important to realize that there is a strong influence of age and ApoE  $\epsilon$ 4 genotype<sup>195, 196</sup>.

For tau, the total tau concentration can be measured in CSF but also the phosphorylated segment. While tau is considered a rather general marker of axonal damage<sup>197</sup>, phosphorylated tau (p-tau) is thought to be more specific for neurofibrillary tangles<sup>198</sup>. Nevertheless, higher CSF concentrations of both total tau (t-tau) and p-tau were associated with more neurofibrillary pathologic abnormalities on autopsy<sup>189, 198</sup>. As an alternative to CSF measurements, p-tau in plasma can also be used as a marker, however the results are not as consistent as in CSF<sup>186</sup>. For measurements of tau the factors of influence are not fully clarified, though ApoE  $\epsilon$ 4 genotype and possibly age and sex appear to associate with higher tau concentrations<sup>199, 200</sup>.

### *Other (potential) biomarkers of brain tissue maintenance*

Although measures of atrophy and cerebral accumulation of misfolded proteins have been investigated extensively, they are far from perfect biomarkers. Both markers represent a relatively late result of flawed processes, and the acquisition

of these markers is costly and currently lacking accuracy. Ideally, biomarkers of brain tissue homeostasis would closely represent ongoing processes in the brain. Regulatory molecules are the most promising candidates identified by translational research. For example miRNAs, which are short non-coding RNA molecules (up to 23 nucleotides) which regulate gene expression post-transcriptionally by repressing translation<sup>201</sup>. Several miRNAs appear to spatially and temporarily regulate replication and differentiation of neurons<sup>171, 202, 203</sup>. However, validation of identified miRNA signals is scarce and many studies have shown discordant results<sup>204</sup>. Other potentially interesting regulatory molecules could be NPs, which were shown to play an essential role in fluid homeostasis and neuro-inflammation<sup>172</sup>. BDNF could also be of interest as a biomarker, though previous research results have been conflicting<sup>173</sup>. Considering the absence of any truly suitable marker reflecting processes of brain homeostasis, broader approaches for identifying biomarkers could be of help. Broad panels of metabolomics and proteomics are commonly utilized to identify new markers of neurodegenerative diseases<sup>205</sup>. This method has several advantages for use as a biomarker; the measurements can be performed in serum, plasma or urine, most platforms are quite robust and a combination of suitable markers can be used for a comprehensive approach<sup>205-207</sup>. However, the different platforms that are available vary greatly in number and type of metabolites and proteins measured, and the limited overlap between the platforms hampers comparison of different studies<sup>205</sup>.

## DISCUSSION

Here, we present the hypothesis that biomarkers of tissue maintenance represent a novel category of biomarkers of ageing, at the interface between biomarkers of the hallmarks of ageing and markers of age-related functional decline and disease. However, these biomarkers should be first measured in ageing studies to assess their validity before they can be used in clinic. A panel of biomarkers for bone, cartilage, muscle, and brain tissue maintenance is proposed and their advantages and limitations for use in (pre)clinical studies are discussed. Established biomarkers are available for bone tissue, but biomarkers for cartilage and muscle tissue are less established and should be investigated in further studies. For brain tissue maintenance, no suitable established biomarkers are currently available. **Figure 2** comprises an overview of the proposed biomarkers.

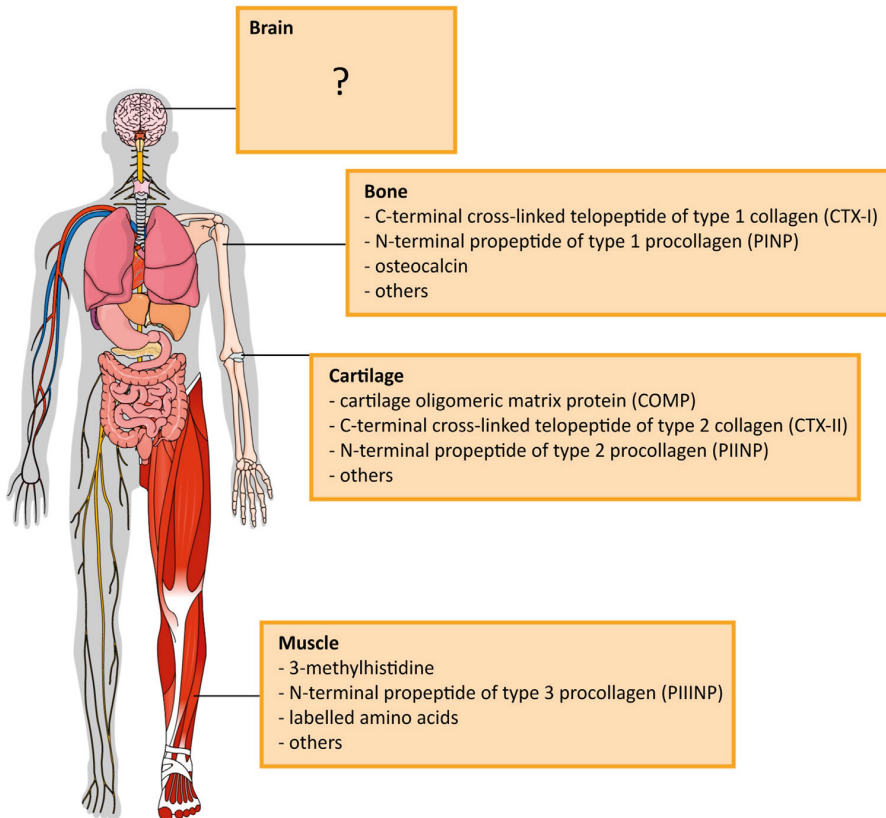
These biomarkers can be measured in future ageing research to determine whether these biomarkers are indeed potential early markers of the ageing process, and in particular age-related diseases including osteoporosis, osteoarthritis, sarcopenia, and neurodegenerative diseases. At this moment it is unknown whether it is beneficial to have elevated levels of biomarkers of

tissue maintenance or reduced levels (assuming that the amount of damage is equal). Higher levels of tissue turnover could indicate that the tissue is capable to regenerate, which is positive for health and lifespan. However, higher levels could also indicate that eventually the capacity of regeneration will decrease faster, leading to earlier stem cell exhaustion, and thus an earlier onset of age-related functional decline. Lower levels of tissue maintenance and repair could indicate that many cells are already in senescence, so fewer cells are able to regenerate, which is undesirable. On the other hand, lower levels could also indicate that the tissue is only regenerating itself when it is really necessary leading to prolonged ability for maintenance and repair, and thus increased lifespan. Moreover, it could be that it is beneficial to have elevated levels at young age but reduced levels at old age or the other way around. To determine which of these scenarios is most likely, the proposed biomarkers could for example be measured in humans with different rates of ageing. Subjects that could be included in these validation studies are healthy individuals of different chronological ages, people showing delayed biological ageing, and people showing accelerated biological ageing. In a group of healthy individuals with the same chronological age, biological age can be quantified by (sub)clinical biomarkers of ageing described by others, since most of these biomarkers are well-defined and highly correlated with ageing. Subsequently, the group could be divided into individuals with poor functional status, i.e. with high biological age, and individuals with good functional status, i.e. with low biological age. Their measured biomarkers of tissue regeneration can then be compared between the groups and linked to biological age.

Another design of a validation study is to include offspring of long-living families, since they are considered to have the genetic propensity to reach old age in good health like their parents, so they are more likely to be biologically younger and to show features of delayed ageing<sup>208</sup>. Studies in offspring of long-living families have shown that these individuals, compared to age-matched controls, are generally healthier and have a lower prevalence of age-related diseases<sup>209</sup>. Previously, we measured several biomarkers of bone tissue turnover in serially sampled blood obtained from a group of 37 middle-aged individuals, comprising offspring of long-living families and age-matched controls, and assessed the variation in serum concentration of these biomarkers over 24 hours<sup>55</sup>. In **Figure 3**, we present the data on markers of bone tissue turnover stratified for members from long-lived families and age-matched controls, as an example towards the use of biomarkers of tissue maintenance in a preclinical study. In this study, three out of the five bone markers seemed to be lower in offspring of long-lived families compared to controls. Given the small sample size of this preliminary study, we propose that larger studies will be required to address this hypothesis in greater depth. Furthermore, as suggested by Burkle *et al.*, people with characteristics of accelerated ageing, for example people with Down's syndrome or Werner's

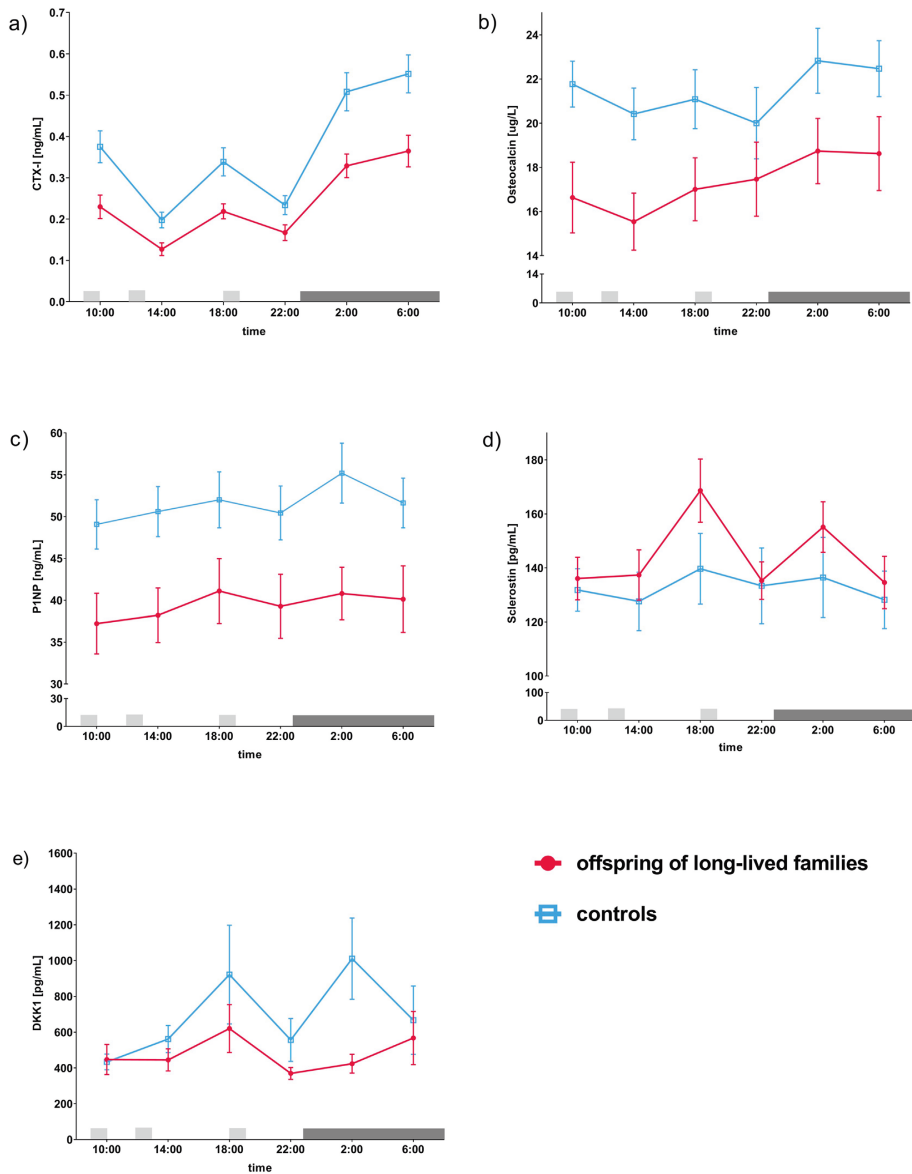
syndrome, and possibly people with obesity, could also be included in biomarker-validation studies <sup>12</sup>. However, before validated biomarkers can be broadly used in research or the clinic, measurement of these biomarkers should be standardized

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**Figure 2.** Overview of proposed biomarkers of tissue maintenance  
Main proposed biomarkers of maintenance of bone, cartilage, and muscle tissue. This selection of biomarkers could be measured in future ageing research to determine whether these biomarkers are indeed potential early markers of the ageing process, and of age-related diseases including osteoporosis, osteoarthritis, and sarcopenia. For brain tissue maintenance no suitable biomarkers are currently available. Figure created in the Mind the Graph platform: [www.mindthegraph.com](http://www.mindthegraph.com).

## Chapter 2



**Figure 3.** Bone markers over 24 h stratified for offspring of long-lived families and controls. The mean (SE) of a) C-terminal cross-linked telopeptide of type 1 collagen (CTX-I), b) osteocalcin, c) N-terminal propeptide of type 1 procollagen (P1NP), d) sclerostin, and e) Dickkopf-related protein 1 (DKK1) are presented every 4 h starting at 10:00 during 24 h for 19 offspring of long-lived families (pink, dark) and 18 controls (blue, light). Light bars represent meal times and dark bars represent the period when the lights were switched off.

Whether reduced or elevated levels of biomarkers of tissue maintenance are beneficial might also be dependent on the context, including the (micro) environment, communication with other tissues and cells, and/or circulating factors. For example, other ageing processes, such as (immuno)senescence and inflammaging, were first seen as detrimental and reducing lifespan, but researchers recently proposed that these processes can also be viewed as adaptive responses leading to enhanced survival<sup>30,211</sup>. Whether these processes are beneficial or detrimental depends on the microenvironment and circulating factors. Also parabiosis experiments demonstrate that circulating factors are important for proper functioning<sup>212,213</sup>. In this review we presented some examples of internal and external factors that influence tissue maintenance, including hormones, inflammatory factors, nutrition, exercise, immobility, etc. For example, sex hormones are related to bone and muscle maintenance, but also growth factors as GH and IGF-1 strongly influence the regenerative capacity of tissues. Furthermore, the state of inflammaging influences many processes in the body, probably including tissue maintenance<sup>214</sup>. This shows that also tissue maintenance might be highly dependent on the (micro)environment and signaling/circulating factors. Therefore, when measuring biomarkers of tissue maintenance, one should consider to include other regulatory factors to get a more comprehensive picture. Moreover, these regulating factors might even be more informative than a single biomarker of tissue maintenance. For example, specific serum miRNA profiles were found to relate to bone pathologies with even a higher predictive power than bone mineral density or bone turnover markers<sup>215</sup>. Therefore, future research could besides validating proposed biomarkers of tissue maintenance, include identification of circulating factors crucial for tissue maintenance.

## FUNDING

This work was supported by the European Commission project THYRAGE (Horizon 2020 research and innovation programme, 666869). E. van der Spoel was supported by a personal PhD grant from the Leiden University Medical Center.

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# CHAPTER 3

Thyroid status and mortality in nonagenarians from long-lived families and the general population

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*Aging (Albany NY) 2017 Oct 25;9(10):2223-2234.*

## ABSTRACT

The relationship between thyroid status and longevity has been investigated extensively. However, data on thyroid status and survival in old age is scarce. In this study we investigated associations of different parameters of thyroid status with mortality in nonagenarians, and whether these associations were different in nonagenarians from long-lived families than in nonagenarians from the general population. In total, 805 nonagenarians from the Leiden Longevity Study and 259 nonagenarians from the Leiden 85-plus Study were followed up to collect mortality data. At baseline, levels of thyrotropin (TSH), free thyroxine (fT4) and free triiodothyronine (fT3) were measured. In nonagenarians from long-lived families and from the general population, associations between thyroid parameters and mortality were similar. We found no interaction between study population and parameters of thyroid status on mortality ( $P$ -values $>0.70$ ). The results from both studies were combined to derive generalizable associations. Hazard ratios (HRs) for the highest tertiles were determined, resulting in TSH HR 0.91 ( $P=0.25$ ), fT4 HR 1.22 ( $P=0.02$ ), fT3 HR 0.74 ( $P=1.31e-4$ ), and fT3/fT4 HR 0.66 ( $P=5.64e-7$ ). In conclusion, a higher ratio of fT3/fT4, higher levels of fT3, and lower levels of fT4 are associated with lower mortality rate in nonagenarians and independent of familial longevity status.

## INTRODUCTION

Thyroid hormone is crucial for growth and development in every stage of life. Without proper tuning of thyroid hormone activity, adverse effects on cognitive function, bone mineralisation, and energy metabolism are common. In case of thyroid hormone deficiency (hypothyroidism), weight gain, mood impairment, and dyslipidaemia are prevalent problems <sup>1</sup>. Thyroid hormone excess (hyperthyroidism) may lead to atrial fibrillation, muscle wasting, weight loss and osteoporosis <sup>2,3</sup>. Even within the normal range (euthyroidism), variations in thyroid status have been associated with cardiovascular risk and body composition <sup>4</sup>.

Circulating thyroid hormone levels are regulated by the hypothalamus-pituitary-thyroid (HPT) axis <sup>5</sup>. First the hypothalamus secretes thyrotropin releasing hormone (TRH), inducing release of thyrotropin (TSH) by the pituitary. TSH then stimulates the thyroid gland to synthesise and secrete thyroid hormones, consisting of the prohormone thyroxine (T4) and the active hormone triiodothyronine (T3). Most of T4 and T3 is bound to binding proteins, resulting in only a small fraction of unbound, free T4 (fT4) and unbound, free T3 (fT3) in the circulation. Subsequently, through negative feedback, the hypothalamus and pituitary adjust secretion of TRH and TSH to circulating levels of fT4. In peripheral tissues, fT4 is converted by tissue specific deiodinases to customize local availability of active T3.

Previously, indications were found of a relationship between lower thyroid status and longevity. In the Leiden 85-plus Study, a prospective population-based study in the city of Leiden in the Netherlands <sup>6</sup>, higher levels of TSH were associated with lower mortality in participants between ages 85 and 90 <sup>7</sup>. Furthermore, in the Leiden Longevity Study, a family study of siblings with exceptional longevity <sup>8,9</sup>, slightly higher levels of circulating TSH and lower levels of fT4 and fT3 were associated with increased familial longevity <sup>10</sup>. The offspring of these old age siblings had higher TSH levels than their partners, while they had similar levels of fT4 and fT3 <sup>11</sup>. Taken together, these studies suggest a different HPT-axis set point as a familial trait of long-lived families. However, data on associations between thyroid status and survival at exceptionally old age are scarce and no data are available on whether these associations are similar in nonagenarians from long-lived families as compared to nonagenarians from the general population.

To address these questions, we assessed and compared the associations between parameters of thyroid status and mortality in two nonagenarian study populations; the Leiden Longevity Study and the Leiden 85-plus Study.

## RESULTS

### Characteristics of study populations

Characteristics of the nonagenarian participants of the Leiden Longevity Study and the Leiden 85-plus Study are presented separately for the 384 men and 680 women in **Table 1**. For the Leiden Longevity Study, male participants were younger (91.4 years) than the female participants (93.6 years,  $P < 0.001$ ). Circulating levels of fT3 were significantly higher in men (4.1 pmol/L) than in women (4.0 pmol/L,  $P = 0.001$ ). The women had lower ratios of fT3/fT4 (0.25) compared to the men (0.26,  $P < 0.001$ ). The other parameters were not significantly different. In the Leiden 85-plus Study, measurements performed at age 90 years were taken as baseline for both men and women. Levels of high-sensitivity C-reactive protein (hsCRP) were significantly different between men and women (men 3.7 mg/L, women 2.8 mg/L,  $P = 0.02$ ). All other measured parameters were similar for men and women.

### Thyroid status and mortality in long-lived families

In **table 2** the associations between thyroid status parameters and mortality rate in the participants of the Leiden Longevity Study are presented. For levels of TSH, no association with mortality rate was found. For levels of fT4, a more than 20 percent increased mortality rate was observed in the highest tertile compared to the lowest, however statistical significance was lost after correction for hsCRP (model 1 hazard ratio (HR) 1.22 (95% CI 1.01-1.48)  $P = 0.04$ , model 2 HR 1.21 (95% CI 0.99-1.45)  $P = 0.06$ ). Higher levels of fT3 were associated with lower mortality in both models (model 1 HR 0.70 (95% CI 0.58-0.85)  $P = 2.33 \times 10^{-4}$ , model 2 HR 0.73 (95% CI 0.60-0.88)  $P = 1.33 \times 10^{-3}$ ). The product of fT4xTSH and the ratio fT4/TSH were not associated with mortality in either model. The highest tertile of the ratio of fT3/fT4 had more than 30 percent reduction in mortality rate compared to the lowest tertile (model 1 HR 0.65 (95% CI 0.54-0.79)  $P = 1.65 \times 10^{-5}$ , model 2 HR 0.68 (95% CI 0.56-0.83)  $P = 1.13 \times 10^{-4}$ ). The results were similar for men and women in the stratified analyses (data not shown).

**Table 1.** Characteristics of the participants of the Leiden Longevity Study and the Leiden 85-plus Study

	Men	Women	P-value
<b>Leiden Longevity Study</b>			
Number of participants	312	493	
Age (years)	91.4 (90.1-93.7)	93.6 (92.2-95.3)	<b>&lt;0.01</b>
Deceased (n (%))	287 (92.0)	462 (93.7)	0.88
TSH (mU/L)	1.6 (1.0-2.4)	1.5 (1.0-2.4)	0.16
fT4 (pmol/L)	15.8 (2.0)	16.1 (2.4)	0.08
fT3 (pmol/L)	4.1 (0.6)	4.0 (0.5)	<b>&lt;0.01</b>
fT4xTSH (pmolxmU)	25.3 (16.3-36.2)	24.1 (15.6-37.5)	0.26
fT4/TSH (pmol/mU)	9.9 (6.2-14.9)	10.8 (6.3-17.2)	0.10
fT3/fT4	0.26 (0.05)	0.25 (0.04)	<b>&lt;0.01</b>
hsCRP (mg/L) <sup>a</sup>	3.0 (1.4-6.6)	2.6 (1.2-5.2)	0.13
<b>Leiden 85-plus Study</b>			
Number of participants	72	187	
Age (years)	90	90	
Deceased (n (%))	71 (98.6)	183 (97.9)	0.16
TSH (mU/L)	1.9 (1.1-3.0)	1.7 (1.0-2.9)	0.46
fT4 (pmol/L)	16.2 (2.5)	16.3 (2.2)	0.80
fT3 (pmol/L)	4.0 (0.5)	4.1 (0.6)	0.27
fT4xTSH (pmolxmU)	30.5 (19.5-47.0)	26.9 (18.1-44.2)	0.41
fT4/TSH (pmol/mU)	8.4 (5.0-15.9)	9.2 (5.2-16.3)	0.49
fT3/fT4	0.25 (0.05)	0.26 (0.04)	0.94
hsCRP (mg/L)	3.7 (2.0-8.3)	2.8 (1.2-5.8)	<b>0.02</b>

Data are presented as median (interquartile range) or mean (standard deviation) where appropriate, unless indicated otherwise.

P-values shown for Mann-Whitney U test, T-Test, or Chi-Square test where appropriate.

<sup>a</sup> data available for 312 men and 492 women.

**Table 2.** Parameters of thyroid status and mortality rate in the Leiden Longevity Study

Leiden Longevity Study participants						
	Median (range)		Hazard ratio (95% CI)			
	Men (n=312)	Women (n=493)	Model 1 (n=805)	Model 2 <sup>a</sup> (n=804)	P-value	P-value
<b>TSH</b>						
Lowest tertile	0.9 (0.1-1.2)	0.7 (0.1-1.1)	1.00 (reference)	1.00 (reference)	0.30	0.24
Middle tertile	1.6 (1.2-2.1)	1.5 (1.1-2.0)	0.92 (0.78-1.08)	0.91 (0.77-1.07)	0.49	0.44
Highest tertile	3.0 (2.1-11.7)	3.0 (2.0-17.3)	0.95 (0.79-1.14)	0.93 (0.77-1.12)		
<b>ft4</b>						
Lowest tertile	13.8 (10.2-14.7)	13.7 (10.1-15.0)	1.00 (reference)	1.00 (reference)	0.30	0.36
Middle tertile	15.6 (14.8-16.6)	16.0 (15.1-16.9)	0.91 (0.75-1.09)	0.92 (0.76-1.10)	<b>0.04</b>	0.06
Highest tertile	17.7 (16.7-22.1)	18.3 (17.0-23.5)	1.22 (1.01-1.48)	1.21 (0.99-1.45)		
<b>ft3</b>						
Lowest tertile	3.6 (2.6-3.8)	3.5 (2.6-3.7)	1.00 (reference)	1.00 (reference)	<b>0.01</b>	<b>0.01</b>
Middle tertile	4.1 (3.9-4.3)	3.9 (3.8-4.1)	0.77 (0.64-0.93)	0.78 (0.65-0.94)	<b>&lt;0.01</b>	<b>&lt;0.01</b>
Highest tertile	4.6 (4.4-5.8)	4.5 (4.2-5.8)	0.70 (0.58-0.85)	0.73 (0.60-0.88)		
<b>ft4xTSH</b>						
Lowest tertile	14.0 (2.0-19.5)	12.3 (1.6-18.2)	1.00 (reference)	1.00 (reference)	0.96	0.93
Middle tertile	25.3 (19.5-32.1)	24.1 (18.2-30.9)	1.00 (0.84-1.18)	0.99 (0.84-1.18)	0.92	0.94
Highest tertile	45.6 (32.2-212.9)	46.9 (31.1-257.5)	1.01 (0.83-1.22)	1.01 (0.83-1.22)		

Table 2. Continued.

Leiden Longevity Study participants						
	Median (range)		Hazard ratio (95% CI)			
	Men (n=312)	Women (n=493)	Model 1 (n=805)	P-value	Model 2 <sup>a</sup> (n=804)	P-value
<b>ft4/TSH</b>						
Lowest tertile	5.2 (1.2-7.5)	4.8 (0.6-7.8)	1.00 (reference)		1.00 (reference)	
Middle tertile	9.9 (7.5-13.0)	10.8 (7.8-14.3)	0.92 (0.77-1.10)	0.35	0.92 (0.77-1.10)	0.34
Highest tertile	18.6 (13.1-136.7)	22.5 (14.3-235.0)	1.07 (0.90-1.29)	0.44	1.08 (0.91-1.29)	0.38
<b>ft3/ft4</b>						
Lowest tertile	0.22 (0.14-0.24)	0.21 (0.12-0.23)	1.00 (reference)		1.00 (reference)	
Middle tertile	0.26 (0.24-0.28)	0.25 (0.23-0.27)	0.73 (0.61-0.88)	<b>&lt;0.01</b>	0.75 (0.63-0.90)	<b>&lt;0.01</b>
Highest tertile	0.31 (0.28-0.41)	0.29 (0.27-0.37)	0.65 (0.54-0.79)	<b>&lt;0.01</b>	0.68 (0.56-0.83)	<b>&lt;0.01</b>

Participants were divided over sex-specific tertiles, which were combined for analyses.

Model 1: adjusted for family number and age

Model 2: adjusted for family number, age and hsCRP

<sup>a</sup> data available for 312 men and 492 women.

**Table 3.** Parameters of thyroid status and mortality rate in the Leiden 85-plus Study

Leiden 85-plus Study participants						
	Median (range)		Hazard ratio (95% CI)			
	Men (n=72)	Women (n=187)	Model 1 (n=259)	P-value	Model 2 (n=259)	P-value
<b>TSH</b>						
Lowest tertile	0.9 (0.1-1.3)	0.8 (0.1-1.2)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Middle tertile	1.9 (1.4-2.7)	1.7 (1.3-2.4)	0.87 (0.64-1.18)	0.38	0.91 (0.67-1.23)	0.53
Highest tertile	3.9 (2.7-7.5)	3.5 (2.4-14.6)	0.86 (0.64-1.17)	0.35	0.87 (0.64-1.17)	0.35
<b>ftT4</b>						
Lowest tertile	13.8 (11.5-14.6)	14.3 (11.2-15.3)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Middle tertile	16.2 (14.7-17.4)	16.2 (15.4-17.1)	1.37 (1.01-1.86)	0.05	1.35 (0.99-1.83)	0.06
Highest tertile	18.9 (17.5-22.2)	18.3 (17.2-23.2)	1.36 (1.00-1.84)	0.05	1.27 (0.93-1.73)	0.14
<b>ftT3</b>						
Lowest tertile	3.6 (2.7-3.8)	3.5 (2.8-3.8)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Middle tertile	4.0 (3.9-4.1)	4.0 (3.9-4.3)	0.77 (0.57-1.06)	0.11	0.82 (0.60-1.12)	0.21
Highest tertile	4.4 (4.2-5.7)	4.7 (4.4-5.9)	0.66 (0.49-0.90)	<b>0.01</b>	0.72 (0.53-0.98)	<b>0.04</b>
<b>ftT4xTSH</b>						
Lowest tertile	15.0 (1.8-23.1)	13.7 (2.0-20.1)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Middle tertile	30.5 (23.2-42.7)	26.9 (20.5-39.0)	1.21 (0.89-1.64)	0.23	1.24 (0.91-1.69)	0.17
Highest tertile	57.4 (43.8-108.2)	57.5 (39.0-210.5)	0.98 (0.72-1.33)	0.89	0.98 (0.72-1.33)	0.88

Table 3. Continued.

Leiden 85-plus Study participants						
	Median (range)		Hazard ratio (95% CI)			
	Men (n=72)	Women (n=187)	Model 1 (n=259)	P-value	Model 2 (n=259)	P-value
<b>ft4/TSH</b>						
Lowest tertile	3.5 (1.8-5.9)	4.6 (0.8-6.4)	1.00 (reference)		1.00 (reference)	
Middle tertile	8.4 (6.1-12.0)	9.2 (6.4-13.7)	0.97 (0.72-1.32)	0.86	1.01 (0.74-1.36)	0.96
Highest tertile	17.3 (12.6-181.0)	21.1 (13.7-165.8)	1.12 (0.83-1.52)	0.46	1.12 (0.83-1.52)	0.46
<b>ft3/ft4</b>						
Lowest tertile	0.21 (0.13-0.23)	0.22 (0.14-0.24)	1.00 (reference)		1.00 (reference)	
Middle tertile	0.25 (0.23-0.27)	0.26 (0.24-0.27)	0.73 (0.54-0.98)	<b>0.04</b>	0.76 (0.56-1.03)	0.07
Highest tertile	0.30 (0.28-0.37)	0.29 (0.27-0.36)	0.55 (0.40-0.75)	<b>&lt;0.01</b>	0.59 (0.43-0.81)	<b>&lt;0.01</b>

Participants were divided over sex-specific tertiles, which were combined for analyses.

Model 1: crude analysis

Model 2: adjusted for hsCRP

### Thyroid status and mortality in the general population

The association between thyroid status parameters and mortality rate in participants of the Leiden 85-plus Study are presented in **table 3**. Similar to the results in the participants of the Leiden Longevity Study, levels of TSH were not associated with mortality. Higher levels of fT4 were associated with an increased mortality rate in both models, though statistical significance was not reached (model 1 HR 1.36 (95% CI 1.00-1.84)  $P=0.05$ , model 2 HR 1.27 (95% CI 0.93-1.73)  $P=0.14$ ). The highest tertile of fT3 had a significantly lower mortality rate than the lowest tertile (model 1 HR 0.66 (95% CI 0.49-0.90)  $P=0.01$ , model 2 HR 0.72 (95% CI 0.53-0.98)  $P=0.04$ ). The fT4xTSH product and the fT4/TSH ratio were not associated with mortality rate. The ratio of fT3/fT4 was associated with a more than 40 percent decrease in mortality rate in both models in the highest compared to the lowest tertile (model 1 HR 0.55 (95% CI 0.40-0.75)  $P=1.44 \times 10^{-4}$ , model 2 HR 0.59 (95% CI 0.43-0.81)  $P=9.66 \times 10^{-4}$ ). The results were similar for men and women in the stratified analyses (data not shown).

### Thyroid status and mortality independent of study population

The results of the stratified analyses on thyroid status and mortality were similar for both nonagenarians of long-lived families and of the general population. When interaction was tested between study population and parameters of thyroid status on mortality, no significant interaction was found (all  $P$ -values  $>0.7$ , **Supplementary table 1**).

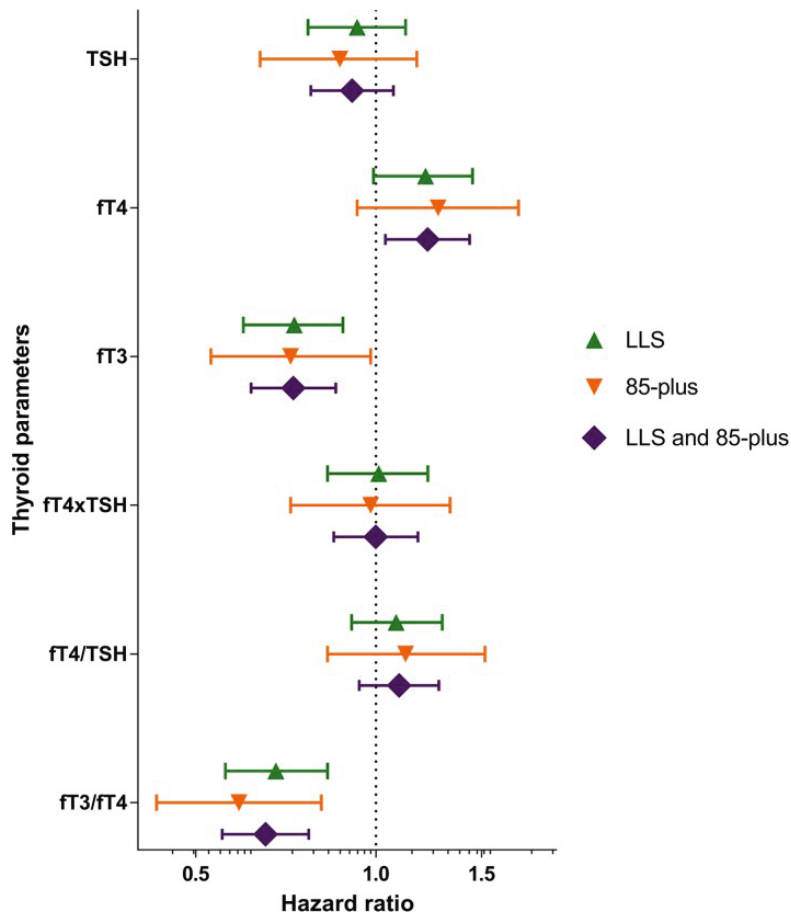
### Generalized results for nonagenarians

Since the associations between thyroid status and mortality were similar in both nonagenarian populations, the results could be combined to provide a generalizable association for nonagenarians. For this purpose, the results for the Leiden Longevity Study and the Leiden 85-plus Study populations were combined, through a fixed-effects inverse-variance weighted analysis on the associations of thyroid parameters and mortality rate with adjustments as described in model 2 (**Supplementary table 2**). The hazard ratios (HRs) of the highest compared to the lowest tertile in both studies and the weighted averages of these HRs are displayed in **Figure 1**. Higher levels of TSH were not associated with mortality rate in either study or in the pooled analysis (HR 0.91, 95% CI 0.78-1.07,  $P=0.25$ ). Higher levels of fT4 were associated with higher mortality rate in both studies, leading to an increase of 22 percent in mortality rate in the highest compared to the lowest fT4 tertile in the pooled analysis (HR 1.22, 1.04-1.43,  $P=0.02$ ). Inversely, higher levels of fT3 were associated with lower mortality rates in both studies, resulting in a decrease in mortality rate of more than 25 percent in the highest compared to the lowest tertile (HR 0.73, 95% CI 0.62-0.86,  $P=1.31 \times 10^{-4}$ ). The product of fT4xTSH and the ratio of fT4/TSH were not associated with mortality rate. Lastly, the fT3/fT4 ratio was associated with a

lower mortality rate in the highest tertiles compared to the lowest in both studies, resulting in a 35 percent lower mortality rate in the pooled analysis (HR 0.65, 95% CI 0.55-0.77,  $P=5.64 \times 10^{-7}$ ).

### Various sensitivity analyses

Sensitivity analyses performed on participants with normal levels of hsCRP (hsCRP < 10 mg/L), participants with mortality within the first two years or past the first year of follow up did not materially change the results (data not shown). The results were similar in participants with TSH levels within the normal range as in the main analyses (data not shown).



**Figure 1.** Mortality rate in the Leiden Longevity Study and the Leiden 85-plus Study.

Hazard ratios and 95% confidence intervals for mortality of the highest sex-specific tertiles compared to the lowest sex-specific tertiles are shown for the Leiden Longevity Study participants, the Leiden 85-plus Study participants and the pooled Hazard Ratios.

LLS = participants of the Leiden Longevity Study, 85-plus = participants of the Leiden 85-plus Study, LLS and 85-plus = combined estimates of the Leiden Longevity Study and the Leiden 85-plus Study

## DISCUSSION

This study assessed the association between parameters of thyroid status and mortality rate in nonagenarians from long-lived families and the general population. In both populations, a higher fT3/fT4 ratio was associated with decreased mortality rate. Although the observed associations for the levels of fT3 and fT4 within the reference range were not identical for the two populations, these showed a similar pattern. When the results of both populations were combined, higher levels of fT3 within the reference were associated with a lower mortality rate, whereas higher levels of fT4 within the reference range were detrimental in old age. Risk of mortality was neither associated with levels of TSH nor with the product of fT4xTSH or the ratio of fT4/TSH. All observed relationships between thyroid status and risk of mortality were consistent in male and female participants and in participants with TSH levels within the normal range.

The ratio of fT3/fT4 can be viewed as an estimate of conversion of fT4 into fT3<sup>12</sup>. Conversion of fT4 takes place by deiodinases in peripheral tissues such as the liver, the kidneys and skeletal muscle as well as inside the thyroid gland<sup>13</sup>. Our result of a lower mortality rate in the participants with the highest ratio of fT3/fT4 in nonagenarians is consistent with the previous finding of Gussekloo *et al.*<sup>7</sup> in participants of the Leiden 85-plus Study aged 85 to 89 years old. One of the possible explanations for this association is that individuals with a lower ratio fT3/fT4 are ill. In times of critical illness and inflammation, conversion of T4 to T3 is inhibited by downregulation of type 1 deiodinase (D1) known as euthyroid sick syndrome<sup>14</sup>. However, when we adjusted for levels of C-reactive protein as a marker of inflammation, the observed association was not attenuated.

The increased risk of mortality with higher levels of fT4 could be a reflection of lower turnover. This could be due to decreased conversion of fT4 by deiodinases, congruent to the observation of the ratio fT3/fT4. Alternatively, higher circulating levels of fT4 might reflect a slower rate of detoxification of thyroid hormones (and possibly other compounds) by the liver and kidney which would be unfavourable for survival. These associations are in line with previous observations<sup>7, 15-18</sup>.

The association between higher circulating levels of fT3 and lower mortality is in line with previous research. A higher BMI was found to be causally related to higher circulating levels of fT3<sup>19</sup>, and higher BMI has also been positively associated with old age survival<sup>20</sup>. The lower risk of mortality with higher levels of fT3 we found was reported by others only in crude analyses when illness was not taken into account<sup>18, 21, 22</sup>. Yet, adjustment for levels of hsCRP did not materially influence the results.

Circulating levels of TSH appear to play no major role in risk of mortality in our nonagenarian populations, which matches the inconclusive results in literature <sup>7,15,17,22-27</sup>. In previously described analyses, decreased mortality with increasing levels of TSH was observed in the prolonged follow-up of the 85-plus Study <sup>5</sup>. However, in participants with TSH levels within the normal range the decrease in mortality was not significant, which is in line with our study in which the participants with extreme values of TSH were excluded.

No relationship was found between mortality risk and the product of  $ft4 \times TSH$ , which is a reflection of resistance to thyroid hormones due to thyroid hormone receptor defects <sup>11,28</sup>. In mice, the influence on mortality depends on the affected receptor type. Defects in the  $\beta$ -receptor cause only marginal increase in mortality whereas  $\alpha$ -receptor defects are associated with severely shortened lifespan <sup>29</sup>. Data on the effects of  $ft4 \times TSH$  in humans are scarce and no data is available for milder variations within populations.

The ratio of  $ft4/TSH$  was also not associated with mortality rate in this study. This ratio combines the levels of  $ft4$  and TSH, resulting in a composite measure which could reflect the responsiveness of the thyroid gland to TSH, a trait previously associated with familial longevity <sup>10,11,30-32</sup>. Possibly the influence of this trait is only found earlier in life, because selection for individuals with this trait has taken place by age 90 years.

The consistency of our results in both nonagenarian populations advocates a universal mechanism of survival in old age via relatively lower levels of  $ft4$  and higher  $ft3$ . Even though nonagenarians from long-lived families exhibit demonstrably lower mortality rate than nonagenarians from the general population <sup>9</sup>, a higher  $ft3/ft4$  ratio appears to be a common trait associated with longevity.

One of the strengths of this study is that we used data from a relatively large population of a rarely investigated age category of nonagenarians. The study participants originated from two different study populations with different study designs and inclusion criteria. This has led to a high heterogeneity in study population, which allows extrapolation of these results to nonagenarians in the general population. Another quality of this study is the use of multiple parameters of thyroid status, which could offer more insight into the interplay of these parameters. These continuous values of proportions are preferable over clinical cut off points for clinical and subclinical thyroid dysfunction for two reasons. Firstly, these continuous variables allow for a more mechanistic approach. Secondly, the diagnostic criteria for thyroid dysfunction and normal ranges of TSH and  $ft4$  differ over time, per country and per laboratory <sup>33,34</sup>. Limitations of

this study are the lack of information on thyroid disease and use of medication affecting the thyroid, as well as general information on chronic and acute illness.

In conclusion, we have found lower mortality rate associated with a higher ratio of fT3/fT4, lower fT4 levels and higher fT3 levels in nonagenarians from long-lived families and from the general population. Based on the results of the present study, associations of thyroid status with old age mortality were not dependent on familial longevity status. To identify the causal mechanisms underlying the observed associations between thyroid status and mortality, future research should focus on biological mechanisms via which thyroid parameters might contribute to longevity.

## **METHODS**

### **Leiden Longevity Study**

As described previously in more detail, the aim of the Leiden Longevity Study was to identify genetic and phenotypical factors contributing to familial longevity<sup>8,9</sup>. Families were included if at least two proband siblings had lived to exceptionally old age, being 89 years or older for men and 91 years or older for women. No exclusion criteria based on health or demographics were applied. The Medical Ethical Committee of the Leiden University Medical Centre approved the study and written informed consent was obtained from all study participants. Between July 2002 and May 2006, 421 Dutch Caucasian families were recruited, comprising 944 nonagenarian participants.

Of these 944 nonagenarians data on TSH, fT4 and fT3 were available for 859 participants. Three participants were excluded from the analyses because serum TSH levels were outside the detection range (two participants with TSH <0.005 mU/L and one participant with TSH >100mU/L). Four participants were excluded because their fT3 level was below the reference range (<2.5 pmol/L), as a possible sign of euthyroid sick syndrome. Lastly, 16 participants were excluded because their fT4 levels were outside the reference range (six participants with <10.0 pmol/L and ten participants with >24.0 pmol/L), because of presumed thyroid disease or medication use affecting the thyroid function. Subsequently, participants with (log transformed) TSH or fT4 or one of the composite measurements ((log transformed) product of fT4xTSH, ratio of (log transformed) fT4/TSH or ratio of fT3/fT4) that deviated more than three standard deviations from the population mean were excluded. Of the included 805 participants, 749 had passed away on the 1<sup>st</sup> of February 2014. The median follow up time was 3.49 years, with interquartile range of 1.53 to 4.97 years.

### **Leiden 85-plus Study**

The Leiden 85-plus Study is a prospective, population-based study aimed at investigating determinants of successful aging <sup>6</sup>. Inclusion criteria were being 85 years old and living in Leiden, the Netherlands, between September 1997 and September 1999. No exclusion criteria based on health were applied. Out of 705 eligible individuals 599 participants were enrolled, since 92 people had refused and 14 were deceased before the assessment visit. Over a follow-up period of five years, participants were assessed annually until death or refusal. The study was approved by the Medical Ethical Committee of the Leiden University Medical Centre and all participants provided oral informed consent.

To investigate a similar age group as the Leiden Longevity Study, the current study used data of the participants of the Leiden 85-plus Study at the age of ninety years. Out of the 599 participants enrolled at 85 years, 52 had refused to continue participation and 270 were deceased before the assessment visit following their 90<sup>th</sup> birthday. From the total of 277 nonagenarian participants who were eligible, three participants did not provide a blood sample and three had missing data on TSH level. One participant was excluded because the serum fT3 level was below the reference range (<2.5 pmol/L) and two participants were excluded for having serum levels of fT4 below the reference range (10.0 pmol/L). Of the remaining 268 participants, those with thyroid status parameters (where appropriate log transformed) that deviated more than three standard deviations from the population mean were excluded. In total, 259 participants were included, of which 254 had passed away on the 1<sup>st</sup> of February 2014. The median follow up time was 3.84 years, with interquartile range of 1.90 to 5.89 years.

### **Chemical analyses**

For both the Leiden Longevity Study and the Leiden 85-plus Study, non-fasted blood samples were collected from all participants between 09:30 hours and 17:00 hours at the assessment visit. Measurements of TSH, fT4 and fT3 were performed using the Modular E170 from Roche, Almere, the Netherlands. The reference ranges at our laboratory are 0.3-4.8 mU/L for TSH (detection range 0.005-100 mU/L), 10.0-24.0 pmol/L for fT4 (detection range 1.3-100 pmol/L) and 2.5-5.5 pmol/L for fT3 (detection range 0.400-50.0 pmol/L). High-sensitivity C-reactive protein (hsCRP) (detection range 0.3-350 mg/L) was measured using Cobas Integra 800 from Roche, Almere, the Netherlands. All coefficients of variation were below 5%. The measurements were performed in a single batch at the Department of Clinical Chemistry of the Leiden University Medical Centre.

### **Composite measurements of thyroid status**

To assess the thyroid status in more detail, three composite measurements were computed. The product of fT4 and TSH (fT4xTSH) was calculated, to allow for

assessment of the sensitivity of the pituitary thyrotrophs to feedback regulation by thyroid hormones as described previously<sup>11,28</sup>. Furthermore, the ratio of fT4 and TSH (fT4/TSH) was calculated, as a measure of responsiveness of the thyroid gland to TSH as proposed by Jansen *et al.*<sup>11</sup>. The ratio of fT3 and fT4 (fT3/fT4) was calculated as a measure of thyroid hormone conversion.

### Statistical analyses

Because of the study design of the Leiden Longevity Study in which the men are younger than the women, all analyses were performed on men and women separately. Means and standard deviations, and where appropriate medians and interquartile ranges, were computed for parameters of thyroid status and age and hsCRP for male and female participants of both the Leiden Longevity Study and the Leiden 85-plus Study. Differences between men and women were tested using the T-test, Chi-Square test, or Mann-Whitney U test. Secondly, the participants of both studies were divided over sex-specific tertiles based on TSH, fT4, fT3, the product of fT4xTSH, the ratio of fT4/TSH, or the ratio of fT3/fT4. Survival analyses were performed using Cox regression on combined tertiles of men and women in both studies and in men and women separately using two statistical models. In model 1 for the Leiden Longevity Study, the analyses were adjusted for differences in age at entry by left truncated analysis and adding age at entry as an independent variable. Furthermore, robust standard errors were used to correct for sibblingship by clustering on family number of the Leiden Longevity Study participants. For the Leiden 85-plus Study, model 1 was a crude analysis. In model 2, hsCRP was added as an independent variable, to adjust for inflammation, for both the Leiden Longevity Study and the Leiden 85-plus Study. The outcomes were presented as hazard ratios (HRs) for tertiles with the lowest tertile as a reference. To formally test whether the associations between parameters of thyroid status and mortality were similar for nonagenarians of long-lived families and the general population, interaction between study population (long-lived families or the general population) and parameters of thyroid status on mortality was tested by adding an interaction term and population of origin as covariates to the model 2 analysis excluding familial clustering. To assess a generalizable result for nonagenarian populations, the results were analyzed using a fixed-effects inverse-variance weighted analysis based on the coefficient for the highest tertile compared to the lowest tertile and its Standard Error in Cox regression. The output was transformed to HR. As sensitivity analyses we performed the same analyses in participants with hsCRP <10 mg/L, in participants who had passed away in the first two years of follow up, in participants who had survived the first year of follow up, and in participants with TSH levels within the normal range. In the analyses values of  $P < 0.05$  were considered significant. For data analyses Statistical Package for the Social Sciences (SPSS) version 23, the Stata Data analysis and Statistical

Software for Windows version 12.0 SE and R version 3.2.4 for Windows were used. The graph was created using GraphPad Prism version 6.05 for Windows.

## **ACKNOWLEDGEMENTS**

We thank all participants of the Leiden Longevity Study and the Leiden 85-plus Study for their consistent cooperation, the secretary staff and the research nurses for their valuable contribution.

## **CONFLICT OF INTEREST STATEMENT**

The authors of this manuscript have no conflict of interest to declare.

## **FUNDING**

This study was funded by the European Commission funded projects THYRAGE (Horizon 2020 research and innovation programme under grant agreement No 666869) and by the Innovation Oriented research Program on Genomics (SenterNovem; IGE01014 and IGE5007); the Netherlands Genomics Initiative/ Netherlands Organization for Scientific Research (NGI/NWO; 05040202 and 050-060-810, NCHA).

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## Supplementary tables

**Table S1.** Interaction between parameters of thyroid status and population of origin on mortality

Parameter of thyroid status	Interaction P-value
TSH	0.70
ft4	0.97
ft3	0.74
ft4xTSH	0.82
ft4/TSH	0.88
ft3/ft4	0.77

Data are presented as P-value for interaction term for parameter of thyroid status and study population on the Cox regression adjusted for age and hsCRP.

**Table S2.** Parameters of thyroid status and mortality rate in nonagenarians

Parameter of thyroid status	Hazard ratio (95% CI)	P-value
TSH	0.91 (0.78-1.07)	0.25
ft4	1.22 (1.04-1.43)	0.02
ft3	0.73 (0.62-0.86)	1.31x10 <sup>-4</sup>
ft4xTSH	1.00 (0.85-1.17)	0.99
ft4/TSH	1.09 (0.94-1.27)	0.26
ft3/ft4	0.65 (0.55-0.77)	5.64x10 <sup>-7</sup>

Data are presented as Hazard ratios for weighted averages of mortality rates of the highest compared to lowest sex-specific tertiles in the Leiden Longevity Study and the Leiden 85-plus Study.



# CHAPTER 4

## Association of Thyroid Dysfunction with Cognitive Function: An Individual Participant Data Analysis

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*JAMA Intern Med.* 2021 Nov 1;181(11):1440-1450.

## ABSTRACT

*Importance:* In clinical guidelines, overt and subclinical thyroid dysfunction are mentioned as causal and treatable factors for cognitive decline. However, scientific literature on these associations shows inconsistent findings.

*Objective:* To assess cross-sectional and longitudinal associations of baseline thyroid dysfunction with cognitive function and dementia.

*Design, Setting and participants:* This multicohort individual participant data analysis assessed 114 267 person-years (median, 1.7-11.3 years) of follow-up for cognitive function and 525 222 person-years (median, 3.8-15.3 years) for dementia between 1989 and 2017.

Analyses on cognitive function included 21 cohorts comprising 38 144 participants. Analyses on dementia included eight cohorts with a total of 2033 cases with dementia and 44 573 controls. Data analysis was performed from December 2016 to January 2021.

*Exposures:* Thyroid function was classified as overt hyperthyroidism, subclinical hyperthyroidism, euthyroidism, subclinical hypothyroidism, and overt hypothyroidism based on uniform thyrotropin cutoff values and study-specific free thyroxine values.

*Main Outcomes and Measures:* The primary outcome was global cognitive function, mostly measured using the Mini-Mental State Examination. Executive function, memory and dementia were secondary outcomes. Analyses were first performed at study level using multivariable linear regression and multivariable Cox regression, respectively. The studies were combined with restricted maximum likelihood meta-analysis. To overcome the use of different scales, results were transformed to standardized mean differences. For incident dementia, hazard ratios were calculated.

*Results:* Among 74 565 total participants, 66,567 (89.3%) participants had normal thyroid function, 577 (0.8%) had overt hyperthyroidism, 2557 (3.4%) had subclinical hyperthyroidism, 4167 (5.6%) had subclinical hypothyroidism, and 697 (0.9%) had overt hypothyroidism. The study-specific median age at baseline varied from 57 to 93 years; 42 847 (57.5%) participants were women. Thyroid dysfunction was not associated with global cognitive function; the largest differences were observed between overt hypothyroidism and euthyroidism—cross-sectionally (-0.06 standardized mean difference in score; 95% CI, -0.20 to 0.08;  $P = .40$ ), and longitudinally (0.11 standardized mean difference higher

decline per year; 95% CI, -0.01 to 0.23,  $P = .09$ ). No consistent associations were observed between thyroid dysfunction and executive function, memory or risk of dementia.

*Conclusions and Relevance:* In this individual participant data analysis of more than 74 000 adults, subclinical hypothyroidism and hyperthyroidism were not associated with cognitive function, cognitive decline or incident dementia. No rigorous conclusions can be drawn regarding the role of overt thyroid dysfunction in risk of dementia. These findings do not support the practice of screening for subclinical thyroid dysfunction in the context of cognitive decline in older adults as recommended in current guidelines.

## INTRODUCTION

Thyroid dysfunction is considered a potentially reversible cause of cognitive decline; hence, thyroid function screening tests are described in guidelines as an essential component of the workup for the diagnosis of dementia<sup>1-3</sup>. Thyroid dysfunction is frequently observed in individuals with suspected dementia<sup>4</sup>. However, the effects of treatment of overt hypothyroidism and hyperthyroidism and subclinical hyperthyroidism on cognitive function are not fully clarified<sup>5-7</sup>. For subclinical hypothyroidism, 4 of 5 recent randomized clinical trials and a meta-analysis on levothyroxine treatment did not find evidence for an improvement in cognitive function<sup>8-13</sup>. Moreover, meta-analyses of observational studies have yielded inconsistent results on associations of subclinical and overt thyroid dysfunction with cognitive impairment and risk of dementia<sup>14-17</sup>. An individual participant data analysis of cohort studies might help clarify the conflicting results of previous studies, as it allows for uniform definitions of thyroid dysfunction and it can assess the differential associations by age groups, sex, and thyroid medication in subgroup analyses<sup>18</sup>. In the present study, we investigated cross-sectional and longitudinal associations of thyroid dysfunction with cognitive function and dementia in an individual participant data analysis of multiple cohorts.

## METHODS

### Study Population

We first approached the coordinating center of the Thyroid Studies Collaboration, a collaborative project of 25 existing longitudinal studies with information on thyroid status<sup>18</sup>. The Medical Ethics Committee of the Leiden University Medical Center waived the need for review owing to the retrospective nature of the study using only previously collected data; no individuals underwent interventions for the present study. Each participant gave informed consent to the original study they participated in, which was oral or written depending on the original study design and legislation at the time of data collection. All 15 Thyroid Studies Collaboration cohorts that had collected data on cognitive function or dementia joined the project. The study designs for all cohorts participating in the current study have been described previously in more detail<sup>19-33</sup>. We approached 14 additional cohorts that were extracted from 4 recent meta-analyses on subclinical thyroid dysfunction and cognitive function or dementia<sup>14-17</sup>. Six of these cohorts consented to collaborating and sharing data<sup>34-39</sup>. Lastly, we included publicly available data of the National Health and Nutrition Examination Survey waves of 1999 to 2002 and 2011 to 2012, which simultaneously collected thyroid and cognitive function among many other parameters<sup>40</sup>.

### Thyroid Function

Thyroid dysfunction was determined biochemically by measurements of thyrotropin and free thyroxine (FT<sub>4</sub>) concentrations in all cohorts. Cohort-specific cutoff values were used for FT<sub>4</sub> levels (**Supplementary table 1**). In accordance with previous projects in the Thyroid Studies Collaboration, participants were classified as euthyroid if thyrotropin level was 0.45 to 4.49 mIU/L<sup>18</sup>. Overt hyperthyroidism was defined as a thyrotropin level less than 0.45 mIU/L and FT<sub>4</sub> level above the reference range. Subclinical hyperthyroidism was defined as a thyrotropin level less than 0.45 mIU/L and FT<sub>4</sub> levels within the reference range, or only as thyrotropin level less than 0.45 mIU/L in absence of an FT<sub>4</sub> measurement (n = 896 among 10 cohorts) because overt hyperthyroidism is rare<sup>41</sup>. A combination of thyrotropin level of 4.50 to 20 mIU/L and FT<sub>4</sub> levels within the reference range was defined as subclinical hypothyroidism. Individuals who had missing FT<sub>4</sub> measurements with mildly elevated thyrotropin levels (4.50- 20 mIU/L) were considered subclinically hypothyroid (n = 523 among 8 cohorts) because chances of overt hypothyroidism in this patient category are low<sup>41</sup>. A thyrotropin level of 20 mIU/L or greater or thyrotropin level of 4.50 mIU/L or greater combined with FT<sub>4</sub> levels below the reference range was defined as overt hypothyroidism.

### Cognitive Function

The primary outcome was global cognitive function, measured by Mini-Mental State Examination (MMSE), Modified Mini-Mental State (3MS) or Severe Cognitive Impairment Rating Scale<sup>42-44</sup>. A difference of 1 point in MMSE score is considered the minimal clinically important difference in individuals without dementia<sup>45</sup>. Executive function and memory were secondary outcomes. For executive function, various tests were used: Digit Symbol Substitution Test, Trail Making Test B, Letter Digit Substitution Test (LDST), Executive Interview 15 and Ruff Figural Fluency Test<sup>46-50</sup>. The minimal clinically important difference for executive function was defined as a difference of 4 points in LDST<sup>51</sup>. Memory was measured using either Rey's Auditory Verbal Learning Test (also referred to as Word Learning Test or Verbal Learning Test), Digit Span Test or Visual Association Test<sup>52-55</sup>. No minimal clinically important difference for memory tests was found in the literature.

### Dementia

Depending on the study design, dementia was diagnosed either in a clinical setting or at a research center. The diagnosis was, at least in part, based on clinical presentation. Studies in which dementia diagnosis was based only on a cutoff point for the MMSE were excluded from this analysis because cognitive function tests are insufficient to diagnose dementia<sup>56</sup>. Prevalence of dementia at baseline was available for 11 cohorts; 431 participants had a diagnosis of dementia at baseline, but only 78 of them were classified as noneuthyroid. Owing to the small number of participants with thyroid dysfunction at baseline, no cross-sectional analyses for dementia were performed.

### **Statistical Analyses**

We used a 2-stage individual participant data analysis approach, which accommodates uniform definitions and analyses for each cohort while keeping complexity to a minimum<sup>18,57</sup>. The first stage consisted of study-level analysis of thyroid dysfunction and cognitive function or dementia conducted on the original datasets with participant-level data. In the second stage, the effect estimates from the first stage were pooled using a random-effects model based on restricted maximum likelihood. Heterogeneity across studies was quantified using the  $I^2$  statistic: less than 40% was considered low heterogeneity; 40% to 75%, moderate heterogeneity; and greater than 75%, high heterogeneity.

For both the cross-sectional and longitudinal analyses between thyroid dysfunction and cognitive function, we used multivariable linear regression models. To facilitate combination of different scales, the results were transformed to standardized mean differences. In the prospective analysis of cognitive decline, we calculated the difference between the last available measurement of cognitive function and baseline cognitive function. The difference was divided by the follow-up time in years to obtain an annual decline, irrespective of duration of follow-up. The annual decline was subsequently standardized, resulting in a standardized mean difference in annual change in cognitive function allowing comparison of changes over time.

The risk of developing dementia during follow-up was assessed using Cox regression models. In these analyses, participants with dementia at baseline were excluded. For studies without precise registration of the date of dementia diagnosis, it was assumed dementia developed halfway between the registration date and the last date that absence of dementia was ascertained.

Thyroid dysfunction (overt hyperthyroidism, subclinical hyperthyroidism, subclinical hypothyroidism, and overt hypothyroidism) was included as a categorical variable with the euthyroid group serving as reference. All analyses were adjusted for age and sex. The longitudinal analyses of cognitive decline were adjusted for baseline cognitive function. Prespecified subgroup analyses were performed by stratification and interaction analysis for sex and for age younger or older than 75 years. Additional analyses were performed with adjustment for educational attainment, though this variable was not available in all cohorts. In sensitivity analyses, participants with missing FT<sub>4</sub> measurements in the subclinical hyperthyroid and subclinical hypothyroid groups were excluded, as were those who used antithyroid medication or thyroid hormone replacement therapy at baseline. Furthermore, we assessed robustness of the associations by pooling the estimates using fixed-effect models and by excluding studies with strata of fewer than 10 participants. To assess whether effects were dependent

on degree of disruption of thyrotropin, analyses were repeated with thyrotropin categories of less than 0.10 mIU/L, 0.10 to 0.44 mIU/L, 4.5 to 6.9 mIU/L, 7.0 to 10 mIU/L and greater than 10 mIU/L, in which participants with thyrotropin between 0.45 and 4.49 mIU/L served as reference. Lastly, instead of using biochemical cutoff points, thyrotropin and FT<sub>4</sub> were analyzed continuously across the full range with cognitive function. Thyrotropin was transformed using the natural logarithm; for both natural log-transformed thyrotropin and FT<sub>4</sub>, models were constructed per standard deviation. Continuous models were performed minimally adjusted by age and sex and with additional adjustment for educational attainment. For sensitivity purposes, the analyses were also conducted excluding the participants who used antithyroid medication or thyroid hormone replacement therapy at baseline. Cohorts with greater than 10% missing measurements for FT<sub>4</sub> were excluded for the continuous analyses on FT<sub>4</sub>. All *P* values were 2-tailed; statistical significance was set at *P* < .05.

Study-level analyses were performed using SPSS Statistics, version 25 (IBM). Effect estimates were pooled and summarized in forest plots using R, version 3.6.1 and metafor package (R Foundation for Statistical Computing)<sup>58</sup>. This study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline.

## RESULTS

### Population Characteristics

Individual participant data on thyroid function and cognitive function and/or dementia were provided by 23 cohorts comprising 74 565 participants. At baseline, 66 567 (89.3%) participants were biochemically classified as euthyroid, 577 (0.8%) as overtly hyperthyroid, 2557 (3.4%) as subclinically hyperthyroid, 4167 (5.6%) as subclinically hypothyroid, and 697 (0.9%) as overtly hypothyroid (**Supplementary Table 1**). The study-specific median age at baseline varied from 57 to 93 years; 42 847 (57.5%) participants were women.

A total of 38 144 participants from 21 cohorts provided data on a measure of cognitive function (**Table**). The median age varied from 58 to 93 years, and 18 089 (47.4%) participants were women. Follow-up for cognitive decline was available for 14 cohorts, with a median follow-up duration varying from 1.7 to 11.3 years, accumulating 114 267 person-years.

Eight cohorts provided follow-up for dementia incidence on 46 606 participants (**Supplementary Table 2**). Among these participants, 28 820 (61.8%) were women, and the median age at baseline was between 57 and 85 years. During follow-up, 2033 (4.4%) cases of incident dementia were identified. Median follow-up duration ranged from 3.8 to 15.3 years, accumulating 525 222 person-years.

**Table 1.** Baseline Characteristics of the 38,144 Participants with Cognitive function measurements in Included Studies

<b>Study (Location)</b>	<b>Population description</b>	<b>Baseline, y</b>	<b>No.</b>	<b>Age, Median (Range), y</b>
Europe				
BELFRAIL cohort study (Belgium)	Adults aged $\geq 80$ y	2008-2009	523	84 (80-102)
BETS (England)	Community-dwelling adults aged $\geq 65$ years	2002-2004	5,845	72 (65-98)
CFAS (England and Wales)	Adults aged $\geq 64$ years	1991-1992	1,015	73 (64-94)
InCHIANTI Study (Italy)	Community dwelling adults	1998-2000	1,187	71 (21-102)
LASA (the Netherlands)	Adults aged $\geq 65$ y	1995-1997	1,266	75 (65-89)
Leiden 85-plus Study (the Netherlands)	Adults aged 85 y	1997-1999	557	85
LLS (the Netherlands)	Long-lived siblings	2002-2005	776	93 (89-103)
PAQUID study (France)	Community dwelling adults aged $\geq 65$ y	1989-1990	407	75 (66-94)
PREVEND study (the Netherlands)	Adults	2003-2006	864	58 (35-82)
PROSPER study (the Netherlands, Ireland, Scotland)	Older community-dwelling adults at high cardiovascular risk	1998-1999	5,775	75 (69-83)
Rotterdam Study (the Netherlands)	Adults aged $\geq 55$ y	1989-1992	1,875	69 (55-93)
SHIP (Germany)	Adults	2002-2006	1,329	69 (60-88)
North America				
CHS (United States)	Community-dwelling adults with Medicare eligibility	1994-1998	3,991	74 (64-98)
HABC Study (United States)	Community dwelling adults aged 70-79 y with Medicare eligibility	1999-2000	2,488	75 (71-82)
MMC (Mexico)	Geriatric outpatients with and without dementia	2004	156	79 (58-98)
MrOS (United States)	Community-dwelling men aged $\geq 65$ y	2000-2002	1,600	73 (65-99)
NHANES 1999-2002 (United States)	Adults	1999-2002	853	70 (60-85)
NHANES 2011-2012 (United States)	Adults	2011-2012	434	68 (60-80)
Australia				
HIMS (Australia)	Men aged $\geq 65$ y	2001-2004	3,168	76 (71-89)
Asia				
KLOSCAD (Republic of Korea)	Adults aged $\geq 60$ y	2010-2017	3,854	70 (61-109)
KLOSHA (Republic of Korea)	Adults aged $\geq 65$ years	2010-2012	181	75 (70-96)
<b>Overall</b>	21 cohorts	1989-2017	38,144	74 (21-109)

Women, No. (%)	Euthyroid participants <sup>a</sup> , No. (%)	Thyroid medication users, No. (%)	Cognitive function		Follow up Duration <sup>d</sup> , Median (Range), y
			Scales	Score <sup>c</sup> , Mean (SD)	
330 (63.1)	453 (86.6)	50 (9.6) <sup>b</sup>	MMSE	26 (4.0)	1.7 (0.5-2.3)
2,972 (50.8)	5,266 (90.1)	0 (0)	MMSE	28 (2.2)	0
518 (51.0)	906 (89.3)	NA	MMSE	28 (2.0)	2.0 (1.9-2.6)
666 (56.1)	1,044 (88.0)	33 (2.8)	MMSE	25 (4.8)	9.0 (2.8-10.0)
650 (51.3)	1,093 (86.3)	26 (2.1)	MMSE, WLT	27 (3.1)	9.9 (2.3-20.8)
369 (66.2)	456 (81.9)	20 (3.6)	MMSE, LDST, VLT	24 (6.3)	5.0 (1.0-5.0)
468 (60.3)	652 (84.0)	NA	MMSE	24 (5.1)	0
234 (57.5)	359 (88.2)	6 (1.5) <sup>b</sup>	MMSE, DSST, VLT	26 (3.5)	11.3 (1.5-27.0)
371 (42.9)	777 (89.9)	NA	RFFT, VAT	64 (25.0)	5.2 (0.8-7.8)
2,984 (51.7)	5,063 (87.7)	256 (4.4)	MMSE, LDST, WLT	28 (1.5)	3.3 (0.8-4.0)
1,155 (61.6)	1,611 (85.9)	46 (2.5) <sup>b</sup>	MMSE	28 (1.7)	10.8 (1.5-21.7)
647 (48.7)	1,008 (75.8)	190 (14.3)	MMSE	28 (3.2)	5.6 (4.3-8.8)
2,356 (59.0)	3,253 (81.5)	401 (10.0) <sup>b</sup>	3MS, DSST	90 (9.9)	5.9 (0.9-7.0)
1,280 (51.4)	2,076 (83.4)	251 (10.1)	3MS, EXIT15	90 (8.9)	8.0 (2.0-13.0)
107 (68.6)	109 (69.9)	12 (7.7)	MMSE	15 (6.5)	0
0 (0)	1,409 (88.1)	122 (7.6) <sup>b</sup>	3MS, TMT	93 (6.4)	4.6 (3.5-5.9)
437 (51.2)	751 (88.0)	91 (10.7)	DSST	42 (18.3)	0
214 (49.3)	405 (93.3)	57 (13.1)	DSST, WLT	45 (17.6)	0
0 (0)	2,897 (91.4)	112 (3.5)	MMSE	28 (1.3)	0
2,152 (55.8)	3,476 (90.2)	NA	SCIRS, TMT, DS	29 (1.7)	3.7 (0.8-7.3)
179 (98.9)	154 (85.1)	NA	MMSE, TMT, DS	24 (3.9)	0
18,089 (47.4)	33,218 (87.1)	1,673 (5.3)			5.4 (0.5-27.0)

Abbreviated study names: BETS, Birmingham Elderly Thyroid Study; CFAS, Cognitive Function and Ageing Study; CHS, Cardiovascular Health Study; HABC, Health, Aging and Body Composition Study; HIMS, Health in Men Study; InCHIANTI, Invecchiare in Chianti Study; KLOSCAD, Korean Longitudinal Study on Cognitive Aging and Dementia; KLOSHA, Korean Longitudinal Study on Health and Aging; LASA, Longitudinal Aging Study Amsterdam; LLS, Leiden Longevity Study; MMC, Mexican Memory Clinic; MrOS, Osteoporotic Fractures in Men Study; NHANES, National Health and Nutrition Examination Survey; PAQUID study, Personnes-Agées QUID study; PREVEND, Prevention of Renal and Vascular End-stage Disease Study; PROSPER, Prospective Study of Pravastatin in the Elderly at Risk; SHIP, Study of Health in Pomerania.

Abbreviated cognition test names: 3MS, Modified Mini-Mental State Examination; DSST, Digit Symbol Substitution Test; EXIT15, The 15-item Executive Interview; LDST, Letter Digit Substitution Test; MMSE, Mini-Mental State Examination; RFFT, Ruff Figural Fluency Test; VAT, Visual Association Test; VLT, Verbal Learning Test; WLT, Word Learning Test.

NA, Data not available.

<sup>a</sup> We used a common definition for biochemical euthyroidism of thyroid-stimulating hormone 0.45-4.49 mIU/L, resulting in different numbers from previous reports

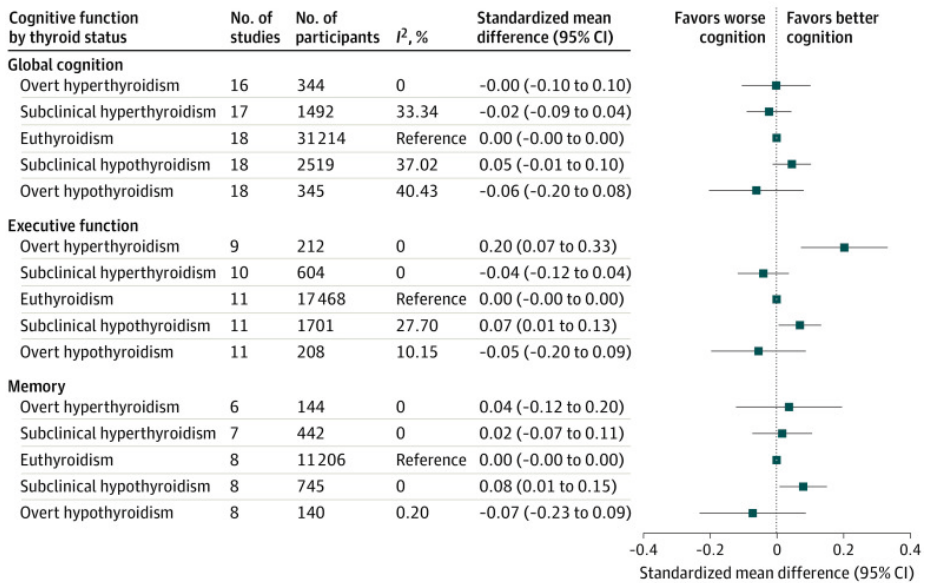
<sup>b</sup> Data on baseline medication use (thyroid replacement therapy, antithyroid drugs) were unavailable for 2 participants of the BELFRAIL Study, 3 participants of the Cardiovascular Health Study, 64 participants of the Osteoporotic Fractures in Men Study, 12 participants of the PAQUID Study, 1 participant of the Rotterdam Study.

<sup>c</sup> Test scores are shown for global cognitive function tests. If no global cognitive function test scores were provided, executive function test scores are shown.

<sup>d</sup> Follow up in years for participants who had a follow up measurement for cognitive function.

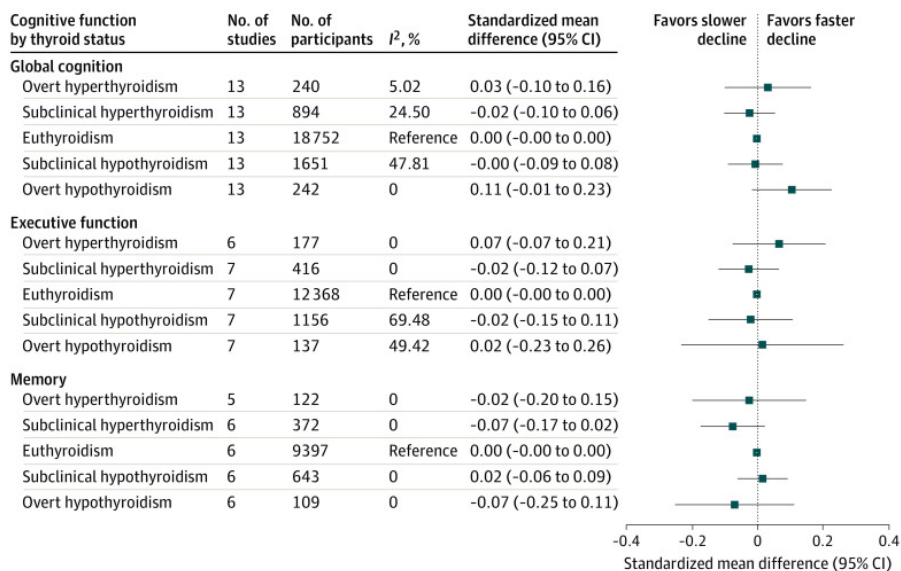
### **Thyroid Dysfunction and Global Cognitive Function**

Cross-sectionally, thyroid dysfunction was not associated with global cognitive function among 18 cohorts (**Figure 1, Supplementary Figure 1**). The largest observed difference was -0.06 standardized mean difference (95% CI, -0.20 to 0.08;  $P = .40$ ) global cognitive function for overt hypothyroidism compared with euthyroidism, which could be interpreted as an approximately 0.1-point lower MMSE score based on the SD for the 2 largest cohorts included.



**Figure 1.** Cross-sectional association between thyroid dysfunction and cognitive function test scores. Standardized mean differences were adjusted for age and sex. Error bars indicate 95% confidence intervals.

No statistically significant association was observed between thyroid dysfunction at baseline and annual change in global cognitive function during follow-up among 13 cohorts (**Figure 2**). Participants with overt hypothyroidism had 0.11 standardized mean difference (95% CI, -0.01 to 0.23;  $P = .09$ ) higher decline per year in global cognitive function than participants who were euthyroid, which translates to approximately 0.1 point on the MMSE scale faster decline per year based on the SD in the largest cohort for this analysis. Additional adjustment for educational attainment did not materially change the results (**Supplementary Figure 2**). Stratification by age and sex did not show any differential effects for global cognitive function (**Supplementary Table 3**).



**Figure 2.** Longitudinal association between thyroid dysfunction and cognitive function test scores. Standardized mean differences were adjusted for age and sex. Error bars indicate 95% confidence intervals.

No statistically significant associations were found when individuals were categorized by severity of thyrotropin abnormality (**Supplementary Figure 3**). Reanalyzing the data with a fixed-effects model or without strata with fewer than 10 participants did not yield different results (**Supplementary Table 4**). Leaving out participants with missing FT4 measurements or those using antithyroid medication or thyroid hormone replacement therapy at baseline also did not change the results. A positive association was found between continuous thyrotropin and global cognition only when thyroid supplementation and antithyroid medication users were excluded (0.028 higher standardized mean difference per SD; 95% CI, 0.003 to 0.053;  $P = .03$ ; **Supplementary Table 5**). No association between continuous FT4 levels and global cognitive function was found. Heterogeneity across studies was low for the cross-sectional main analyses ( $I^2 = 0\%$ -40%), while heterogeneity was low to moderate for the longitudinal and sensitivity analyses ( $I^2 = 0\%$ -70%).

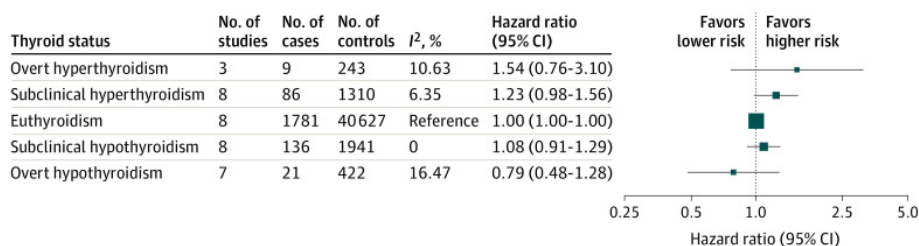
### Thyroid Dysfunction and Executive Function and Memory

No negative association was observed cross-sectionally between thyroid dysfunction and executive function or memory among 11 and 8 cohorts respectively (**Figure 1, Supplementary Figure 4 and 5**). Participants with overt hyperthyroidism had 0.20 standardized mean difference (95% CI, 0.07 to 0.33;  $P = .002$ ) higher executive function score compared with participants who were

euthyroid; transformed, this would account for 1.6 more correct substitutions within 60 seconds for the LDST based on the largest cohort in this analysis. In both executive function and memory, participants with subclinical hypothyroidism performed better than participants who were euthyroid (executive function: 0.07 standardized mean difference; 95% CI, 0.01 to 0.13;  $P = .03$ ; memory: 0.08 standardized mean difference; 95% CI, 0.01 to 0.15;  $P = .03$ ). Longitudinally, no association was found between thyroid dysfunction at baseline and decline in executive function among 7 cohorts or memory among 6 cohorts; all differences were smaller than 0.1 standardized mean difference (**Figure 2**). Additional adjustment for educational attainment did not materially change the results (**Supplementary Figure 2**). No statistically significant interaction with sex or age was present ( $P > .05$  for all; supporting data in **Supplementary Table 3**). Using a fixed-effects model or excluding strata with fewer than 10 participants did not change the results for executive function or memory (**Supplementary Table 4**). The association of subclinical hypothyroidism and better executive function was attenuated when participants with missing fT<sub>4</sub> measurements were left out, while the association with memory was unchanged. The positive association between overt hyperthyroidism and executive function disappeared when participants using thyroid medication were removed. No association was found when individuals were categorized by severity of thyrotropin abnormality or when thyrotropin was analyzed continuously (**Supplementary Figure 3, Supplementary Table 5**). Continuous analysis of FT<sub>4</sub> levels showed a positive association with executive function (0.019 higher standardized mean difference per SD; 95% CI, 0.002 to 0.036;  $P = .03$ ), which was attenuated when participants using thyroid medication were left out. Heterogeneity across studies was low for the cross-sectional main analyses ( $I^2 = 0\%-40\%$ ), while heterogeneity was low to moderate for the longitudinal analyses ( $I^2 = 0\%-70\%$ ) and up to high heterogeneity in the sensitivity analyses ( $I^2 \leq 73\%$ ).

### Thyroid Dysfunction and Dementia

Cross-sectional analysis of thyroid dysfunction and dementia were unfeasible owing to few participants who were not euthyroid with dementia at baseline (78 participants among 11 cohorts). In longitudinal analyses among 12 cohorts, no association was found between thyroid dysfunction and incident dementia (**Figure 3, Supplementary Figure 6**). The hazard ratio of dementia ranged from 1.54 (95% CI, 0.76 to 3.10) for overt hyperthyroidism to 0.79 (95% CI, 0.48 to 1.28) for overt hypothyroidism. Continuous analysis of thyrotropin and FT<sub>4</sub> also did not provide evidence for an association; hazard ratio, 0.96 per SD increase of natural log-transformed thyrotropin (95% CI, 0.91 to 1.02;  $P = .16$ ); hazard ratio, 1.05 per SD increase of FT<sub>4</sub> (95% CI, 0.98 to 1.13;  $P = .16$ ). Heterogeneity between studies was low ( $I^2 = 0\%-40\%$ ).



**Figure 3.** Longitudinal association between thyroid dysfunction and incident dementia. Hazard Ratios were adjusted for age and sex. Error bars indicate 95% confidence intervals.

## DISCUSSION

In this individual participant data analysis of 74 565 participants from 23 cohorts, there was no association between subclinical thyroid dysfunction and cognitive function, cognitive decline, or the onset of dementia. Owing to uncertainty of the results for overt hypothyroidism and hyperthyroidism, no rigorous conclusions can be drawn regarding the association between overt thyroid dysfunction and cognitive decline and dementia.

While prior study-level meta-analyses also reported no association between subclinical hypothyroidism and cognitive function, cognitive decline, or dementia, they were limited by heterogeneity in definitions of thyroid dysfunction and choices of covariates in the statistical models<sup>14-17</sup>. Because we performed an individual participant data analysis, we could standardize definitions of thyroid function categories and of cognitive function and cognitive decline and standardize the statistical models. By addressing these limitations and reaching the same results, the present study provides the strongest observational evidence to date suggesting that subclinical hypothyroidism is not associated with cognitive function or cognitive decline.

Various studies and 2 meta-analyses did show an association between subclinical or overt hyperthyroidism or low thyrotropin level within the reference range and a higher risk of dementia<sup>14,17,20,26,59-61</sup>. Although our findings for subclinical and overt hyperthyroidism and dementia did not reach statistical significance, they are directionally consistent with the literature. Despite combining 8 cohorts comprising more than 45 000 participants, the number of individuals with subclinical and overt hyperthyroidism and the number of individuals who developed dementia during follow-up are limited. Therefore, we cannot exclude a higher risk of dementia in individuals with hyperthyroidism. In addition, individuals with overt hyperthyroidism had a slightly higher rate of cognitive decline, though not statistically significant. Considering the existing

literature and the other results in the present study, the observed cross-sectional association between overt hyperthyroidism and better executive function was most likely a chance finding. Moreover, the observed difference in executive function was less than half the minimal clinically important difference, making it a clinically insignificant finding regardless of the *P* value.

Higher vulnerability among subgroups has been proposed; younger adults and women might be more susceptible to cognitive dysregulation associated with thyroid dysfunction<sup>16,62</sup>. Moreover, cognitive decline might only be present in individuals with more extreme values of thyrotropin<sup>21,63</sup>, or variation in FT<sub>4</sub> instead of thyrotropin levels could be associated with dementia risk<sup>22</sup>. In the present multicohort study, we did not observe differential associations for participants younger and older than 75 years or for men and women, nor any association with variation in FT<sub>4</sub> level or more extreme values of thyrotropin. Therefore, subgroup associations reported in prior studies might not be generalizable outside the original cohorts.

As mentioned before, all but 1 randomized clinical trial on levothyroxine treatment for subclinical hypothyroidism also did not provide evidence for improvement of cognitive function<sup>8-12</sup>. Moreover, both undertreatment and overtreatment with levothyroxine are common, estimated at 27% and 14%, respectively<sup>64</sup>. Overtreatment is associated with increased risk of atrial fibrillation and atherosclerosis<sup>65,66</sup> and, via cerebrovascular damage, might be associated with increased risk of cognitive decline. Therefore, screening for subclinical thyroid dysfunction in older adults to prevent cognitive impairment and dementia does not appear to be effective.

The current individual participant data analysis has several strengths. The use of individual participant data from cohorts from all over the globe enhances generalization while allowing standardized definitions and relevant subgroup analyses. All but 5 of the included studies had a median age of 70 years or older, which is essential but often not the case in research concerning outcomes that are most relevant for older adults<sup>67</sup>. The present study approached cognition comprehensively; we assessed multiple domains of cognitive function, cross-sectionally and longitudinally, and incidence of dementia.

### **Limitations**

Some limitations need to be acknowledged. Thyroid function categorization was based on biochemical characteristics. For 20% to 30% of the participants who were categorized as subclinical hypothyroid or hyperthyroid, we could not confirm subclinical thyroid dysfunction owing to the absence of FT<sub>4</sub> measurement. This may have led to some misclassification, yet sensitivity analyses excluding those

participants with missing FT<sub>4</sub> data yielded similar results. We could not include educational attainment in our main analysis because of 5 out of 18 cohorts did not collect this data. Even though the sensitivity analyses with adjustment for educational attainment yielded similar results as the main analysis, education is a possible confounder which could not be accounted for. For most cohorts, only 1 measurement of thyroid function was available, which is why only baseline thyroid function was used in the present individual participant data analysis. This study could therefore not capture any changes in cognitive function that might occur at the transition of one thyroid status to another. Moreover, for the vast majority of study participants, a maximum of 2 measurements of cognitive function was available, which precluded advanced modeling of change over time including non-linear trajectories. In addition, the interpretation of longitudinal studies of cognitive function can be complicated by practice effects<sup>68</sup>. Standardization of change over time might not fully alleviate this; hence, residual practice effects may still be present. Furthermore, because dementia is clinically difficult to diagnose, some misclassification could have occurred, which may have led to an underestimation of the association. In addition, the number of incident dementia cases in the included cohort-studies was low; we therefore cannot rule out a clinically relevant association between thyroid dysfunction and risk of dementia. The heterogeneity between studies may have been increased by the use of different cognitive function tests, different durations of follow-up, differences in age and sex distribution, different lifestyles across continents, and different inclusion criteria. As heterogeneity was expected a priori, we performed all meta-analyses with random effects. Nonetheless, results for fixed-effects meta-analyses were not materially different. The observed heterogeneity was larger in the longitudinal analyses heterogeneity ( $I^2 = 0\%-70\%$ ) than in the cross-sectional analyses ( $I^2 = 0\%-40\%$ ), likely owing to the additional variation of follow-up duration. We hypothesize that the minor differences in  $I^2$  estimates between different cross-sectional analyses are attributable to differences in sample size per exposure. Because individuals with thyroid disease generally receive medical treatment, we cannot address the question of whether long-term untreated hyperthyroidism or hypothyroidism is associated with cognitive function and dementia risk. Moreover, these results only apply to objectifiable cognitive decline, which is not synonymous with the more subjective cognitive complaints.

## CONCLUSIONS

In this individual participant data analysis combining the individual participant data of 74 565 participants from 23 cohorts, subclinical thyroid dysfunction was not associated with cognitive function, cognitive decline, or risk of dementia. Hence, it is unlikely that treatment for otherwise undetected subclinical thyroid dysfunction would improve cognitive function. Moreover, the chance

of overtreatment is considerable, which increases the risk of atrial fibrillation, atherosclerosis and cerebral infarction and thereby might increase the risk of cognitive decline. Whether treatment of overt hypothyroidism or hyperthyroidism is associated with cognitive decline and risk of dementia remains uncertain. Existing clinical guidelines that prescribe screening of subclinical thyroid dysfunction for prevention of cognitive decline or dementia should therefore be revisited.

### **Conflicts of interest**

HJG has received travel grants and speakers honoraria from Fresenius Medical Care, Neuraxpharm, Servier and Janssen Cilag as well as research funding from Fresenius Medical Care.

### **Acknowledgements and funding**

We thank all study participants for making this study possible. We thank dr. Lilia Cárdenas-Ibarra for providing data from the Mexican Memory Clinic.

This work was supported by the European Commission project THYRAGE (Horizon 2020 research and innovation program, 666869) .

The Thyroid Studies Collaboration is funded by a grant from the Swiss National Science foundation (SNSF 320030-172676 to NR).

This research was supported by contracts HHSN268201200036C, HHSN268200800007C, HHSN268201800001C, N01HC55222, N01HC85079, N01HC85080, N01HC85081, N01HC85082, N01HC85083, N01HC85086, 75N92021D00006, and grants U01HL080295 and U01HL130114 from the National Heart, Lung, and Blood Institute (NHLBI), with additional contribution from the National Institute of Neurological Disorders and Stroke (NINDS). Additional support was provided by R01AG023629 from the National Institute on Aging (NIA). ARC received additional support from R01AG027058, LHK from R01AG15928 and OLL from R01AG20098. A full list of principal CHS investigators and institutions can be found at CHS-NHLBI.org. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

This research was supported by National Institute on Aging (NIA) Contracts N01-AG-6-2101; N01-AG-6-2103; N01-AG-6-2106; NIA grant R01-AG028050, and NINR grant R01-NR012459. This research was funded in part by the Intramural Research Program of the NIH, National Institute on Aging.

The Trøndelag Health Study (The HUNT Study) is a collaboration between HUNT Research Centre (Faculty of Medicine and Health Sciences, NTNU, Norwegian University of Science and Technology), Trøndelag County Council, Central Norway Regional Health Authority, and the Norwegian Institute of Public Health. Information on incident dementia diagnoses for the HUNT study population was provided by Nord-Trøndelag Hospital Trust.

The InCHIANTI study was supported as a “targeted project” (ICS110.1/RF97.71) by the Italian Ministry of Health and in part by the U.S. National Institute on Aging (Contracts: 263 MD 9164 and 263 MD 821336); supported in part by the Intramural Research Program of the National Institute on Aging, National Institutes of Health, Baltimore, Maryland.

This study was supported by a grant from the Korean Health Technology R&D Project, Ministry of Health and Welfare, Republic of Korea (grant no. HI09C1379 [A092077]).

The Longitudinal Aging Study Amsterdam is supported by a grant from the Netherlands Ministry of Health Welfare and Sports, Directorate of Long-Term Care.

The Osteoporotic Fractures in Men (MrOS) Study is supported by National Institutes of Health funding. The following institutes provide support: the National Institute on Aging (NIA), the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS), the National Center for Advancing Translational Sciences (NCATS), and NIH Roadmap for Medical Research under the following grant numbers: U01 AG027810, U01 AG042124, U01 AG042139, U01 AG042140, U01 AG042143, U01 AG042145, U01 AG042168, U01 AR066160, and UL1 TR000128. MHS is supported by NIH NCATS UL1TR002369, The Oregon Clinical and Translational Institute.

The PREVEND study was funded by the Dutch Kidney Foundation ([www.nierstichting.nl](http://www.nierstichting.nl); grant E.033). This study was partly funded by the Dutch Alzheimer Foundation ([www.alzheimer-nederland.nl](http://www.alzheimer-nederland.nl)). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

The Radiation Effects Research Foundation (RERF), Hiroshima and Nagasaki, Japan is a public interest foundation funded by the Japanese Ministry of Health, Labour and Welfare (MHLW) and the US Department of Energy (DOE). This publication was supported by RERF Research Protocol RP-A2-16. The views of the authors do not necessarily reflect those of the two governments.

SHIP is part of the Community Medicine Research Network of the University Medicine Greifswald, which is supported by the German Federal State of Mecklenburg-West Pomerania.

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## Chapter 4

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**Supplementary material for “Association of Thyroid Dysfunction with Cognitive Function: An Individual Participant Data Analysis”**

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**Supplementary Table 1.** Reference ranges for free thyroxine and distribution of biochemical thyroid status at baseline for 23 included cohorts

Study name	Reference range fT4	Overt hyperthyroidism,		Subclinical hyperthyroidism,		Euthyroidism,		Subclinical hypothyroidism,		Overt hypothyroidism,	
		No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
BELFRAIL	0.9 to 1.8 ng/dL	2 (0.4)	53 (10.1)	453 (86.6)	4 (0.8)	11 (2.1)					
BETS	9.0 to 20.0 pmol/L	21 (0.4)	219 (3.7)	5,266 (90.1)	311 (5.3)	28 (0.5)					
CFAS	13.0 to 23.0 pmol/L	7 (0.7)	23 (2.3)	906 (89.3)	39 (3.8)	40 (3.9)					
CHS	0.7 to 1.7 ng/dL	69 (1.7)	113 (2.8)	3,253 (81.5)	528 (13.2)	28 (0.7)					
Health ABC	0.8 to 1.8 ng/dL	7 (0.3)	72 (2.9)	2,076 (83.4)	309 (12.4)	24 (1.0)					
HIMS	10.0 to 23.0 pmol/L	12 (0.3)	37 (1.0)	3,239 (91.2)	251 (7.1)	12 (0.3)					
HUNT	8.0 to 20.0 pmol/L	210 (0.6)	938 (2.8)	31,218 (92.0)	1,309 (3.9)	240 (0.7)					
InCHIANTI Study	0.77 to 2.19 ng/dL	16 (1.3)	86 (7.2)	1,044 (88.0)	33 (2.8)	8 (0.7)					
KLOSCAD	0.89 to 1.76 ng/dL	31 (0.7)	175 (3.9)	4,019 (89.3)	221 (4.9)	57 (1.3)					
KLOSHA	0.89 to 1.76 ng/dL	0 (0.0)	0 (0.0)	154 (85.1)	18 (9.9)	9 (5.0)					
LASA	11.0 to 22.0 pmol/L	11 (0.9)	82 (6.5)	1,093 (86.3)	71 (5.6)	9 (0.7)					
Leiden 85-plus Study	13.0 to 23.0 pmol/L	3 (0.5)	23 (4.1)	456 (81.9)	35 (6.3)	40 (7.2)					
LLS	10.0 to 24.0 pmol/L	5 (0.6)	53 (6.8)	652 (84.0)	59 (7.6)	7 (0.9)					
Mexican Memory Clinic	12.0 to 23.0 pmol/L	0 (0.0)	5 (3.2)	109 (69.9)	36 (23.1)	6 (3.8)					
MrOS	0.70 to 1.85 ng/dL	2 (0.1)	30 (1.9)	1,409 (88.1)	148 (9.3)	11 (0.7)					
NHANES 1999-2002 <sup>a</sup>	69.5 to 164.7 nmol/L	5 (0.6)	30 (3.5)	751 (88.0)	52 (6.1)	15 (1.8)					
NHANES 2011-2012	0.6 to 1.6 ng/dL	2 (0.5)	10 (2.3)	405 (93.3)	15 (3.5)	2 (0.5)					
PAQUID study	16.0 to 29.0 pmol/L	3 (0.7)	20 (4.9)	359 (88.2)	17 (4.2)	8 (2.0)					
PREVEND study	9.14 to 23.81 pmol/L	1 (0.1)	30 (3.5)	777 (89.9)	52 (6.0)	4 (0.5)					
PROSPER study	12.0 to 18.0 pmol/L	109 (1.9)	127 (2.2)	5,063 (87.7)	443 (7.7)	33 (0.6)					
RERF	0.8 to 2.5 ng/dL	0 (0.0)	46 (3.1)	1,245 (84.6)	102 (6.9)	79 (5.4)					

**Supplementary Table 1.** Continued.

Study name	Reference range fT4	Overt hyperthyroidism,		Subclinical hyperthyroidism,		Euthyroidism,		Subclinical hypothyroidism,		Overt hypothyroidism,	
		No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Rotterdam Study	11.07 to 24.97 pmol/L	13 (0.7)	120 (6.4)	1,612 (85.9)	108 (5.8)	24 (1.3)					
SHIP	8.3 to 18.9 pmol/L	48 (3.6)	265 (19.9)	1,008 (75.8)	6 (0.5)	2 (0.2)					
<b>Overall</b>		577 (0.8)	2,557 (3.4)	66,567 (89.3)	4,167 (5.6)	697 (0.9)					

Abbreviated study names: BETS, Birmingham Elderly Thyroid Study; CFAS, Cognitive Function and Ageing Study; CHS, Cardiovascular Health Study; Health ABC, Health, Aging and Body Composition Study; HIMS, Health in Men Study; HUNT, Trøndelag Health Study; InCHIANTI, Invecchiare in Chianti Study; KLOSCAD, Korean Longitudinal Study on Cognitive Aging and Dementia; KLOSHA, Korean Longitudinal Study on Health and Aging; LASA, Longitudinal Aging Study Amsterdam; LLS, Leiden Longevity Study; MrOS, Osteoporotic Fractures in Men Study; NHANES, National Health and Nutrition Examination Survey; PAQUID study, Personnes-Agées QUID study; PREVENI, Prevention of Renal and Vascular End-stage Disease Study; PROSPER, Prospective Study of Pravastatin in the Elderly at Risk; RERF, Radiation Effects Research Foundation; SHIP, Study of Health in Pomerania.

<sup>a</sup>Only total thyroxine was available

**Supplementary Table 2.** Baseline Characteristics of the 46,606 Participants Included for Analyses on Incident Dementia

Study (Location)	Population description	Baseline, y	No.	Age, Median (Range), y	Women, No. (%)	Euthyroid participants <sup>a</sup> , No. (%)	Thyroid medication users <sup>b</sup> , No. (%)	Cases with dementia, No. (%)	Follow up Duration <sup>c</sup> , Median (Range), y
Europe									
HUNT Study (Norway)	Adults	1995-1997	33,915	57 (19-99)	23,276 (68.6)	31,218 (92.0)	1,548 (4.6)	547 (1.6)	14.1 (0.1-15.3)
LASA (the Netherlands)	Adults aged ≥65 y	1995-1997	1,051	74 (65-88)	571 (54.3)	902 (85.8)	22 (2.1)	133 (12.7)	9.9 (1.3-16.5)
Leiden 85-plus Study (the Netherlands)	Adults aged 85 y	1997-1999	483	85	314 (65.0)	394 (81.6)	16 (3.3)	64 (13.3)	5.0 (0.5-5.0)

Supplementary Table 2. Continued.

Study (Location)	Population description	Baseline, y	No.	Age, Median (Range), y	Women, No. (%)	Euthyroid participants <sup>a</sup> , No. (%)	Thyroid medication users <sup>b</sup> , No. (%)	Cases with dementia, No. (%)	Follow up Duration <sup>c</sup> , Median (Range), y
PAQUID study (France)	Community dwelling older adults $\geq 65$ y	1989-1990	358	74 (66-94)	200 (55.9)	322 (89.9)	5 (1.4) <sup>b</sup>	119 (33.2)	9.0 (0.2-27.0)
Rotterdam Study (the Netherlands)	Adults aged $\geq 55$ y	1989-1992	1,865	69 (55-93)	1,149 (61.6)	1,601 (85.8)	46 (2.5) <sup>b</sup>	350 (18.8)	15.3 (0.9-21.3)
Australia									
HIMS(Australia)	Men aged $\geq 65$ y	2001-2004	3,549	76 (71-89)	0 (0)	3,237 (91.2)	123 (3.5)	479 (13.5)	11.4 (0.1-14.1)
Asia									
KLOSCAD (Republic of Korea)	Adults aged $\geq 60$ y	2010-2017	3,913	69 (59-94)	2,228 (56.9)	3,489 (89.2)	NA	134 (3.4)	3.8 (0.3-7.5)
RERF (Japan)	Atomic bomb survivors	2000-2003	1,472	74 (56-97)	1,082 (73.5)	1,245 (84.6)	94 (6.4) <sup>b</sup>	207 (14.1)	8.0 (0.3-10.8)
<b>Overall</b>	8 cohorts	1989-2017	46,606	74 (19-99)	28,820 (61.8)	42,408 (91.0)	1,854 (4.3)	2,033 (4.4)	9.5 (0.1-27.0)

Abbreviated study names: HIMS, Health in Men Study; HUNT, Trøndelag Health Study; KLOSCAD, Korean Longitudinal Study on Cognitive Aging and Dementia; LASA, Longitudinal Aging Study Amsterdam; PAQUID study, Personnes-Agées QUID study; RERF Study, Radiation Effects Research Foundation. NA, Data not available.

<sup>a</sup> We used a common definition for biochemical euthyroidism of thyroid-stimulating hormone 0.45-4.49 mU/L, resulting in different numbers from previous reports  
<sup>b</sup> Data on baseline medication use (thyroid replacement therapy, antithyroid drugs) were unavailable for 12 participants of the PAQUID Study, 1 participant of the RERF Study, 1 participant of the Rotterdam Study.

<sup>c</sup> Follow up for incident cases was ended at date of diagnosis or midway between the last wave without and first wave with dementia.

**Supplementary Table 3.** Cross-sectional associations between Thyroid Dysfunction and Cognitive Function Test Scores stratified by Age and Sex

	Overt Hypothyroidism	Subclinical Hypothyroidism	Euthyroidism	Subclinical Hypothyroidism	Overt Hypothyroidism	No. with Overt Hypothyroidism/ Subclinical Hypothyroidism/ Euthyroidism/ Subclinical Hypothyroidism/ Overt Hypothyroidism
<b>Global cognitive function</b>						
All	-0.00 (-0.10;0.10)	-0.02 (-0.09;0.04)	0 (Ref)	0.05 (-0.01;0.10)	-0.06 (-0.20;0.08)	344/1492/31,214/2519/345
Sex						
Men	0.03 (-0.19;0.25)	-0.03 (-0.10;0.05)	0 (Ref)	0.03 (-0.05;0.12)	-0.02 (-0.18; 0.15)	95/587/16,933/1155/123
Women	-0.00 (-0.13;0.12)	-0.04 (-0.12;0.05)	0 (Ref)	0.08 (0.03;0.14)	-0.10 (-0.26; 0.07)	259/905/14,279/1364/222
Age, years						
<75	0.04 (-0.08;0.15)	-0.03 (-0.08;0.03)	0 (Ref)	0.04 (-0.02;0.10)	-0.05 (-0.18; 0.09)	192/793/17,074/1186/157
≥75	-0.05 (-0.22;0.13)	-0.01 (-0.11;0.10)	0 (Ref)	0.06 (-0.01;0.12)	-0.09 (-0.28; 0.10)	152/699/14,140/1333/188
<b>Executive function</b>						
All	0.20 (0.07;0.33)	-0.04 (-0.12;0.04)	0 (Ref)	0.07 (0.01;0.13)	-0.05 (-0.20; 0.09)	212/604/17,468/1701/208
Sex						
Men	-0.02 (-0.41;0.36)	-0.04 (-0.15;0.08)	0 (Ref)	0.05 (-0.03;0.13)	-0.05 (-0.24; 0.15)	45/215/9003/736/87
Women	0.26 (0.11;0.41)	-0.04 (-0.14;0.07)	0 (Ref)	0.10 (0.03;0.16)	-0.04 (-0.27; 0.19)	167/389/8463/965/121
Age, years						
<75	0.27 (0.10;0.45)	-0.02 (-0.12;0.07)	0 (Ref)	0.02 (-0.04;0.09)	0.03 (-0.16;0.21)	120/350/10086/844/97
≥75	0.07 (-0.16;0.30)	-0.06 (-0.18;0.06)	0 (Ref)	0.12 (0.05;0.19)	-0.15 (-0.36;0.06)	92/254/7382/857/111
<b>Memory</b>						
All	0.04 (-0.12;0.20)	0.02 (-0.07;0.11)	0 (Ref)	0.08 (0.01;0.15)	-0.07 (-0.23; 0.09)	144/442/11,206/745/140
Sex						
Men	-0.04 (-0.37;0.28)	0.04 (-0.11;0.20)	0 (Ref)	0.18 (0.04;0.31)	0.03 (-0.25;0.30)	34/156/5397/262/45

Supplementary Table 3. Continued.

	Overt Hypothyroidism	Subclinical Hypothyroidism	Euthyroidism	Subclinical Hypothyroidism	Overt Hypothyroidism	No. with Overt Hypothyroidism/ Subclinical Hypothyroidism/ Euthyroidism/ Overt Hypothyroidism
Women	0.06 (-0.12;0.25)	0.00 (-0.11;0.11)	0 (Ref)	0.03 (-0.07;0.12)	-0.12 (-0.33;0.09)	110/286/5807/483/95
Age, years						
<75	-0.01 (-0.22;0.20)	0.08 (-0.04;0.19)	0 (Ref)	0.04 (-0.05;0.14)	-0.04 (-0.27;0.19)	78/256/6599/413/64
≥75	0.09 (-0.16;0.33)	-0.07 (-0.21;0.07)	0 (Ref)	0.13 (0.02;0.24)	-0.08 (-0.32;0.17)	66/186/4607/332/76

Supplementary Table 4. Sensitivity analyses of cross-sectional associations between Thyroid Dysfunction and Cognitive Function Test Scores

	Global cognitive function		Executive function		Memory	
	N cases/N controls	SMD (95% CI)	N cases/N controls	SMD (95% CI)	N cases/N controls	SMD (95% CI)
<b>Overt Hypothyroidism</b>						
Random-effects model	344/30951	-0.00 (-0.11; 0.10)	212/16989	0.20 (0.07; 0.33)	144/10674	0.04 (-0.12; 0.20)
Fixed-effects model	344/30951	-0.00 (-0.11; 0.10)	212/16989	0.20 (0.07; 0.33)	144/10674	0.04 (-0.12; 0.20)
Excluding strata <10 participants	315/24643	0.02 (-0.09; 0.13)	192/11275	0.23 (0.09; 0.36)	138/9291	0.02 (-0.14; 0.19)
Excluding participants using thyroid medication at baseline	165/25080	-0.09 (-0.32; 0.15)	79/11447	0.07 (-0.19; 0.33)	77/6397	-0.05 (-0.27; 0.17)
<b>Subclinical Hypothyroidism</b>						
Random-effects model	1492/31060	-0.02 (-0.09; 0.05)	604/17348	-0.04 (-0.12; 0.04)	442/11054	0.02 (-0.07; 0.11)
Fixed-effects model	1492/31060	-0.03 (-0.08; 0.03)	604/17348	-0.04 (-0.12; 0.04)	442/11054	0.02 (-0.07; 0.11)
Excluding strata <10 participants	1487/30951	-0.02 (-0.09; 0.05)	604/17348	-0.04 (-0.12; 0.04)	442/11054	0.02 (-0.07; 0.11)

Supplementary Table 4. Continued.

	Global cognitive function		Executive function		Memory	
	N cases/N controls	SMD (95% CI)	N cases/N controls	SMD (95% CI)	N cases/N controls	SMD (95% CI)
Excluding participants using thyroid medication at baseline	1018/25186	-0.08 (-0.18; 0.01)	264/12494	-0.08 (-0.20; 0.04)	216/6772	0.07 (-0.06; 0.21)
Excluding participants with missing free thyroxine	1148/31060	-0.03 (-0.11; 0.05)	494/17348	-0.03 (-0.12; 0.05)	353/11054	0.03 (-0.08; 0.13)
<b>Subclinical Hypothyroidism</b>						
Random-effects model	2519/31214	0.05 (-0.01; 0.10)	1701/17468	0.07 (0.01; 0.14)	745/11206	0.08 (0.01; 0.15)
Fixed-effects model	2519/31214	0.05 (0.01; 0.09)	1701/17468	0.08 (0.03; 0.12)	745/11206	0.08 (0.01; 0.15)
Excluding strata <10 participants	2509/29753	0.04 (-0.02; 0.10)	1701/17468	0.07 (0.01; 0.14)	745/11206	0.08 (0.01; 0.15)
Excluding participants using thyroid medication at baseline	2002/25186	0.04 (-0.02; 0.10)	1280/12494	0.09 (0.01; 0.16)	479/6772	0.08 (-0.01; 0.17)
Excluding participants with missing free thyroxine	1995/31214	0.01 (-0.06; 0.08)	1259/17468	0.03 (-0.03; 0.09)	515/11206	0.10 (0.02; 0.18)
<b>Overt Hypothyroidism</b>						
Random-effects model	345/31214	-0.06 (-0.20; 0.08)	208/17468	-0.05 (-0.20; 0.09)	140/11206	-0.07 (-0.23; 0.09)
Fixed-effects model	345/31214	-0.08 (-0.19; 0.02)	208/17468	-0.06 (-0.19; 0.07)	140/11206	-0.07 (-0.23; 0.09)
Excluding strata <10 participants	296/26797	-0.06 (-0.23; 0.11)	187/15831	-0.08 (-0.23; 0.07)	109/8601	-0.09 (-0.30; 0.12)
Excluding participants using thyroid medication at baseline	208/25186	-0.05 (-0.25; 0.15)	122/12494	-0.06 (-0.30; 0.17)	66/6772	-0.20 (-0.51; 0.10)

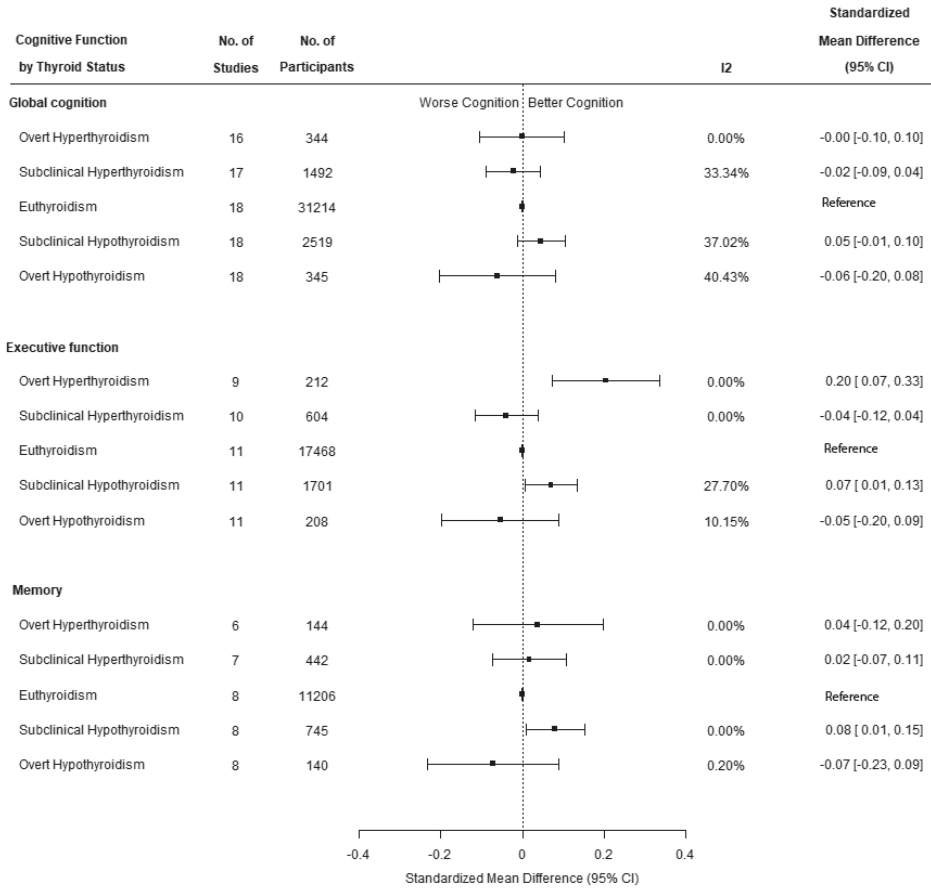
**Supplementary Table 5.** Association between full range TSH and ft4 and Cognitive Function Test Scores

	TSH			ft4		
	N	Per SD of lnTSH	I <sup>2</sup>	N	Per SD	I <sup>2</sup>
<b>Global cognition</b>						
Minimally adjusted	35,901	0.012 (-0.006; 0.030)	63.56%	22,514	0.006 (-0.007; 0.018)	0.00%
Adjusted for education	26,725	0.016 (-0.001; 0.033)	48.37%	14,845	0.002 (-0.014; 0.018)	7.94%
Excluding participants using thyroid medication	28,571	<b>0.028 (0.003; 0.053)</b>	70.49%	15,973	-0.000 (-0.021; 0.020)	25.97%
<b>Executive function</b>						
Minimally adjusted	20,192	0.018 (-0.002; 0.038)	45.48%	11,042	<b>0.019 (0.002; 0.036)</b>	0.00%
Adjusted for education	20,165	0.011 (-0.005; 0.027)	29.24%	11,018	0.015 (-0.001; 0.032)	0.00%
Excluding participants using thyroid medication	14,238	0.040 (-0.001; 0.081)	73.11%	5,681	0.003 (-0.028; 0.034)	4.44%
<b>Memory</b>						
Minimally adjusted	12,673	0.006 (-0.011; 0.023)	0.16%	5,598	0.012 (-0.013; 0.037)	0.00%
Adjusted for education	12,656	0.000 (-0.016; 0.016)	0.19%	5,581	0.014 (-0.009; 0.036)	0.00%
Excluding participants using thyroid medication	7,607	-0.012 (-0.047; 0.024)	29.67%	804	0.012 (-0.069; 0.094)	0.00%

For the continuous associations of ft4 with cognition cohorts with >10% missing data were excluded; Health ABC, LASA, Mexican Memory Clinic cohort, PAQUID, PROSPER, Rotterdam Study, SHIP. NHANES 1999-2002 was excluded because only total T4 was measured.

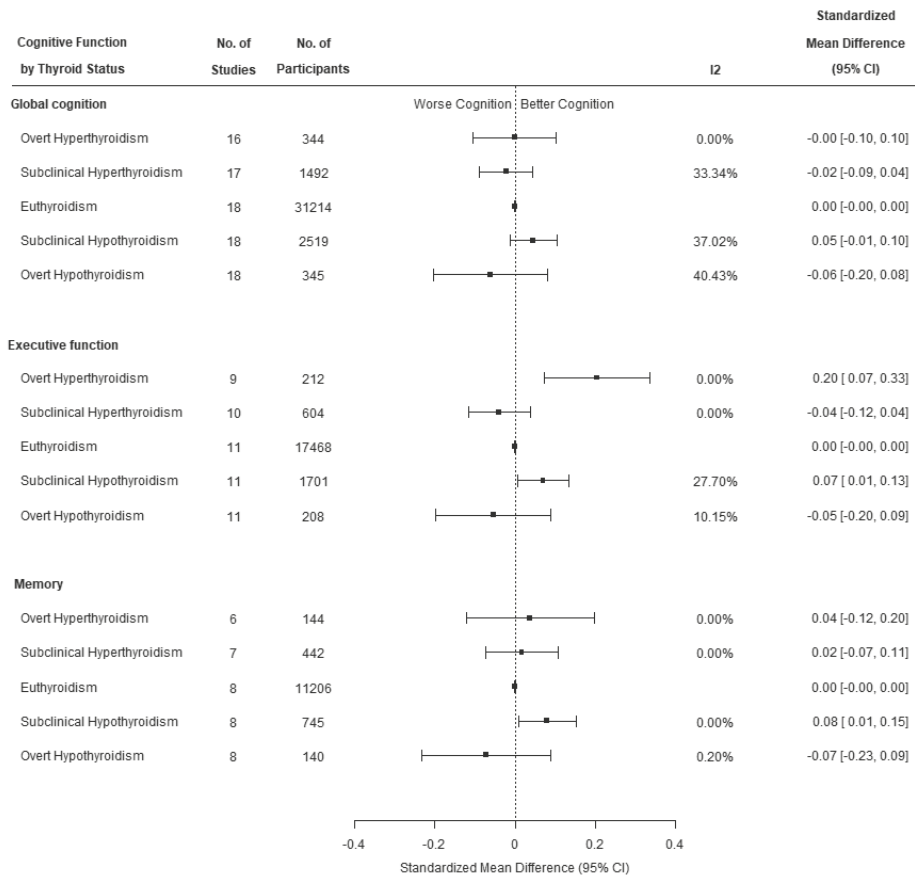
Data on educational attainment was not available for BETS, CFAS, LLS, MMC and SHIP.

Data on medication use was unavailable for CFAS, KLOSCAD, KLOSHA, LLS and PREVEND.

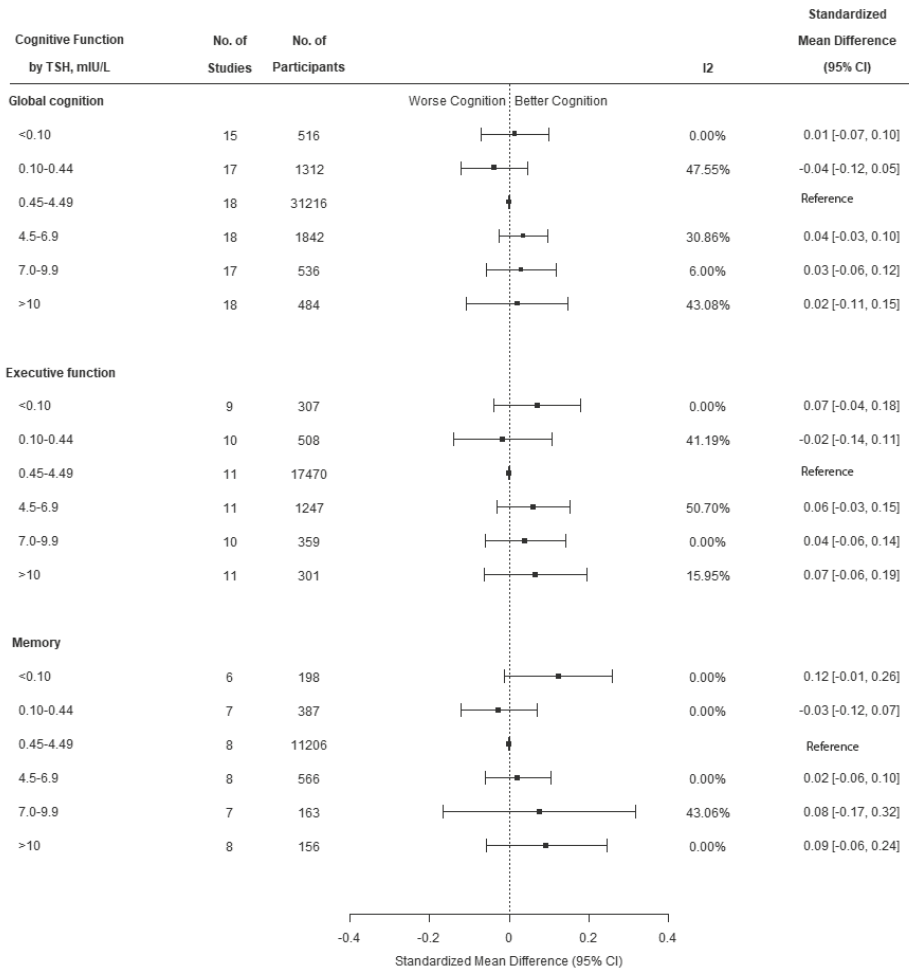


**Supplementary Figure 1.** Cross-sectional association between Thyroid Dysfunction and Global Cognitive Function

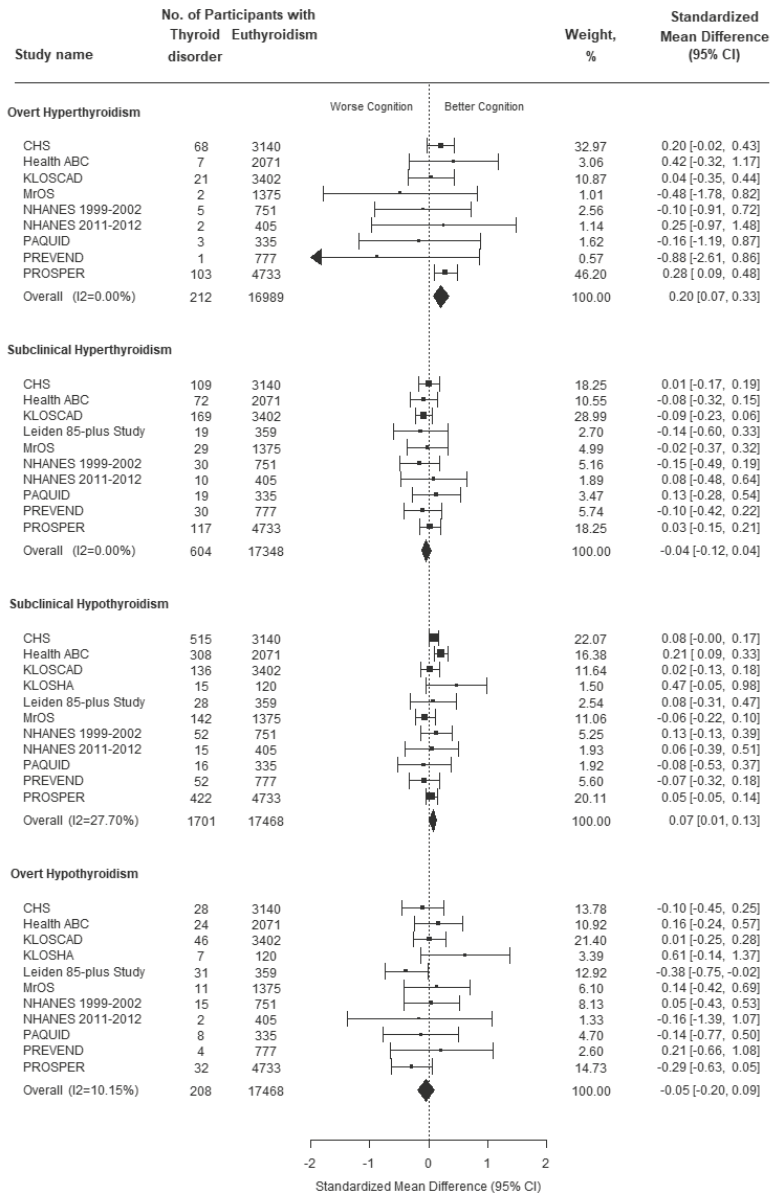
Standardized mean differences (SMDs) were adjusted for age and sex. Error bars indicate 95% confidence intervals. Abbreviated study names: BETS, Birmingham Elderly Thyroid Study; CFAS, Cognitive Function and Ageing Study; CHS, Cardiovascular Health Study; Health ABC, Health, Aging and Body Composition Study; HIMS, Health in Men Study; InCHIANTI, Invecchiare in Chianti Study; KLOSCAD, Korean Longitudinal Study on Cognitive Aging and Dementia; KLOSHA, Korean Longitudinal Study on Health and Aging; LASA, Longitudinal Aging Study Amsterdam; LLS, Leiden Longevity Study; MMC, Mexican Memory Clinic; MrOS, Osteoporotic Fractures in Men Study; PAQUID study, Personnes-Agées QUID study; PROSPER, Prospective Study of Pravastatin in the Elderly at Risk; SHIP, Study of Health in Pomerania.



**Supplementary Figure 2.** Cross-sectional association between Thyroid Dysfunction and Cognitive Function Test Scores additionally adjusted for education. Standardized mean differences (SMDs) were adjusted for age, sex and educational attainment. Error bars indicate 95% confidence intervals.

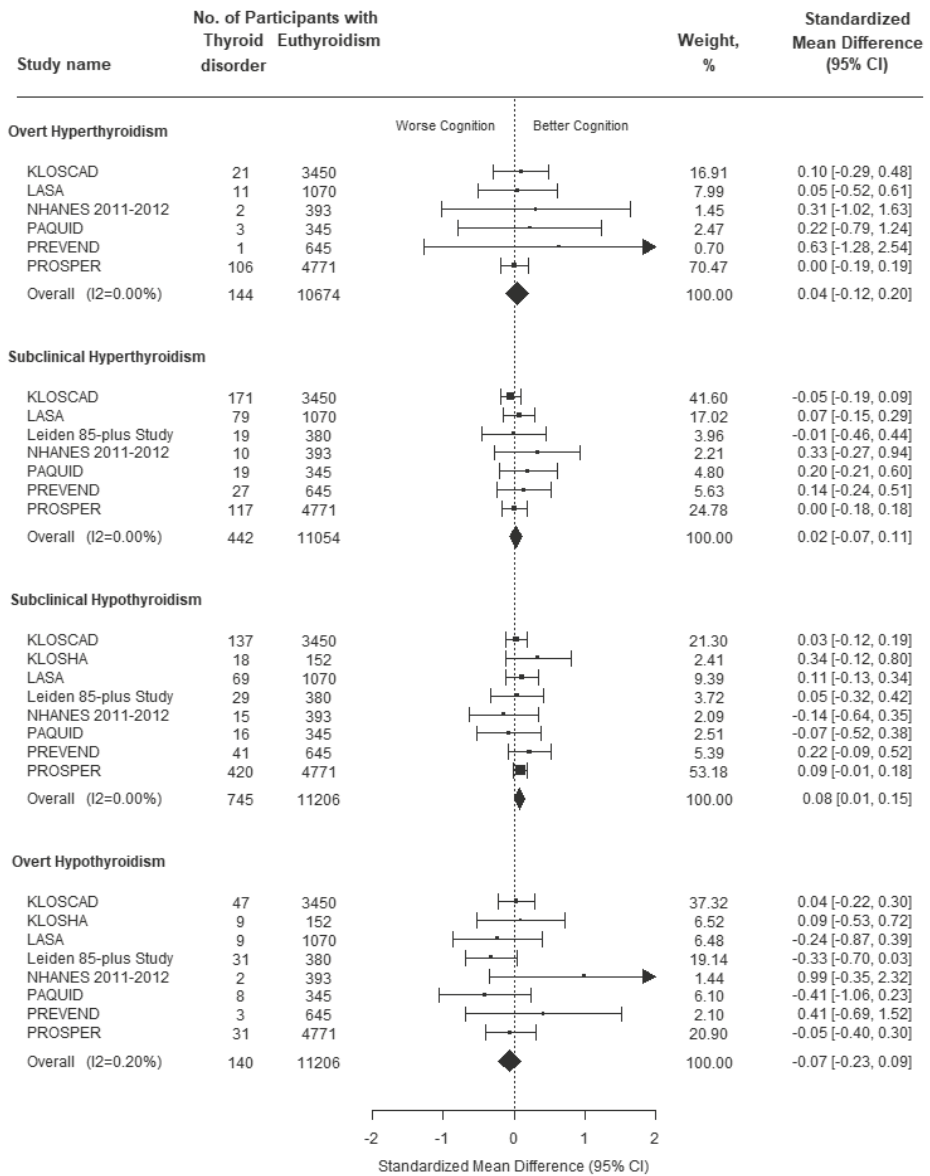


**Supplementary Figure 3.** Cross-sectional association between categorized TSH and Cognitive Function Test Scores  
 Standardized mean differences (SMDs) were adjusted for age and sex. Error bars indicate 95% confidence intervals.

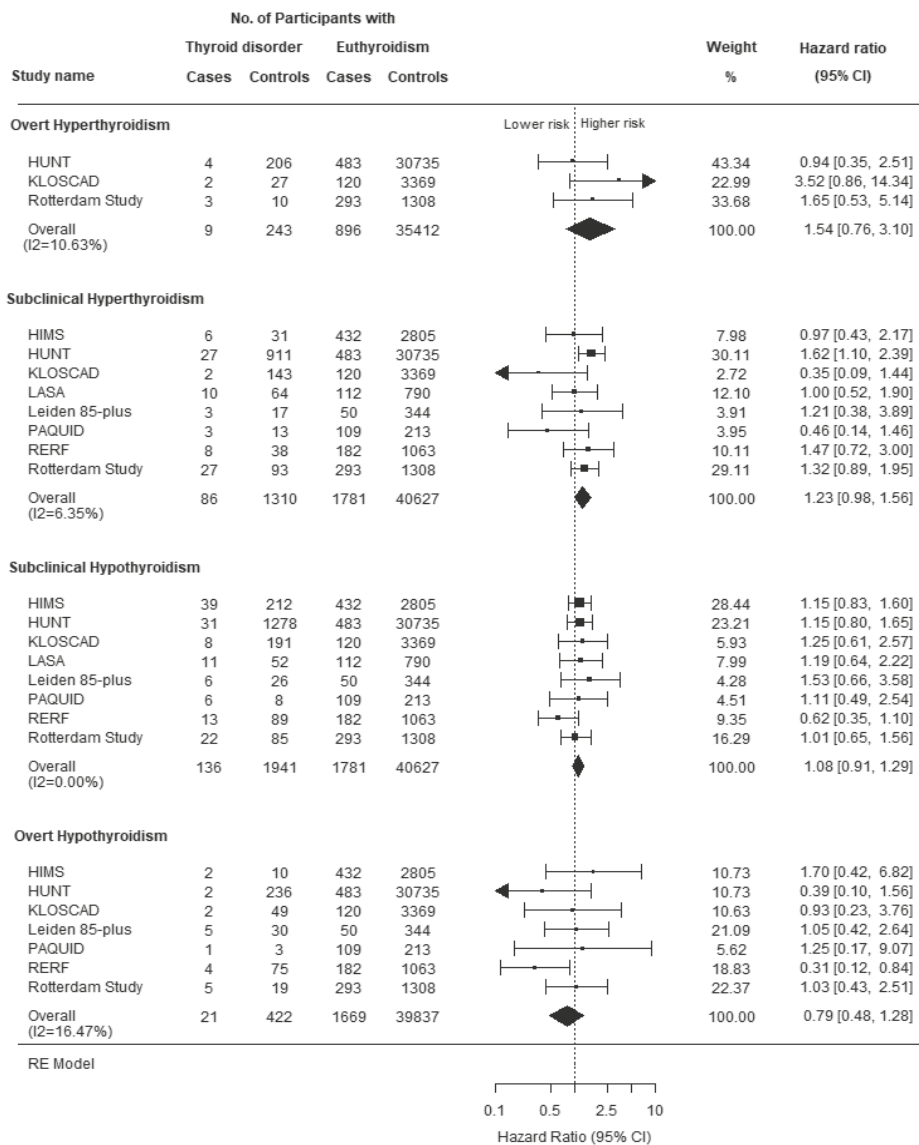


**Supplementary Figure 4.** Cross-sectional association between Thyroid Dysfunction and Executive Function

Standardized mean differences (SMDs) were adjusted for age and sex. Error bars indicate 95% confidence intervals. Abbreviated study names: CHS, Cardiovascular Health Study; Health ABC, Health, Aging and Body Composition Study; Korean Longitudinal Study on Cognitive Aging and Dementia; KLOSCAD, Korean Longitudinal Study on Health and Aging; MrOS, Osteoporotic Fractures in Men Study; NHANES, National Health and Nutrition Examination Survey; PAQUID study, Personnes-Agées QUID study; PREVEND, Prevention of Renal and Vascular End-stage Disease Study; PROSPER, Prospective Study of Pravastatin in the Elderly at Risk.



**Supplementary Figure 5.** Cross-sectional association between Thyroid Dysfunction and Memory. Standardized mean differences (SMDs) were adjusted for age and sex. Error bars indicate 95% confidence intervals. Abbreviated study names: KLOSCAD, Korean Longitudinal Study on Cognitive Aging and Dementia; KLOSHA, Korean Longitudinal Study on Health and Aging; LASA, Longitudinal Aging Study Amsterdam; LLS, Leiden Longevity Study; MMC, Mexican Memory Clinic; MrOS, Osteoporotic Fractures in Men Study; NHANES, National Health and Nutrition Examination Survey; PAQUID study, Personnes-Agées QUID study; PREVEND, Prevention of Renal and Vascular End-stage Disease Study; PROSPER, Prospective Study of Pravastatin in the Elderly at Risk.



**Supplementary Figure 6.** Longitudinal Association between Thyroid Dysfunction and Incident Dementia

Hazard Ratios (HRs) were adjusted for age and sex. Error bars indicate 95% confidence intervals. Abbreviated study names: HIMS, Health in Men Study; HUNT, Trøndelag Health Study; KLOSCAD, Korean Longitudinal Study on Cognitive Aging and Dementia; LASA, Longitudinal Aging Study Amsterdam; PAQUID study, Personnes-Agées QUID study; RERF, Radiation Effects Research Foundation.





# CHAPTER 5

Thyroid Stimulating Hormone and Bone Mineral Density: Evidence From a Two-Sample Mendelian Randomization Study and a Candidate Gene Association Study

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*J Bone Miner Res 2018 Jul;33(7):1318-1325.*

## ABSTRACT

With population aging, prevalence of low bone mineral density (BMD) and associated fracture risk are increased. To determine whether low circulating thyroid stimulating hormone (TSH) levels within the normal range are causally related to BMD, we conducted a two-sample Mendelian randomization (MR) study. Furthermore, we tested whether common genetic variants in the TSH receptor (*TSHR*) gene and genetic variants influencing expression of *TSHR* (eQTLs) are associated with BMD. For both analyses, we used summary-level data of genome-wide association studies (GWAS) investigating BMD of the femoral neck (N=32,735) and the lumbar spine (N=28,498) in cohorts of European ancestry from the Genetic Factors of Osteoporosis (GEFOS) Consortium. For the MR study, we selected 20 genetic variants that were previously identified for circulating TSH levels in a GWAS meta-analysis (N=26,420). All independent genetic instruments for TSH were combined in analyses for both femoral neck and lumbar spine BMD. In these studies, we found no evidence that a genetically determined 1 standard deviation (SD) decrease in circulating TSH concentration was associated with femoral neck BMD (0.003 SD decrease in BMD per SD decrease of TSH, 95% C.I. -0.053; 0.048, P=0.92) or lumbar spine BMD (0.010 SD decrease in BMD per SD decrease of TSH, 95% C.I. -0.069; 0.049, P=0.73). A total of 706 common genetic variants have been mapped to the *TSHR* locus and expression loci for *TSHR*. However, none of these genetic variants were associated with BMD at the femoral neck or lumbar spine. In conclusion, we found no evidence for a causal effect of circulating TSH on BMD, nor did we find any association between genetic variation at the *TSHR* locus or expression thereof and BMD.

## INTRODUCTION

Bone is a dynamic tissue that undergoes continuous remodeling to maintain its strength and integrity<sup>1</sup>. When bone remodeling is uncoupled and resorption exceeds formation, bone mineral density (BMD) progressively decreases and ultimately leads to osteoporosis<sup>2</sup>. To develop therapies that are more effective and accompanied by fewer side effects than current treatments, further research into the molecular mechanisms underlying the pathogenesis of osteoporosis is required.

One of these potential underlying mechanisms is thyroid status. Briefly, thyroid status is a composite measure of circulating thyroid stimulating hormone (thyrotropin, TSH) and free thyroxine (fT4). In healthy individuals, circulating levels of TSH and fT4 are regulated by the hypothalamic-pituitary-thyroid-axis (HPT-axis) via feedforward and negative feedback mechanisms<sup>3</sup>. Therefore, circulating levels of TSH and fT4 are inversely related. However, the exact combination of circulating concentrations of TSH and fT4 is determined by the HPT-axis set point, which is unique for each individual<sup>4</sup>. Thyroid hormones have a critical role in adult bone turnover and maintenance<sup>5</sup>. Hyperthyroidism (TSH concentration below the normal reference range and fT4 circulating level above the normal reference range) is associated with low BMD and an increased risk of fracture, and is an established cause of secondary osteoporosis<sup>6</sup>. Furthermore, a similar relationship has been reported in individuals with subclinical hyperthyroidism (reduced circulating concentration of TSH but circulating fT4 within the normal reference range)<sup>7,8</sup>, and in euthyroid individuals with a relatively low TSH and relatively high fT4 within the normal reference range<sup>9,10</sup>. Accordingly, individuals with subclinical hyperthyroidism had increased bone loss during prospective follow-up compared to euthyroid individuals<sup>11</sup>. In addition to effects of thyroid hormone on bone, some studies have suggested direct effects of TSH on bone<sup>12,13</sup>. However, others have not confirmed these findings<sup>14,15</sup>. Therefore, it remains unclear whether observed changes in bone mass and strength result from increased fT4 levels alone, or whether the associated decrease in TSH also contributes.

Mendelian randomization (MR) can be used to determine whether an association is causal, as it eliminates confounding and reverse causation. MR analysis uses genetic variants associated with an exposure as instrumental variables instead of direct measurements of the exposure<sup>16</sup>. Because genetic traits are inherited independently according to Mendel's second law, the determinant is randomly distributed in the population and independent of the outcome. In the present study, this assumes that genes associated with thyroid status and genes associated with BMD are inherited independently. Thus, analogous to randomized

clinical trials, by using MR analysis the exposure can be assigned randomly and so associations between exposure (thyroid status) and outcome (BMD) can be investigated in the absence of confounding and reverse causation. MR studies can be performed on circulating TSH and fT4 levels within the normal reference range<sup>17</sup>, as these measures of thyroid status have been shown to be partly and independently genetically determined in large-scale genome-wide association studies (GWAS)<sup>18, 19</sup>. Even though TSH and fT4 levels are highly correlated, different genetic variants were associated with circulating levels of TSH than with fT4<sup>18</sup>. The genetic independence of these traits highlights the individuality of the HPT-axis set point, and allow for separate analyses of TSH and fT4.

In the present study, we aimed to investigate whether thyroid status is causally associated with BMD through a two-sample Mendelian randomization study. However, due to the limited number of genetic instruments available for fT4 concentration in the largest meta-analysis to date on BMD (i.e. lack of statistical power), only the relationship between TSH and BMD could be investigated rigorously. Additionally, to investigate the TSH receptor, which mediates TSH action in target cells, we explored the *TSHR* gene in a candidate gene study to determine whether genetic variation at this locus or expression thereof is associated with BMD.

## **MATERIALS AND METHODS**

### **Genetic variants for TSH**

We selected single nucleotide polymorphisms (SNPs) for all genetic loci independently associated with circulating levels of TSH ( $p$  value  $< 5 \times 10^{-8}$ ) identified by the largest GWAS meta-analysis to date<sup>18</sup>. All participants included in the GWAS were of European ancestry, and individuals with known thyroid pathologies, taking thyroid medication, who underwent thyroid surgery and with circulating levels of TSH  $< 0.4$  mIU/L or  $> 4.0$  mIU/L were excluded from the analyses. For comparability of the different cohorts, the circulating levels of TSH were log transformed, and standardised to Z-values. Due to these transformations, the additive beta estimates of the SNPs can be interpreted as the per-allele standard deviation (SD) change in logTSH concentration. In total, 20 loci for TSH were identified in 26,420 participants. Overall, the mean age of participants ranged from 42.5 to 79.0 years, 44 percent of the participants were men. As an illustration, the descriptives of the two largest included cohorts (PROSPER and SardinIA) were as follows. In PROSPER, 49.1% were men and the mean age was 75.3 years with 3.4 years SD. In the SardinIA cohort, 46.9% were men and the mean age was 42.5 with 17.7 years SD. The mean TSH concentration was 1.9 mIU/L and 1.7 mIU/L respectively (SD was 0.8 mIU/L for both cohorts).

### Data sources and outcome definition

We used publicly available data from the largest meta-analysis to date on BMD from the Genetic Factors of Osteoporosis (GEFOS) consortium<sup>20</sup>, which identified novel loci for BMD at the femoral neck, lumbar spine and forearm, sites of the three most common osteoporotic fractures. Forearm BMD data were not used in the present study, because of the relatively low number of participants (N = 8,143). The meta-analysis on femoral neck BMD comprised 32,735 participants from nine cohorts of European ancestry and the meta-analysis on lumbar spine BMD comprised 28,498 participants from eight cohorts of European ancestry. The mean age in the participating cohorts ranged from 17.7 to 80.2 years, and 34 percent of the participants in the meta-analysis were men. From these data, we extracted the per-allele beta estimates of the SNPs previously identified in relation to circulating levels of TSH on femoral neck BMD and lumbar spine BMD, accompanied by the standard errors and the effect alleles.

### Power calculation

The statistical power for the MR analyses for TSH on BMD was calculated using a publicly available power calculator<sup>21</sup>. For the femoral neck BMD and the lumbar spine BMD there was sufficient power (femoral neck power = 0.85, and lumbar spine power = 0.80) to detect a causal association with a coefficient of 0.07 SD of BMD per decrease of one SD of TSH when using the data from GEFOS<sup>20</sup>.

### Statistical analyses

Methods for Mendelian randomization analysis of summary-level data have been described<sup>17, 22, 23</sup>. Briefly, associations between individual genetic instruments for circulating levels of TSH and BMD were estimated, after taking into account multiple testing via Bonferroni correction based on the number of genetic instruments tested. We combined effects of the individual genetic instruments using inverse-variance weighted (IVW) analyses<sup>23</sup>, resulting in a weighted mean estimate of the effect of genetically determined 1-SD decrease in circulating level of TSH on BMD of the femoral neck and the lumbar spine in SD. However, this method could suffer from bias, because of potential pleiotropic effects of the genetic variants on other apparently unrelated phenotypes. If genetic variants have pleiotropic effects that influence outcome (eg, BMD) via alternative pathways, the observed associations can be biased. Therefore, MR-Egger regression<sup>24</sup> was conducted as sensitivity analysis to account for potential pleiotropy and to formally test the presence of directional pleiotropy. Additionally, we performed weighted median estimator (WME) analyses<sup>25</sup>, which estimate a weighted median effect of genetically determined 1-SD decrease in circulating level of TSH on BMD. Similarity of the IVW and WME effect estimates indicates that the results are robust<sup>25</sup>. We also performed additional sensitivity analyses to account for possible regression dilution of analyses. In two-sample MR analyses,

the reliability of the results depends on the precision of the previously measured association between the genetic variants and the exposure (ie, circulating TSH concentration). If the reported effect sizes for TSH do not reflect the true effect of the genetic variants, the association between the genetically determined levels of TSH and BMD will be erroneous. One of the available tests to assess the resulting imprecision of the MR is  $I^2_{GX}$ -statistic<sup>26</sup>. Preferably the  $I^2_{GX}$ -statistic is close to 1, but an  $I^2_{GX}$ -statistic  $\geq 0.9$  is still acceptable<sup>26</sup>. If the  $I^2_{GX}$ -statistic is lower, the effect estimate is likely to be diluted, which means the observed association is an underestimation of the true effect. This type of bias can be corrected by simulation extrapolation (SIMEX)<sup>27</sup>. This method simulates estimates of the investigated association with increasing imprecision to extrapolate a more precise estimate.

The combined effects of the genetic variants were calculated using the codes in R that were provided online by the authors<sup>24-26</sup>. Results are presented as the mean effect per 1-SD genetically determined decrease in circulating TSH level together with the 95% confidence interval (CI); a two-sided  $p$  value of less than 0.05 was considered statistically significant.

### **Candidate gene association study on the TSH receptor**

To investigate whether variation in the TSH receptor gene (*TSHR*) is associated with BMD, we conducted a candidate gene association study using the same publicly available summary level data of the GEFOS consortium<sup>20</sup>. SNPs were indexed if located in the *TSHR* gene or within 50,000 base pairs up- or downstream. Additionally, previously reported SNPs influencing expression of *TSHR* (expression quantitative loci [eQTLs]) were included in the study. We excluded SNPs with a minor allele frequency (MAF) lower than 5% or if the SNP was absent from the GEFOS datasets. To determine an appropriate threshold for statistical significance, we based the correction factor on the number of independent genetic variants, meaning those not in linkage disequilibrium (LD). The number of independent genetic variants was calculated using the web-based tool LDlink (considering an  $R^2 < 0.4$ )<sup>28</sup>. A cutoff of  $R^2 < 0.4$  was chosen, to limit the number of independent variants to a minimum, resulting in a smaller chance of false negative results. A  $-\log(P\text{-value})$  plot was constructed using R package ggplot2<sup>29</sup> for both femoral neck and lumbar spine BMD, with a Bonferroni-corrected significance threshold ( $p = 0.05/\text{number of independent variants}$ ) and a nominal threshold ( $p = 0.05$ ).

## RESULTS

### Effect of individual genetic instruments for TSH

The associations between individual genetic instruments for circulating concentration of TSH and BMD are summarised in **Table 1**. Of the 20 SNPs previously associated with circulating TSH level, 19 were available in the BMD datasets; for rs6885099 in *PDE8B* we used rs2046045 as a proxy SNP ( $R^2 = 1.00$ ,  $D' = 1.00$ ). None of the individual genetic instruments for circulating concentration of TSH were associated with femoral neck BMD ( $p$  values  $>0.05$ ) (**Figure 1A**) or lumbar spine BMD ( $p$  values  $>0.05$ ) (**Figure 1B**).

### Combined effect of the genetic instruments for TSH

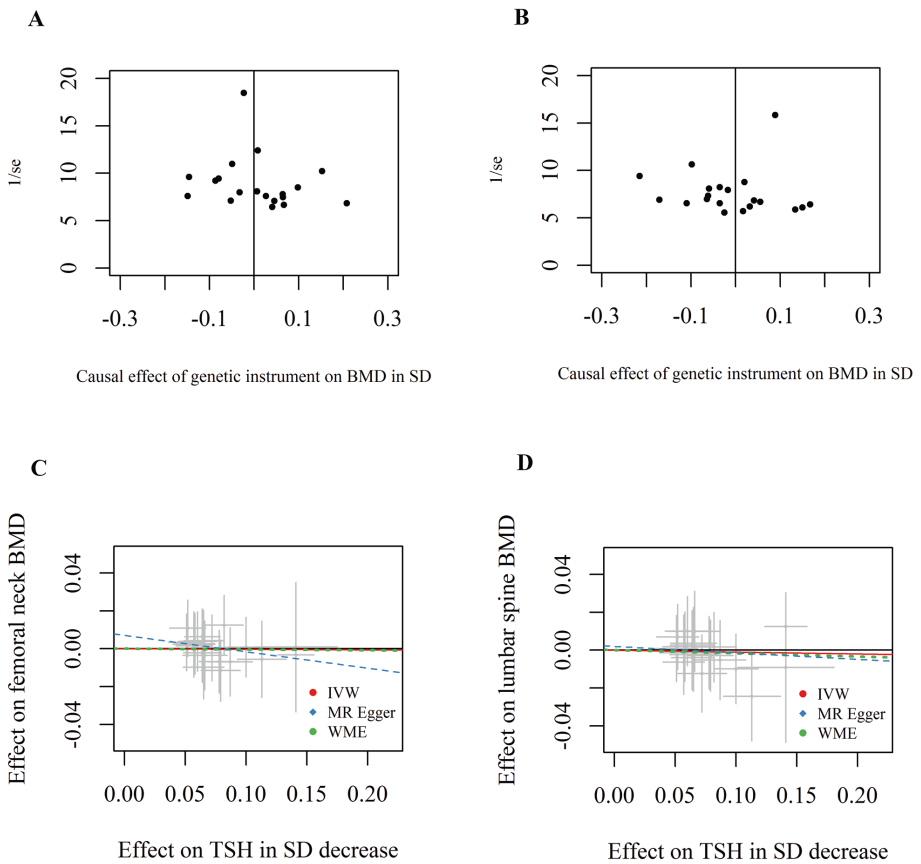
Using the inverse-variance weighted analysis (**Table 2**), we found no evidence for an association between genetically determined lower circulating levels of TSH and femoral neck BMD (0.003 SD decrease in BMD per SD decrease in TSH; 95% CI, -0.053 to 0.048;  $p = 0.92$ ) (**Figure 1C**) or lumbar spine BMD (0.010 SD decrease in BMD per SD decrease in TSH; 95% CI, -0.069 to 0.049;  $p = 0.73$ ) (**Figure 1D**). The estimates from MR-Egger regression and WME analyses were consistent with these results. As the  $I^2_{GX}$ -statistic of the combined genetic variants for TSH was 0.81, we performed additional simulation extrapolation (SIMEX) of the MR-Egger estimate, which did not materially change the observations. Moreover, MR-Egger did not indicate the presence of directional pleiotropy given the absence of evidence of deviation of the regression line from the intercept.

**Table 1.** Associations of individual genetic instruments for TSH with BMD of the femoral neck and the lumbar spine

Gene	SNP	Chromosome	Position	Effect allele	EAF	Effect on TSH in SD	F-Statistic	Femoral neck BMD in SD	Lumbar spine BMD in SD
NR3C2	rs10032216	4	149669506	T	0.781	0.087	63	0.0069	0.0052
FGF7	rs10519227	15	49746364	A	0.245	-0.072	43	0.0005	-0.0123
CAPZB	rs10799824	1	19841174	A	0.161	-0.113	89	-0.0056	-0.0244
ITPK1	rs11624776	14	93595591	A	0.660	-0.064	34	-0.0021	0.0026
VEGFA	rs11755845	6	43904780	T	0.266	-0.065	42	-0.0097	-0.0023
IGFBP5	rs13015993	2	217625523	A	0.736	0.078	61	0.0068	0.0014
MBIP	rs1537424	14	36574018	T	0.608	-0.052	33	0.0108	0.0069
GLIS3	rs1571583	9	4267209	A	0.249	0.057	32	-0.0023	0.0015
PRDM11	rs17723470	11	45227567	T	0.279	-0.065	42	0.0042	0.0036
MIR1179	rs17776563	15	89119104	A	0.322	-0.060	36	0.0039	0.0100
NFIA	rs334699	1	61620496	A	0.052	-0.141	45	0.0009	-0.0091
MAF/LOC440389	rs3813582	16	79749353	T	0.674	0.082	67	-0.0125	-0.0016
INSR	rs4804416	19	7223848	T	0.569	-0.057	40	0.0015	-0.0063
ABO	rs657152	9	136139265	A	0.343	0.058	42	-0.0026	-0.0018
PDE8B	rs6885099	5	76530349	A	0.594	-0.141	245	-0.0033	0.0125
PDE10A	rs753760	6	166046483	C	0.691	0.100	100	-0.0008	0.0099
NRG1	rs7825175	8	32416274	A	0.210	-0.066	36	-0.0035	0.0099
VEGFA	rs9472138	6	43811762	T	0.285	-0.079	62	-0.0115	-0.0028
SASH1	rs9497965	6	148521292	T	0.415	0.051	32	-0.0034	-0.0008
SOX9	rs9915657	17	70127536	T	0.541	-0.064	51	0.0063	-0.0040

Abbreviations: SNP, single nucleotide polymorphism; EAF, effect allele frequency; TSH, thyroid stimulating hormone; SD, standard deviation; BMD, bone mineral density.

Data presented as beta coefficients per effect allele.



**Figure 1.** The effect of genetic instruments for TSH levels on BMD

Results are presented as the beta coefficients.

In panel **A** and **B**: The X-axis presents the per-allele effect on BMD for each individual genetic variant; the Y-axis presents the 1/Standard Error (1/SE) for each effect estimate. The association between 20 individual genetic variants for TSH levels and (**A**) Femoral neck BMD in standard deviations, (**B**) Lumbar spine BMD in standard deviations.

In panel **C** and **D**: The X-axis presents the decrease in TSH in standard deviations; the Y-axis presents the effect on BMD. The modeled association between genetic instruments for TSH and BMD using Inverse-Variance Weighted analysis (IVW), MR Egger and Weighted Median Estimator (WME) are shown for (**C**) Femoral neck BMD in standard deviations, (**D**) Lumbar spine BMD in standard deviations.

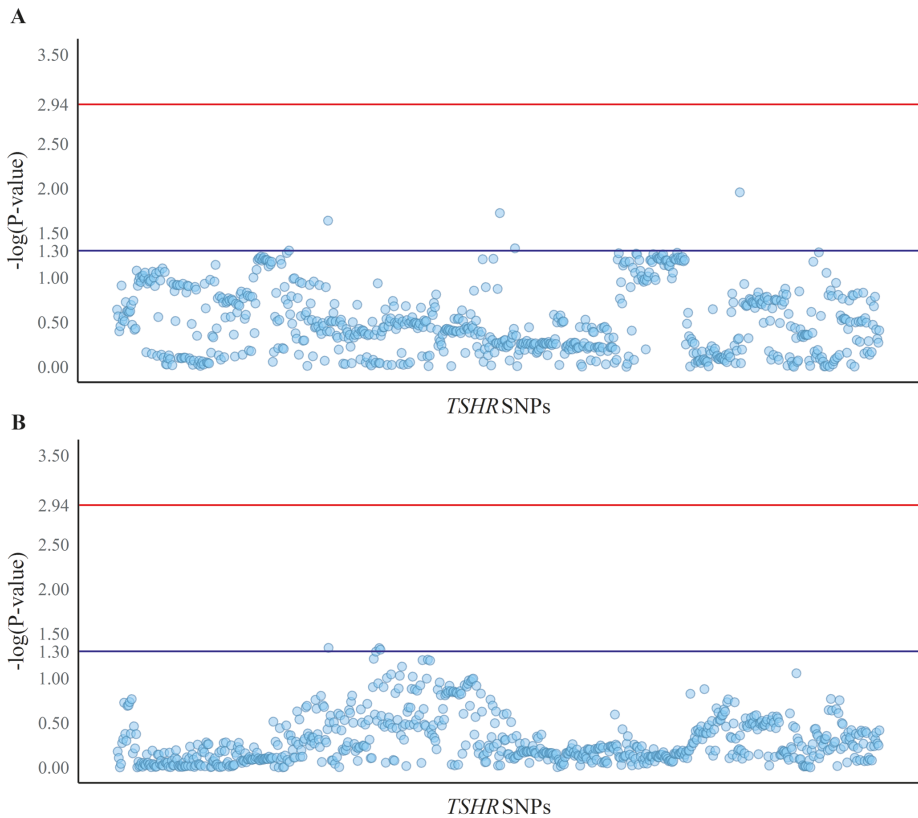
**Table 2.** Mendelian randomization estimates for TSH on BMD

	Femoral neck BMD in SD	P-value	Lumbar spine BMD in SD	P-value
<b>Inverse-Variance Weighted</b>	0.00 (-0.05; 0.05)	0.92	-0.01 (-0.07; 0.05)	0.73
<b>MR-Egger</b>				
<i>Estimate</i>	-0.09 (-0.23; 0.08)	0.28	-0.03 (-0.20; 0.15)	0.71
<i>Intercept</i>	0.01 (-0.01; 0.02)	0.15	0.00 (-0.01; 0.02)	0.75
<b>MR-Egger+SIMEX</b>				
<i>Estimate</i>	-0.10 (-0.13; 0.03)	0.16	-0.04 (-0.21; 0.14)	0.68
<i>Intercept</i>	0.01 (0.00; 0.02)	0.15	0.00 (-0.01; 0.02)	0.75
<b>Weighted Median</b>	0.00 (-0.08; 0.07)	0.90	-0.02 (-0.10; 0.07)	0.67

Abbreviations: BMD, bone mineral density; SD, standard deviation; TSH, thyroid stimulating hormone; SIMEX, simulation extrapolation. Data presented as beta coefficients with 95% confidence interval per standard deviation decrease of serum level thyrotropin (TSH).

### Common genetic variants in the TSH receptor locus and expression loci

A total of 755 common SNPs were mapped either in the *TSHR* locus or in eQTLs. In the GEFOS dataset, 706 of the mapped SNPs were available, amounting to 44 independent loci (**Supplementary Table 1**).  $-\log(p \text{ value})$  plots are shown for the *TSHR* SNPs and BMD of the femoral neck (**Figure 2A**) and the lumbar spine (**Figure 2B**). At the nominal significance of  $p < 0.05$ , five SNPs were associated with femoral neck BMD and three with lumbar spine BMD. However, none of these associations remained statistically significant following Bonferroni correction for multiple testing.



**Figure 2.**  $-\log(P)$  plot of the candidate gene analysis. Results are presented as the  $-\log$  of the  $P$ -value for each SNP organized by position number. The lower horizontal line at 1.30 corresponds to the  $-\log$  of  $P$ -value=0.05 indicating nominal significance, the upper horizontal line at 2.94 corresponds to the  $-\log$  of  $P$ -value= $1.14 \times 10^{-3}$  indicating the Bonferroni-corrected threshold of statistical significance. The association between *TSHR* SNPs and (A) Femoral neck BMD, (B) Lumbar spine BMD

## DISCUSSION

We used Mendelian randomization to determine whether lower circulating levels of TSH within the normal range are causally associated with reduced BMD. Despite interrogating the largest publicly available GWAS meta-analyses,<sup>20</sup> we were unable to demonstrate an association between genetic instruments for circulating levels of TSH and femoral neck or lumbar spine BMD. Furthermore, no significant association was found between common genetic variants within the *TSHR* gene or expression regulating regions thereof and BMD. Thus, we found no evidence for a causal relationship between lower circulating levels of

TSH within the normal range and reduced BMD, or for any association between genetic variance in the *TSHR* gene or *TSHR* expression and BMD.

These findings add to previous research regarding the role of TSH in the skeleton, which has yielded contrasting results. In osteoblasts of rodent and of human origin TSH receptors were identified<sup>30</sup>, although no expression of the TSH $\alpha$  or  $\beta$  subunits was observed<sup>31</sup>. The reported effects of TSH on osteoblasts *in vitro* are contradictory as inhibition<sup>32</sup>, stimulation<sup>33-35</sup> and no effect<sup>31,36</sup> of TSH on differentiation and function have all been observed. Furthermore, in human osteoblasts, TSH receptor expression and cAMP responses to TSH are low, making physiologically relevant actions of TSH unlikely<sup>37</sup>. In osteoclasts the findings have been more consistent, with the majority reporting TSH inhibiting osteoclast formation and function whilst others have shown no effect<sup>32,35,36</sup>.

*In vivo*, thyroid hormone treated *TSHR*-knockout mice displayed decreased BMD and bone strength<sup>32,38</sup>, but importantly, this phenotype also reflects the consequences of profound congenital hypothyroidism and delayed thyroid hormone replacement<sup>31</sup>. Consistent with this, adult rodents, treated with TSH doses insufficient to alter systemic T3 or T4 level, showed suppressed bone resorption and increased formation<sup>35,39,40</sup>. By contrast, a similar skeletal phenotype of delayed bone development<sup>31</sup> was reported in two contrasting mouse models for congenital hypothyroidism (i) *Pax8*-knockout mice with no T4 or T3 but grossly elevated TSH in the presence of a fully functional TSH receptor and (ii) *TSHR*-knockout mice with no T4 or T3 but grossly elevated TSH in the absence of a functional TSH receptor. Although these results do not support a predominant role for the TSH receptor in bone, the effects of TSH could be masked by the severely reduced T4 and T3 levels.

Human observational studies have shown strong indications for an association between higher thyroid status within and outside the normal range and lower BMD<sup>6,8,9</sup>. Importantly, in observational studies in humans, no conclusions can be drawn on relative roles of TSH or thyroid hormones because they are maintained by the HPT axis in a physiological reciprocal relationship<sup>41</sup>. In genetic studies investigating the relationship between TSH and BMD, the non-synonymous Asp727Glu polymorphism in the human *TSHR* gene (rs1991517) has been associated with higher mean BMD in two studies<sup>42,43</sup>. However, this observation has not been replicated by other studies and no other common *TSHR* genetic variants have been associated with BMD.

In this study we investigated the effect of circulating TSH levels, within the normal range, on BMD in the absence of confounding, by using genetic variants associated with circulating TSH level as instrumental variables in a two-sample

Mendelian randomization analysis using summary level data. This highly efficient method allows for large sample sizes to be used, but has the disadvantage that stratified analyses, for example by sex, age or menopausal status, are not possible. Analyses in specific subgroups such as postmenopausal women would also have been of interest, due to their increased risk of developing osteoporosis<sup>44</sup>. Furthermore, in previous observational studies stronger associations between thyroid status and BMD were observed in women compared to men<sup>8, 45</sup>. Therefore, we cannot conclude that no association between TSH levels and BMD is present in more vulnerable subgroups. Nonetheless, in the general population as a whole, we found no causal association between TSH and BMD. Another potential limitation of our study is overlap between GWAS meta-analyses of thyroid function parameters and GWAS meta-analyses of BMD; three out of nine cohorts (Framingham Heart Study, TwinsUK study and Rotterdam Study) were included in both studies. If weak instruments were used, this overlap in study populations could lead to bias<sup>46</sup>. Since all genetic instruments were selected from among the top hits of the largest published GWAS on thyroid function to date, the instrument strength was assumed to be sufficient based on previous studies<sup>47</sup>. Therefore, potential effects of weak instrument bias can be expected to be negligible<sup>46</sup>. Furthermore, the genetic instrument identification and the Mendelian randomization were performed in cohorts of European ancestry which may limit generalizability to non-European populations. A potential limitation of our combined genetic variants for circulating TSH level could be that they also reflect the circulating levels of fT4, due to the reciprocal physiological relationship between TSH and fT4 in healthy individuals<sup>41</sup>. However, the GWAS that identified the variants for TSH had identified different genetic variants for fT4<sup>18</sup>. Reciprocal associations of TSH SNPs with fT4 were assessed in sensitivity analyses, yet, as stated by the authors, the study was underpowered to detect any statistically significant associations<sup>18</sup>. Even though no certain conclusions can be drawn, the results of the sensitivity analysis did not imply strong reciprocal associations with circulating fT4 levels for the SNPs associated with circulating TSH. Therefore, the results for the MR study on lower circulating TSH might be influenced by slightly higher levels of fT4, yet these effects appear to be small. A final limitation of using genetic variants for TSH identified by this GWAS meta-analyses is the euthyroid state of the included participants. Because circulating TSH levels in clinical thyroid dysfunction are unlike the individual set point<sup>48</sup>, we cannot extrapolate our results to individuals with TSH outside the reference range. Thus, our results are only applicable to adults with circulating TSH levels within the reference range.

In addition, we investigated the association of common genetic variants in the *TSHR* gene locus and the expression loci with BMD in a candidate gene study. For this analysis we used 706 common genetic variants (44 independent loci), which covered the majority of the common variation in *TSHR* and the eQTLs of

this gene. A limitation of this method is the unknown effect of the tested genetic variants inside the *TSHR* gene on the TSH receptor and on thyroid status, yet we observed no indication for biologically relevant associations between this gene and BMD. Furthermore, an important limitation is the absence of 49 SNPs mapped to the *TSHR* gene in the summary-level data we used. Nevertheless, we found no association between common variation of the *TSHR* locus and BMD despite using the largest human dataset available for BMD of the femoral neck and lumbar spine.

### **Conclusion**

In summary, we found no evidence that circulating TSH levels in the normal range are causally associated with BMD nor did we find any association between common genetic variation in the *TSHR* gene or expression of *TSHR* and BMD. Therefore, the associations found in observational studies between low circulating TSH and lower BMD are possibly related to the reciprocal higher levels of fT4, due to residual confounding or reverse causality. In clinical treatment of thyroid disease, treatment is aimed at normalization of TSH levels into the normal range and alleviation of symptoms. Based on our current results, we found no indications for inappropriateness of current guidelines aimed at restoration of TSH within the normal reference range with regard to bone health. In future research, better genetic tools for fT4 levels are required to further interpret the effects of thyroid status on BMD. Additionally, more clinical end points could be investigated resulting in greater clinical applicability.

### **DISCLOSURES**

Dr. van Heemst reports grants from the European Commission, during the conduct of the study. All other co-authors have nothing to disclose.

### **ACKNOWLEDGMENTS**

This study was supported by the European Commission-funded project THYRAGE (Horizon 2020 research and innovation programme under grant agreement No 666869). We thank the GEFOS consortium for making the summary statistics data files of their meta-analyses available and the authors of the GWAS meta-analysis of Porcu and colleagues<sup>18</sup> for their work on genetic variants associated with thyroid-related traits.

Authors'roles: Study design: NAvV, RN, DvH. Data analysis: NAvV, RN and JBvK. Data interpretation: NAvV, RN, JBvK, RGJW, JHDB, GRW and DvH. Supervision: DvH. Drafting manuscript: NAvV, RN, RGJW, JHDB, GRW and DvH. All authors have critically revised the manuscript content and approved of the final version

of the submitted manuscript. All authors take responsibility for the integrity of the data analysis.

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## SUPPLEMENTARY MATERIAL

Supplementary Table 1. Associations between SNPs in the TSHR locus and eQTLs and BMD of the femoral neck and lumbar spine

Gene	SNP	Chrom	Position	Minor allele	MAF	Femoral neck BMD				Lumbar spine BMD			
						Beta	SE	P-value	-log (P-value)	Beta	SE	P-value	-log (P-value)
TSHR	rs2122253	14	81127338	G	0.149	-0.0133	0.0108	0.2290	0.6401	-0.0057	0.0126	0.6620	0.1792
TSHR	rs1197384	14	81127514	T	0.149	-0.0121	0.0108	0.2722	0.5651	-0.0035	0.0126	0.7850	0.1051
TSHR	rs6574598	14	81131831	C	0.133	-0.0094	0.0109	0.3995	0.3985	0.0000	0.0127	0.9973	0.0012
TSHR	rs59093673	14	81140458	A	0.141	-0.0104	0.0109	0.3495	0.4565	-0.0014	0.0127	0.9114	0.0403
TSHR	rs8019534	14	81169470	C	0.382	-0.0147	0.0093	0.1227	0.9112	0.0071	0.0112	0.5344	0.2721
TSHR	rs1197381	14	81170713	A	0.409	0.0080	0.0076	0.3001	0.5228	-0.0064	0.0088	0.4809	0.3180
TSHR	rs2371336	14	81171177	C	0.382	-0.0087	0.0077	0.2722	0.5651	0.0121	0.0090	0.1885	0.7247
TSHR	rs35178479	14	81171888	C	0.409	0.0079	0.0076	0.3080	0.5115	-0.0073	0.0088	0.4199	0.3769
TSHR	rs2590484	14	81172484	T	0.458	0.0102	0.0075	0.1877	0.7266	-0.0060	0.0088	0.5020	0.2993
TSHR	rs2619673	14	81174902	C	0.383	-0.0093	0.0077	0.2377	0.6239	0.0117	0.0090	0.2041	0.6902
TSHR	rs2596108	14	81175607	T	0.382	-0.0093	0.0077	0.2383	0.6229	0.0118	0.0090	0.2013	0.6962
TSHR	rs12878836	14	81175896	A	0.383	-0.0092	0.0077	0.2455	0.6100	0.0122	0.0090	0.1852	0.7324
TSHR	rs36055485	14	81186946	C	0.456	0.0091	0.0075	0.2387	0.6221	-0.0073	0.0088	0.4180	0.3789
TSHR	rs2060596	14	81187126	A	0.406	-0.0100	0.0077	0.2030	0.6926	0.0126	0.0090	0.1716	0.7656
TSHR	rs8007834	14	81276643	A	0.176	-0.0094	0.0096	0.3389	0.4699	0.0045	0.0112	0.6953	0.1579
TSHR	rs327450	14	81292286	C	0.154	-0.0141	0.0103	0.1812	0.7418	-0.0116	0.0120	0.3455	0.4615
TSHR	rs17111153	14	81298251	T	0.163	-0.0085	0.0096	0.3841	0.4155	0.0058	0.0112	0.6124	0.2130
TSHR	rs58625998	14	81298274	A	0.242	-0.0078	0.0085	0.3729	0.4284	0.0082	0.0100	0.4242	0.3724
TSHR	rs11625199	14	81372745	A	0.490	-0.0133	0.0075	0.0834	1.0787	0.0000	0.0088	0.9957	0.0019
TSHR	rs759916	14	81373641	C	0.401	0.0121	0.0077	0.1236	0.9081	-0.0016	0.0090	0.8644	0.0633
TSHR	rs759917	14	81373831	G	0.401	0.0126	0.0077	0.1079	0.9670	-0.0003	0.0090	0.9778	0.0097
TSHR	rs4016442	14	81373937	A	0.490	-0.0127	0.0075	0.0990	1.0043	0.0006	0.0088	0.9451	0.0245
TSHR	rs6574608	14	81374822	C	0.489	0.0122	0.0075	0.1120	0.9508	-0.0013	0.0088	0.8872	0.0520

Supplementary Table 1. Continued.

Gene	SNP	Chrom	Position	Minor allele	MAF	Femoral neck BMD				Lumbar spine BMD			
						Beta	SE	P-value	-log (P-value)	Beta	SE	P-value	-log (P-value)
TSHR	rs2122253	14	81127338	G	0.149	-0.0133	0.0108	0.2290	0.6401	-0.0057	0.0126	0.6620	0.1792
TSHR	rs7157845	14	81375305	G	0.489	-0.0128	0.0075	0.0959	1.0182	0.0002	0.0088	0.9819	0.0079
TSHR	rs10142753	14	81376490	T	0.489	0.0128	0.0075	0.0950	1.0225	-0.0012	0.0088	0.8928	0.0492
TSHR	rs722906	14	81376855	A	0.490	-0.0126	0.0075	0.1023	0.9900	0.0008	0.0088	0.9265	0.0332
TSHR	rs2010847	14	81376921	A	0.490	-0.0131	0.0075	0.0878	1.0566	0.0006	0.0088	0.9482	0.0231
TSHR	rs12885819	14	81377524	G	0.097	0.0055	0.0132	0.6856	0.1639	-0.0058	0.0153	0.7132	0.1468
TSHR	rs6574609	14	81377553	G	0.427	-0.0122	0.0076	0.1165	0.9337	0.0013	0.0089	0.8822	0.0544
TSHR	rs7153979	14	81378061	A	0.401	0.0128	0.0077	0.1043	0.9817	-0.0007	0.0090	0.9396	0.0271
TSHR	rs7154132	14	81378082	C	0.401	0.0125	0.0077	0.1104	0.9569	-0.0010	0.0090	0.9156	0.0383
TSHR	rs7154373	14	81378239	C	0.401	0.0126	0.0077	0.1083	0.9652	-0.0001	0.0090	0.9941	0.0026
TSHR	rs35765671	14	81378554	T	0.098	0.0049	0.0132	0.7156	0.1453	-0.0072	0.0153	0.6474	0.1888
TSHR	rs17615020	14	81378754	T	0.489	-0.0132	0.0075	0.0862	1.0646	0.0009	0.0088	0.9162	0.0380
TSHR	rs7140452	14	81379868	C	0.427	-0.0128	0.0076	0.1000	0.9999	0.0007	0.0089	0.9404	0.0267
TSHR	rs7147306	14	81380066	T	0.436	-0.0119	0.0076	0.1244	0.9053	-0.0002	0.0089	0.9852	0.0065
TSHR	rs6574610	14	81380130	G	0.489	-0.0131	0.0075	0.0893	1.0492	0.0003	0.0088	0.9726	0.0121
TSHR	rs55634365	14	81381104	A	0.097	0.0043	0.0132	0.7517	0.1240	-0.0056	0.0153	0.7198	0.1428
TSHR	rs12896436	14	81382533	G	0.193	-0.0108	0.0097	0.2769	0.5576	0.0042	0.0113	0.7199	0.1428
TSHR	rs12434318	14	81382572	A	0.435	-0.0139	0.0079	0.0853	1.0689	0.0010	0.0089	0.9100	0.0410
TSHR	rs4899780	14	81383229	A	0.426	-0.0125	0.0076	0.1076	0.9682	0.0018	0.0089	0.8438	0.0738
TSHR	rs7144634	14	81383427	T	0.054	-0.0054	0.0153	0.7309	0.1361	-0.0012	0.0178	0.9494	0.0225
TSHR	rs4903947	14	81383901	A	0.490	0.0135	0.0075	0.0790	1.1025	-0.0005	0.0088	0.9523	0.0212
TSHR	rs34611247	14	81384289	G	0.097	0.0036	0.0132	0.7888	0.1030	-0.0063	0.0154	0.6885	0.1621
TSHR	rs12050416	14	81385568	T	0.426	-0.0134	0.0076	0.0864	1.0634	0.0014	0.0089	0.8760	0.0575
TSHR	rs1013890	14	81386625	G	0.054	-0.0014	0.0157	0.9315	0.0308	0.0024	0.0176	0.8949	0.0482

Supplementary Table 1. Continued.

Gene	SNP	Chrom	Position	Minor allele	MAF	Femoral neck BMD				Lumbar spine BMD			
						Beta	SE	P-value	-log (P-value)	Beta	SE	P-value	-log (P-value)
TSHR	rs2122253	14	81127338	G	0.149	-0.0133	0.0108	0.2290	0.6401	-0.0057	0.0126	0.6620	0.1792
TSHR	rs12586908	14	81387338	A	0.109	0.0009	0.0121	0.9421	0.0259	-0.0021	0.0140	0.8809	0.0551
TSHR	rs77951617	14	81387913	C	0.054	-0.0039	0.0153	0.8043	0.0946	-0.0004	0.0178	0.9814	0.0082
TSHR	rs35728625	14	81388215	A	0.097	0.0044	0.0132	0.7456	0.1275	-0.0065	0.0154	0.6813	0.1667
TSHR	rs17615156	14	81388568	C	0.053	-0.0035	0.0153	0.8203	0.0860	0.0002	0.0178	0.9901	0.0043
TSHR	rs1016699	14	81389124	G	0.426	-0.0124	0.0076	0.1121	0.9503	0.0018	0.0089	0.8422	0.0746
TSHR	rs1025252	14	81389135	A	0.062	-0.0008	0.0142	0.9568	0.0192	-0.0012	0.0165	0.9443	0.0249
TSHR	rs1025253	14	81389266	G	0.404	0.0122	0.0077	0.1193	0.9235	-0.0006	0.0090	0.9501	0.0223
TSHR	rs759918	14	81390128	C	0.487	0.0113	0.0075	0.1416	0.8489	-0.0025	0.0088	0.7783	0.1089
TSHR	rs2059719	14	81390791	C	0.198	-0.0101	0.0096	0.3063	0.5139	0.0054	0.0112	0.6392	0.1944
TSHR	rs759919	14	81391078	T	0.195	-0.0121	0.0077	0.1217	0.9146	0.0021	0.0089	0.8146	0.0890
TSHR	rs8022411	14	81391185	G	0.404	0.0122	0.0077	0.1207	0.9184	-0.0005	0.0089	0.9563	0.0194
TSHR	rs10140915	14	81391413	C	0.053	-0.0041	0.0151	0.7920	0.1013	0.0003	0.0176	0.9870	0.0057
TSHR	rs4903948	14	81392124	T	0.403	0.0121	0.0077	0.1223	0.9125	-0.0006	0.0090	0.9475	0.0234
TSHR	rs116875278	14	81392483	A	0.053	-0.0040	0.0153	0.7985	0.0977	-0.0007	0.0178	0.9697	0.0133
TSHR	rs10132220	14	81392502	A	0.053	-0.0039	0.0152	0.8020	0.0958	0.0002	0.0177	0.9892	0.0047
TSHR	rs4903949	14	81392513	A	0.488	0.0121	0.0075	0.1174	0.9304	-0.0013	0.0088	0.8852	0.0530
TSHR	rs117857224	14	81392561	C	0.053	-0.0042	0.0153	0.7881	0.1034	-0.0007	0.0178	0.9676	0.0143
TSHR	rs78060889	14	81392888	T	0.053	-0.0042	0.0153	0.7879	0.1035	-0.0007	0.0178	0.9676	0.0143
TSHR	rs2371456	14	81393496	A	0.426	-0.0113	0.0076	0.1464	0.8344	0.0026	0.0089	0.7718	0.1125
TSHR	rs2371457	14	81393703	A	0.403	0.0121	0.0077	0.1227	0.9111	-0.0005	0.0089	0.9599	0.0178
TSHR	rs12433134	14	81393926	T	0.425	-0.0120	0.0076	0.1250	0.9032	0.0023	0.0089	0.7979	0.0980
TSHR	rs60407794	14	81394116	C	0.098	0.0034	0.0132	0.8000	0.0969	-0.0086	0.0153	0.5831	0.2342
TSHR	rs10150391	14	81394123	G	0.053	-0.0029	0.0152	0.8517	0.0697	0.0007	0.0177	0.9670	0.0146

Supplementary Table 1. Continued.

Gene	SNP	Chrom	Position	Minor allele	MAF	Femoral neck BMD				Lumbar spine BMD			
						Beta	SE	P-value	-log (P-value)	Beta	SE	P-value	-log (P-value)
TSHR	rs2122253	14	81127338	G	0.149	-0.0133	0.0108	0.2290	0.6401	-0.0057	0.0126	0.6620	0.1792
TSHR	rs2162547	14	81394526	T	0.193	-0.0097	0.0097	0.3311	0.4800	0.0042	0.0113	0.7154	0.1454
TSHR	rs12590770	14	81394624	A	0.110	0.0008	0.0120	0.9479	0.0232	-0.0033	0.0139	0.8148	0.0890
TSHR	rs2217175	14	81394797	C	0.053	-0.0029	0.0151	0.8509	0.0701	0.0003	0.0176	0.9880	0.0053
TSHR	rs8005883	14	81395771	G	0.403	0.0120	0.0077	0.1274	0.8950	-0.0006	0.0089	0.9499	0.0223
TSHR	rs8017038	14	81397574	T	0.425	-0.0116	0.0076	0.1359	0.8668	0.0027	0.0089	0.7667	0.1154
TSHR	rs78218384	14	81397830	C	0.095	-0.0100	0.0128	0.4478	0.3489	-0.0091	0.0150	0.5543	0.2563
TSHR	rs28787628	14	81398044	T	0.062	-0.0011	0.0142	0.9383	0.0276	-0.0018	0.0165	0.9152	0.0385
TSHR	rs8021757	14	81398357	G	0.062	-0.0026	0.0142	0.8568	0.0671	-0.0015	0.0165	0.9314	0.0309
TSHR	rs8003193	14	81398506	C	0.062	-0.0005	0.0142	0.9746	0.1112	-0.0008	0.0165	0.9620	0.0168
TSHR	rs58078360	14	81399304	G	0.151	0.0010	0.0104	0.9233	0.0347	-0.0053	0.0120	0.6657	0.1767
TSHR	rs56757452	14	81399586	C	0.160	0.0017	0.0101	0.8691	0.0609	-0.0060	0.0117	0.6169	0.2098
TSHR	rs2195101	14	81399692	A	0.151	0.0007	0.0103	0.9441	0.0250	-0.0051	0.0120	0.6768	0.1696
TSHR	rs2217176	14	81400050	C	0.402	0.0123	0.0077	0.1172	0.9310	-0.0004	0.0089	0.9674	0.0144
TSHR	rs72693043	14	81400093	C	0.204	-0.0117	0.0093	0.2204	0.6568	0.0071	0.0109	0.5226	0.2819
TSHR	rs12878041	14	81400425	G	0.159	0.0014	0.0102	0.8906	0.0503	-0.0072	0.0118	0.5514	0.2586
TSHR	rs12878167	14	81400427	T	0.159	0.0012	0.0102	0.9053	0.0432	-0.0072	0.0118	0.5480	0.2612
TSHR	rs10132871	14	81401059	C	0.150	0.0011	0.0104	0.9165	0.0379	-0.0037	0.0120	0.7666	0.1154
TSHR	rs728444	14	81402760	C	0.402	0.0127	0.0077	0.1058	0.9757	-0.0001	0.0090	0.9950	0.0022
TSHR	rs2371460	14	81403312	T	0.061	-0.0037	0.0091	0.6944	0.1584	0.0003	0.0106	0.9793	0.0091
TSHR	rs56343573	14	81403518	T	0.228	-0.0069	0.0089	0.4508	0.3460	-0.0020	0.0104	0.8529	0.0691
TSHR	rs7159065	14	81403729	C	0.229	-0.0066	0.0089	0.4667	0.3310	-0.0020	0.0104	0.8511	0.0700
TSHR	rs4899781	14	81403850	T	0.442	-0.0123	0.0076	0.1105	0.9564	0.0024	0.0088	0.7897	0.1026
TSHR	rs6574611	14	81404019	A	0.442	-0.0139	0.0076	0.0720	1.1425	0.0008	0.0088	0.9326	0.0303

Supplementary Table 1. Continued.

Gene	SNP	Chrom	Position	Minor allele	MAF	Femoral neck BMD				Lumbar spine BMD			
						Beta	SE	P-value	-log (P-value)	Beta	SE	P-value	-log (P-value)
TSHR	rs2122253	14	81127338	G	0.149	-0.0133	0.0108	0.2290	0.6401	-0.0057	0.0126	0.6620	0.1792
TSHR	rs6574612	14	81404074	T	0.228	-0.0082	0.0090	0.3712	0.4304	-0.0010	0.0104	0.9229	0.0349
TSHR	rs7143744	14	81404742	C	0.412	0.0107	0.0077	0.1701	0.7694	-0.0002	0.0089	0.9788	0.0093
TSHR	rs7149702	14	81404972	C	0.376	-0.0027	0.0080	0.7450	0.1278	-0.0038	0.0090	0.6769	0.1695
TSHR	rs4903954	14	81405614	C	0.412	0.0109	0.0076	0.1615	0.7917	-0.0001	0.0089	0.9904	0.0042
TSHR	rs35298517	14	81405743	G	0.147	0.0023	0.0104	0.8321	0.0798	-0.0057	0.0121	0.6466	0.1894
TSHR	rs3742722	14	81405846	C	0.412	0.0107	0.0076	0.1697	0.7702	-0.0003	0.0089	0.9744	0.0113
TSHR	rs17111237	14	81406341	G	0.203	-0.0125	0.0093	0.1899	0.7214	0.0069	0.0109	0.5338	0.2726
TSHR	rs12587883	14	81406514	G	0.091	0.0030	0.0105	0.7810	0.1074	-0.0055	0.0121	0.6561	0.1831
TSHR	rs17111246	14	81406683	A	0.212	-0.0101	0.0092	0.2803	0.5523	0.0070	0.0107	0.5253	0.2796
TSHR	rs12892825	14	81406730	C	0.412	0.0102	0.0076	0.1915	0.7179	-0.0010	0.0089	0.9142	0.0390
TSHR	rs2888046	14	81407048	G	0.412	0.0104	0.0077	0.1844	0.7342	-0.0015	0.0089	0.8712	0.0599
TSHR	rs2114703	14	81407169	T	0.412	0.0107	0.0076	0.1704	0.7685	-0.0002	0.0089	0.9818	0.0080
TSHR	rs2162548	14	81407456	C	0.412	0.0105	0.0076	0.1810	0.7423	-0.0005	0.0089	0.9530	0.0209
TSHR	rs11625251	14	81407891	G	0.087	-0.0146	0.0131	0.2748	0.5610	-0.0090	0.0153	0.5634	0.2492
TSHR	rs3742721	14	81407921	G	0.412	0.0106	0.0077	0.1756	0.7555	-0.0003	0.0089	0.9701	0.0132
TSHR	rs6574613	14	81408100	A	0.406	0.0105	0.0077	0.1818	0.7405	-0.0006	0.0089	0.9493	0.0226
TSHR	rs6574615	14	81408165	G	0.218	-0.0074	0.0094	0.4364	0.3601	0.0009	0.0105	0.9345	0.0294
TSHR	rs3742720	14	81408304	G	0.147	0.0032	0.0105	0.7638	0.1170	-0.0051	0.0121	0.6813	0.1667
TSHR	rs8009260	14	81408591	G	0.423	0.0101	0.0076	0.1959	0.7080	-0.0031	0.0089	0.7346	0.1339
TSHR	rs17111256	14	81408671	A	0.212	-0.0109	0.0092	0.2451	0.6107	0.0059	0.0107	0.5922	0.2275
TSHR	rs72693050	14	81409634	C	0.203	-0.0122	0.0093	0.1996	0.6999	0.0070	0.0109	0.5313	0.2746
TSHR	rs57104434	14	81409791	T	0.218	-0.0117	0.0091	0.2061	0.6859	0.0051	0.0106	0.6345	0.1976
TSHR	rs4899782	14	81410002	T	0.416	0.0112	0.0076	0.1531	0.8151	-0.0018	0.0089	0.8443	0.0735

Supplementary Table 1. Continued.

Gene	SNP	Chrom	Position	Minor allele	MAF	Femoral neck BMD				Lumbar spine BMD			
						Beta	SE	P-value	-log (P-value)	Beta	SE	P-value	-log (P-value)
TSHR	rs2122253	14	81127338	G	0.149	-0.0133	0.0108	0.2290	0.6401	-0.0057	0.0126	0.6620	0.1792
TSHR	rs4899783	14	81410262	T	0.416	0.0114	0.0076	0.1432	0.8440	-0.0013	0.0089	0.8887	0.0513
TSHR	rs67231034	14	81410653	T	0.366	-0.0027	0.0078	0.7300	0.1367	-0.0019	0.0090	0.8330	0.0794
TSHR	rs67065081	14	81410660	C	0.416	0.0107	0.0077	0.1704	0.7685	-0.0018	0.0089	0.8470	0.0721
TSHR	rs11627019	14	81410932	T	0.417	0.0088	0.0082	0.2900	0.5377	-0.0036	0.0089	0.6946	0.1583
TSHR	rs11627929	14	81411026	T	0.417	0.0094	0.0082	0.2611	0.5832	-0.0014	0.0089	0.8765	0.0572
TSHR	rs10136404	14	81411299	G	0.417	0.0113	0.0076	0.1477	0.8305	-0.0022	0.0089	0.8130	0.0899
TSHR	rs11623416	14	81411347	G	0.417	0.0110	0.0076	0.1590	0.7986	-0.0023	0.0089	0.8048	0.0943
TSHR	rs2371461	14	81411757	G	0.366	-0.0035	0.0077	0.6550	0.1838	-0.0027	0.0090	0.7677	0.1148
TSHR	rs12895801	14	81412117	C	0.416	0.0109	0.0076	0.1629	0.7881	-0.0022	0.0089	0.8084	0.0924
TSHR	rs35847336	14	81412494	C	0.147	0.0046	0.0105	0.6681	0.1752	-0.0028	0.0122	0.8254	0.0834
TSHR	rs17111270	14	81413039	G	0.417	0.0109	0.0077	0.1640	0.7852	-0.0021	0.0089	0.8142	0.0893
TSHR	rs7145224	14	81413293	C	0.437	-0.0127	0.0076	0.1000	1.0002	0.0048	0.0088	0.5947	0.2257
TSHR	rs7144208	14	81413489	G	0.353	-0.0065	0.0078	0.4185	0.3783	0.0023	0.0091	0.8045	0.0945
TSHR	rs72693053	14	81413849	G	0.205	-0.0123	0.0093	0.1946	0.7109	0.0033	0.0108	0.7655	0.1161
TSHR	rs8012343	14	81413969	A	0.431	-0.0135	0.0076	0.0816	1.0881	0.0037	0.0088	0.6840	0.1650
TSHR	rs7544072	14	81414285	C	0.435	-0.0144	0.0076	0.0633	1.1989	0.0024	0.0088	0.7945	0.0999
TSHR	rs7615911	14	81414351	T	0.435	-0.0145	0.0076	0.0610	1.2146	0.0019	0.0088	0.8324	0.0797
TSHR	rs7615941	14	81414408	G	0.435	-0.0145	0.0076	0.0616	1.2101	0.0020	0.0088	0.8285	0.0817
TSHR	rs10146516	14	81414747	A	0.435	-0.0146	0.0076	0.0583	1.2345	0.0022	0.0088	0.8053	0.0941
TSHR	rs10146348	14	81414819	G	0.435	-0.0143	0.0076	0.0645	1.1904	0.0023	0.0088	0.7953	0.0995
TSHR	rs10135774	14	81414913	C	0.435	-0.0145	0.0076	0.0614	1.2116	0.0020	0.0088	0.8209	0.0857
TSHR	rs17626988	14	81415002	A	0.433	-0.0146	0.0076	0.0592	1.2279	0.0023	0.0088	0.8006	0.0966
TSHR	rs2217177	14	81415154	C	0.435	-0.0144	0.0076	0.0632	1.1991	0.0024	0.0088	0.7884	0.1033

Supplementary Table 1. Continued.

Gene	SNP	Chrom	Position	Minor allele	MAF	Femoral neck BMD				Lumbar spine BMD			
						Beta	SE	P-value	-log (P-value)	Beta	SE	P-value	-log (P-value)
TSHR	rs2122253	14	81127338	G	0.149	-0.0133	0.0108	0.2290	0.6401	-0.0057	0.0126	0.6620	0.1792
TSHR	rs2195103	14	81415462	C	0.435	-0.0143	0.0076	0.0636	1.1965	0.0022	0.0088	0.8037	0.0949
TSHR	rs2217178	14	81415557	T	0.435	-0.0143	0.0076	0.0635	1.1969	0.0022	0.0088	0.8087	0.0922
TSHR	rs10149689	14	81415800	G	0.435	-0.0138	0.0076	0.0748	1.1263	0.0028	0.0088	0.7564	0.1213
TSHR	rs2114705	14	81415926	A	0.434	-0.0142	0.0076	0.0658	1.1817	0.0024	0.0088	0.7933	0.1005
TSHR	rs12050278	14	81416038	C	0.435	-0.0139	0.0076	0.0713	1.1471	0.0022	0.0088	0.8037	0.0949
TSHR	rs12050077	14	81416064	A	0.435	-0.0142	0.0076	0.0663	1.1788	0.0025	0.0088	0.7796	0.1081
TSHR	rs12050279	14	81416153	C	0.071	-0.0024	0.0150	0.8779	0.0566	0.0175	0.0167	0.3067	0.5133
TSHR	rs12586161	14	81416170	G	0.144	0.0044	0.0105	0.6808	0.1670	-0.0005	0.0121	0.9693	0.0136
TSHR	rs12050078	14	81416354	A	0.261	-0.0096	0.0087	0.2757	0.5596	0.0068	0.0101	0.5139	0.2891
TSHR	rs8009120	14	81417218	A	0.422	0.0113	0.0076	0.1481	0.8293	-0.0026	0.0089	0.7789	0.1085
TSHR	rs12050350	14	81417771	C	0.261	-0.0090	0.0087	0.3082	0.5112	0.0060	0.0101	0.5604	0.2515
TSHR	rs12890859	14	81418088	C	0.144	0.0053	0.0105	0.6181	0.2089	0.0000	0.0121	0.9969	0.0014
TSHR	rs55751898	14	81419263	G	0.269	-0.0090	0.0085	0.2975	0.5265	0.0092	0.0099	0.3629	0.4402
TSHR	rs4903955	14	81419789	T	0.435	-0.0144	0.0076	0.0632	1.1992	0.0020	0.0088	0.8269	0.0825
TSHR	rs7154269	14	81420307	G	0.436	-0.0140	0.0076	0.0702	1.1535	0.0027	0.0088	0.7655	0.1160
TSHR	rs12590277	14	81420487	A	0.144	0.0054	0.0105	0.6154	0.2109	0.0000	0.0122	0.9975	0.0011
TSHR	rs12590236	14	81420604	C	0.144	0.0051	0.0105	0.6312	0.1998	0.0002	0.0121	0.9840	0.0070
TSHR	rs2371462	14	81420774	T	0.420	0.0119	0.0076	0.1271	0.8957	-0.0031	0.0089	0.7337	0.1345
TSHR	rs8022139	14	81421345	T	0.166	-0.0137	0.0099	0.1762	0.7540	-0.0113	0.0116	0.3389	0.4700
TSHR	rs8022600	14	81421423	T	0.436	-0.0150	0.0076	0.0529	1.2766	0.0020	0.0088	0.8233	0.0844
TSHR	rs8009058	14	81422868	C	0.169	-0.0130	0.0099	0.1983	0.7026	-0.0092	0.0115	0.4328	0.3637
TSHR	rs2268451	14	81424118	G	0.438	-0.0152	0.0076	0.0498	1.3031	0.0015	0.0088	0.8659	0.0625
TSHR	rs2268452	14	81424136	A	0.268	-0.0098	0.0085	0.2588	0.5871	0.0079	0.0099	0.4347	0.3618

Supplementary Table 1. Continued.

Gene	SNP	Chrom	Position	Minor allele	MAF	Femoral neck BMD				Lumbar spine BMD			
						Beta	SE	P-value	-log (P-value)	Beta	SE	P-value	-log (P-value)
TSHR	rs2122253	14	81127338	G	0.149	-0.0133	0.0108	0.2290	0.6401	-0.0057	0.0126	0.6620	0.1792
TSHR	rs45585237	14	81424318	A	0.153	-0.0148	0.0104	0.1621	0.7903	-0.0083	0.0121	0.5041	0.2975
TSHR	rs726019	14	81425412	T	0.097	-0.0130	0.0130	0.3286	0.4834	-0.0071	0.0147	0.6353	0.1971
TSHR	rs726020	14	81425425	G	0.368	-0.0130	0.0078	0.1031	0.9868	0.0027	0.0091	0.7733	0.1117
TSHR	rs726018	14	81425428	A	0.368	-0.0131	0.0078	0.1017	0.9927	0.0027	0.0091	0.7742	0.1112
TSHR	rs12892567	14	81427552	C	0.153	0.0083	0.0102	0.4215	0.3752	0.0079	0.0118	0.5151	0.2882
TSHR	rs61981273	14	81427579	T	0.298	0.0062	0.0086	0.4828	0.3162	-0.0122	0.0100	0.2352	0.6286
TSHR	rs28699303	14	81427675	T	0.097	-0.0176	0.0125	0.1693	0.7714	-0.0088	0.0147	0.5557	0.2551
TSHR	rs76339876	14	81428254	C	0.067	0.0052	0.0149	0.7330	0.1349	0.0187	0.0172	0.2889	0.5392
TSHR	rs179243	14	81428564	T	0.368	-0.0127	0.0078	0.1120	0.9508	0.0029	0.0091	0.7599	0.1193
TSHR	rs179244	14	81429103	A	0.107	-0.0135	0.0120	0.2708	0.5674	-0.0067	0.0140	0.6413	0.1930
TSHR	rs179245	14	81429463	G	0.368	-0.0126	0.0078	0.1156	0.9371	0.0029	0.0091	0.7565	0.1212
TSHR	rs2284715	14	81429871	T	0.153	0.0071	0.0105	0.5076	0.2945	0.0079	0.0118	0.5104	0.2921
TSHR	rs2284716	14	81430845	G	0.153	0.0072	0.0105	0.5053	0.2965	0.0086	0.0117	0.4765	0.3220
TSHR	rs179247	14	81432546	G	0.498	0.0086	0.0075	0.2628	0.5803	-0.0111	0.0087	0.2127	0.6723
TSHR	rs179248	14	81433038	C	0.497	0.0093	0.0078	0.2430	0.6143	-0.0114	0.0087	0.2033	0.6918
TSHR	rs150611635	14	81433675	A	0.090	-0.0005	0.0137	0.9739	0.0115	-0.0125	0.0150	0.4168	0.3801
TSHR	rs2284718	14	81434889	G	0.261	-0.0091	0.0086	0.3028	0.5189	0.0078	0.0101	0.4475	0.3492
TSHR	rs179249	14	81435199	C	0.366	-0.0124	0.0078	0.1205	0.9192	0.0053	0.0091	0.5693	0.2446
TSHR	rs179250	14	81435483	A	0.098	-0.0164	0.0123	0.1947	0.7106	-0.0098	0.0144	0.5042	0.2974
TSHR	rs2284719	14	81435598	A	0.261	-0.0092	0.0086	0.2975	0.5266	0.0076	0.0101	0.4597	0.3375
TSHR	rs77549221	14	81435625	G	0.090	-0.0040	0.0129	0.7598	0.1193	-0.0112	0.0149	0.4646	0.3330
TSHR	rs179251	14	81435760	C	0.366	-0.0127	0.0078	0.1109	0.9550	0.0057	0.0091	0.5383	0.2690
TSHR	rs179252	14	81435985	G	0.491	0.0070	0.0075	0.3622	0.4410	-0.0121	0.0087	0.1747	0.7577

Supplementary Table 1. Continued.

Gene	SNP	Chrom	Position	Minor allele	MAF	Femoral neck BMD					Lumbar spine BMD				
						Beta	SE	P-value	-log (P-value)	Beta	SE	P-value	-log (P-value)		
TSHR	rs2122253	14	81127338	G	0.149	-0.0133	0.0108	0.2290	0.6401	-0.0057	0.0126	0.6620	0.1792		
TSHR	rs7154821	14	81436171	T	0.125	0.0101	0.0107	0.3561	0.4484	0.0156	0.0124	0.2197	0.6582		
TSHR	rs72693057	14	81436493	A	0.261	-0.0090	0.0086	0.3086	0.5106	0.0083	0.0101	0.4224	0.3742		
TSHR	rs179253	14	81437253	G	0.098	-0.0121	0.0128	0.3525	0.4529	-0.0101	0.0144	0.4899	0.3099		
TSHR	rs179254	14	81437340	C	0.366	-0.0123	0.0078	0.1212	0.9164	0.0050	0.0091	0.5920	0.2277		
TSHR	rs179255	14	81437602	A	0.491	0.0065	0.0075	0.3947	0.4038	-0.0126	0.0087	0.1570	0.8042		
TSHR	rs179256	14	81438070	T	0.095	-0.0106	0.0131	0.4289	0.3676	-0.0025	0.0147	0.8674	0.0618		
TSHR	rs35789224	14	81438111	G	0.126	0.0108	0.0107	0.3236	0.4900	0.0162	0.0124	0.2016	0.6955		
TSHR	rs2110695	14	81438498	T	0.125	0.0103	0.0107	0.3474	0.4591	0.0160	0.0124	0.2097	0.6784		
TSHR	rs77783364	14	81439116	T	0.093	-0.0024	0.0128	0.8534	0.0689	-0.0104	0.0148	0.4923	0.3078		
TSHR	rs179257	14	81439266	A	0.365	-0.0122	0.0078	0.1275	0.8944	0.0059	0.0091	0.5252	0.2797		
TSHR	rs11850464	14	81439462	T	0.271	-0.0073	0.0085	0.3987	0.3994	0.0091	0.0099	0.3740	0.4271		
TSHR	rs150259843	14	81439610	G	0.081	-0.0402	0.0173	0.0231	1.6370	-0.0388	0.0190	0.0456	1.3409		
TSHR	rs36193909	14	81439642	A	0.121	0.0117	0.0111	0.3046	0.5162	0.0134	0.0129	0.3121	0.5058		
TSHR	rs12589960	14	81439672	A	0.127	0.0091	0.0106	0.4023	0.3955	0.0142	0.0124	0.2632	0.5798		
TSHR	rs179258	14	81441293	C	0.105	-0.0124	0.0120	0.3133	0.5040	-0.0030	0.0140	0.8370	0.0773		
TSHR	rs179259	14	81441353	A	0.105	-0.0126	0.0120	0.3067	0.5132	-0.0030	0.0140	0.8356	0.0780		
TSHR	rs2268453	14	81441998	A	0.120	0.0131	0.0111	0.2496	0.6028	0.0134	0.0129	0.3128	0.5048		
TSHR	rs179260	14	81442055	T	0.095	-0.0166	0.0126	0.1974	0.7046	-0.0017	0.0147	0.9093	0.0413		
TSHR	rs59431750	14	81442069	G	0.261	-0.0090	0.0086	0.3097	0.5090	0.0093	0.0101	0.3681	0.4341		
TSHR	rs179261	14	81442234	C	0.105	-0.0098	0.0126	0.4488	0.3479	-0.0043	0.0141	0.7645	0.1166		
TSHR	rs179262	14	81442348	T	0.415	0.0060	0.0082	0.4727	0.3254	-0.0105	0.0089	0.2497	0.6025		
TSHR	rs2268456	14	81442410	T	0.126	0.0063	0.0132	0.6422	0.1923	-0.0001	0.0147	0.9948	0.0022		
TSHR	rs179263	14	81442515	G	0.415	0.0079	0.0077	0.3132	0.5042	-0.0103	0.0089	0.2605	0.5842		

Supplementary Table 1. Continued.

Gene	SNP	Chrom	Position	Minor allele	MAF	Femoral neck BMD				Lumbar spine BMD			
						Beta	SE	P-value	-log (P-value)	Beta	SE	P-value	-log (P-value)
TSHR	rs2122253	14	81127338	G	0.149	-0.0133	0.0108	0.2290	0.6401	-0.0057	0.0126	0.6620	0.1792
TSHR	rs2284720	14	81443167	G	0.261	-0.0092	0.0086	0.2969	0.5274	0.0090	0.0101	0.3844	0.4153
TSHR	rs2110696	14	81443368	C	0.354	-0.0077	0.0079	0.3430	0.4648	0.0045	0.0092	0.6303	0.2005
TSHR	rs79058377	14	81444032	A	0.092	-0.0013	0.0128	0.9226	0.0350	-0.0068	0.0148	0.6532	0.1849
TSHR	rs2284721	14	81444085	C	0.127	0.0081	0.0106	0.4560	0.3410	0.0133	0.0124	0.2945	0.5309
TSHR	rs76285753	14	81444130	G	0.092	-0.0011	0.0128	0.9322	0.0305	-0.0071	0.0148	0.6415	0.1928
TSHR	rs2284722	14	81444367	A	0.354	-0.0071	0.0079	0.3777	0.4228	0.0053	0.0092	0.5744	0.2408
TSHR	rs4903956	14	81444480	C	0.354	-0.0067	0.0079	0.4087	0.3886	0.0060	0.0092	0.5216	0.2826
TSHR	rs2160214	14	81444588	C	0.127	0.0076	0.0106	0.4815	0.3174	0.0153	0.0123	0.2275	0.6430
TSHR	rs2215981	14	81444967	A	0.410	0.0061	0.0077	0.4380	0.3585	-0.0107	0.0089	0.2444	0.6119
TSHR	rs4411444	14	81445108	G	0.485	-0.0043	0.0078	0.5940	0.2262	0.0119	0.0088	0.1835	0.7363
TSHR	rs1035144	14	81445121	C	0.483	0.0009	0.0075	0.9103	0.0408	-0.0127	0.0088	0.1563	0.8060
TSHR	rs1035145	14	81445245	A	0.120	0.0114	0.0111	0.3164	0.4998	0.0133	0.0129	0.3148	0.5019
TSHR	rs5002906	14	81445299	C	0.358	-0.0058	0.0079	0.4686	0.3292	0.0057	0.0092	0.5456	0.2632
TSHR	rs77647068	14	81445576	C	0.358	-0.0063	0.0079	0.4390	0.3576	0.0042	0.0092	0.6573	0.1822
TSHR	rs72693068	14	81445738	T	0.354	-0.0068	0.0079	0.4030	0.3947	0.0050	0.0092	0.5932	0.2268
TSHR	rs1003150	14	81445835	T	0.094	-0.0165	0.0126	0.2018	0.6952	-0.0029	0.0147	0.8456	0.0728
TSHR	rs72693069	14	81445862	G	0.358	-0.0063	0.0079	0.4320	0.3645	0.0048	0.0092	0.6108	0.2141
TSHR	rs72693070	14	81445931	C	0.354	-0.0072	0.0079	0.3751	0.4258	0.0051	0.0092	0.5904	0.2289
TSHR	rs8003515	14	81446242	C	0.485	-0.0017	0.0075	0.8260	0.0830	0.0109	0.0087	0.2229	0.6520
TSHR	rs8003402	14	81446306	T	0.120	0.0095	0.0111	0.4001	0.3979	0.0133	0.0129	0.3147	0.5022
TSHR	rs72693072	14	81446407	T	0.354	-0.0070	0.0079	0.3878	0.4114	0.0054	0.0092	0.5688	0.2451
TSHR	rs72693073	14	81446557	A	0.354	-0.0068	0.0079	0.3983	0.3998	0.0054	0.0092	0.5704	0.2438
TSHR	rs72693075	14	81446568	A	0.261	-0.0070	0.0087	0.4290	0.3676	0.0101	0.0101	0.3298	0.4818

Supplementary Table 1. Continued.

Gene	SNP	Chrom	Position	Minor allele	MAF	Femoral neck BMD				Lumbar spine BMD			
						Beta	SE	P-value	-log (P-value)	Beta	SE	P-value	-log (P-value)
TSHR	rs2122253	14	81127338	G	0.149	-0.0133	0.0108	0.2290	0.6401	-0.0057	0.0126	0.6620	0.1792
TSHR	rs77932265	14	81446788	T	0.093	-0.0017	0.0128	0.8939	0.0487	-0.0082	0.0148	0.5877	0.2309
TSHR	rs2300516	14	81446908	G	0.483	-0.0021	0.0075	0.7809	0.1074	0.0117	0.0088	0.1931	0.7142
TSHR	rs2300517	14	81447745	C	0.127	0.0083	0.0106	0.4439	0.3527	0.0133	0.0124	0.2948	0.5305
TSHR	rs3783951	14	81448096	A	0.354	-0.0064	0.0079	0.4309	0.3656	0.0065	0.0092	0.4899	0.3099
TSHR	rs75488731	14	81450246	A	0.093	0.0012	0.0129	0.9258	0.0335	-0.0041	0.0149	0.7883	0.1033
TSHR	rs77542443	14	81450256	A	0.093	0.0006	0.0129	0.9641	0.0159	-0.0042	0.0149	0.7836	0.1059
TSHR	rs2284723	14	81450743	A	0.078	0.0130	0.0126	0.3117	0.5063	0.0230	0.0147	0.1255	0.9015
TSHR	rs12101255	14	81451052	T	0.339	-0.0017	0.0078	0.8350	0.0783	0.0176	0.0092	0.0607	1.2171
TSHR	rs74064796	14	81451102	A	0.261	-0.0069	0.0086	0.4377	0.3588	0.0117	0.0101	0.2549	0.5936
TSHR	rs12101261	14	81451229	T	0.339	-0.0010	0.0078	0.8975	0.0470	0.0183	0.0092	0.0504	1.2974
TSHR	rs59711583	14	81451330	G	0.261	-0.0076	0.0087	0.3916	0.4072	0.0107	0.0101	0.3006	0.5220
TSHR	rs190607719	14	81451548	T	0.077	0.0207	0.0129	0.1159	0.9361	0.0243	0.0150	0.1142	0.9425
TSHR	rs8003061	14	81451956	A	0.339	-0.0009	0.0079	0.9062	0.0428	0.0187	0.0092	0.0458	1.3388
TSHR	rs17111346	14	81452172	A	0.339	-0.0009	0.0079	0.9106	0.0407	0.0186	0.0092	0.0478	1.3208
TSHR	rs72693078	14	81453113	A	0.262	-0.0067	0.0086	0.4476	0.3491	0.0115	0.0101	0.2644	0.5778
TSHR	rs72693080	14	81453719	A	0.261	-0.0074	0.0087	0.4027	0.3950	0.0101	0.0101	0.3297	0.4819
TSHR	rs72693081	14	81453862	G	0.078	0.0118	0.0126	0.3610	0.4425	0.0230	0.0147	0.1259	0.8998
TSHR	rs2024427	14	81454236	T	0.261	-0.0081	0.0087	0.3593	0.4445	0.0097	0.0101	0.3499	0.4560
TSHR	rs2024428	14	81454436	C	0.094	-0.0156	0.0126	0.2278	0.6424	-0.0022	0.0147	0.8861	0.0525
TSHR	rs2284725	14	81454539	C	0.078	0.0135	0.0126	0.2916	0.5352	0.0246	0.0146	0.0993	1.0029
TSHR	rs34277709	14	81454564	T	0.135	-0.0006	0.0113	0.9556	0.0197	-0.0134	0.0131	0.3166	0.4995
TSHR	rs2284726	14	81454578	G	0.415	0.0073	0.0077	0.3523	0.4531	-0.0100	0.0089	0.2750	0.5606
TSHR	rs2284727	14	81454684	C	0.268	-0.0088	0.0085	0.3106	0.5078	0.0099	0.0099	0.3274	0.4850

Supplementary Table 1. Continued.

Gene	SNP	Chrom	Position	Minor allele	MAF	Femoral neck BMD				Lumbar spine BMD			
						Beta	SE	P-value	-log (P-value)	Beta	SE	P-value	-log (P-value)
TSHR	rs2122253	14	81127338	G	0.149	-0.0133	0.0108	0.2290	0.6401	-0.0057	0.0126	0.6620	0.1792
TSHR	rs2284728	14	81454848	T	0.078	0.0137	0.0126	0.2870	0.5421	0.0253	0.0146	0.0905	1.0434
TSHR	rs36093037	14	81455306	T	0.136	0.0007	0.0113	0.9547	0.0201	-0.0122	0.0131	0.3605	0.4431
TSHR	rs7143071	14	81455409	T	0.094	-0.0172	0.0126	0.1823	0.7392	-0.0033	0.0147	0.8267	0.0827
TSHR	rs4903960	14	81455707	G	0.088	-0.0169	0.0132	0.2096	0.6786	-0.0032	0.0153	0.8408	0.0753
TSHR	rs58838870	14	81456605	C	0.078	0.0129	0.0127	0.3201	0.4947	0.0219	0.0147	0.1465	0.8342
TSHR	rs55945219	14	81456613	C	0.261	-0.0078	0.0087	0.3765	0.4242	0.0094	0.0101	0.3630	0.4401
TSHR	rs10145099	14	81456694	T	0.412	0.0078	0.0077	0.3222	0.4919	-0.0096	0.0089	0.2933	0.5328
TSHR	rs59103962	14	81456889	T	0.078	0.0124	0.0126	0.3376	0.4716	0.0227	0.0147	0.1298	0.8867
TSHR	rs59627467	14	81456897	C	0.078	0.0136	0.0126	0.2900	0.5375	0.0251	0.0146	0.0935	1.0290
TSHR	rs35523135	14	81457068	C	0.137	0.0010	0.0113	0.9283	0.0323	-0.0104	0.0131	0.4359	0.3606
TSHR	rs28414437	14	81457257	C	0.347	-0.0031	0.0078	0.6968	0.1569	0.0167	0.0091	0.0741	1.1301
TSHR	rs17544968	14	81457303	T	0.268	-0.0088	0.0085	0.3095	0.5093	0.0096	0.0099	0.3451	0.4621
TSHR	rs17111361	14	81457406	C	0.137	0.0007	0.0117	0.9503	0.0222	-0.0092	0.0130	0.4898	0.3100
TSHR	rs17545038	14	81457572	C	0.268	-0.0084	0.0085	0.3339	0.4764	0.0099	0.0099	0.3262	0.4865
TSHR	rs6574616	14	81457583	T	0.088	-0.0169	0.0132	0.2085	0.6809	-0.0007	0.0154	0.9637	0.0160
TSHR	rs6574617	14	81457615	A	0.412	0.0085	0.0080	0.2996	0.5235	-0.0108	0.0089	0.2388	0.6220
TSHR	rs2371463	14	81457788	A	0.476	-0.0007	0.0075	0.9292	0.0319	0.0112	0.0088	0.2117	0.6743
TSHR	rs724169	14	81457856	T	0.268	-0.0085	0.0085	0.3245	0.4888	0.0098	0.0099	0.3334	0.4770
TSHR	rs724170	14	81457940	A	0.476	-0.0011	0.0075	0.8887	0.0512	0.0112	0.0088	0.2132	0.6712
TSHR	rs2300518	14	81458516	T	0.078	0.0130	0.0126	0.3128	0.5047	0.0227	0.0147	0.1314	0.8815
TSHR	rs2300520	14	81459186	A	0.078	0.0139	0.0126	0.2778	0.5563	0.0249	0.0146	0.0958	1.0187
TSHR	rs72693090	14	81459753	G	0.268	-0.0084	0.0085	0.3319	0.4790	0.0099	0.0099	0.3287	0.4832
TSHR	rs72693091	14	81459840	G	0.078	0.0128	0.0126	0.3210	0.4935	0.0226	0.0147	0.1329	0.8766

Supplementary Table 1. Continued.

Gene	SNP	Chrom	Position	Minor allele	MAF	Femoral neck BMD				Lumbar spine BMD			
						Beta	SE	P-value	-log (P-value)	Beta	SE	P-value	-log (P-value)
TSHR	rs2122253	14	81127338	G	0.149	-0.0133	0.0108	0.2290	0.6401	-0.0057	0.0126	0.6620	0.1792
TSHR	rs11845052	14	81459924	T	0.268	-0.0086	0.0085	0.3185	0.4968	0.0106	0.0099	0.2939	0.5318
TSHR	rs8022899	14	81460128	A	0.078	0.0126	0.0126	0.3279	0.4843	0.0223	0.0147	0.1380	0.8603
TSHR	rs72693093	14	81460147	T	0.244	-0.0079	0.0087	0.3697	0.4322	0.0096	0.0101	0.3543	0.4506
TSHR	rs11159479	14	81460651	T	0.413	0.0074	0.0077	0.3448	0.4624	-0.0103	0.0090	0.2600	0.5850
TSHR	rs66487278	14	81461187	A	0.078	0.0126	0.0126	0.3274	0.4849	0.0234	0.0147	0.1198	0.9214
TSHR	rs7145701	14	81461348	A	0.094	-0.0160	0.0127	0.2177	0.6621	-0.0021	0.0148	0.8896	0.0508
TSHR	rs2160215	14	81461472	C	0.346	-0.0024	0.0078	0.7606	0.1188	0.0173	0.0091	0.0628	1.2021
TSHR	rs56389234	14	81461616	A	0.412	0.0072	0.0077	0.3590	0.4449	-0.0112	0.0090	0.2205	0.6565
TSHR	rs113713483	14	81461632	G	0.268	-0.0090	0.0085	0.3022	0.5197	0.0099	0.0099	0.3311	0.4801
TSHR	rs113036954	14	81461716	G	0.268	-0.0088	0.0085	0.3136	0.5037	0.0096	0.0099	0.3429	0.4648
TSHR	rs11850285	14	81461979	T	0.078	0.0132	0.0126	0.3061	0.5141	0.0246	0.0146	0.1008	0.9965
TSHR	rs1023586	14	81462283	C	0.346	-0.0024	0.0078	0.7671	0.1152	0.0174	0.0091	0.0619	1.2084
TSHR	rs12184983	14	81462556	C	0.135	-0.0003	0.0113	0.9816	0.0081	-0.0110	0.0131	0.4115	0.3857
TSHR	rs4903961	14	81462649	G	0.346	-0.0026	0.0078	0.7476	0.1263	0.0173	0.0091	0.0632	1.1992
TSHR	rs2268457	14	81462772	T	0.261	-0.0080	0.0087	0.3655	0.4371	0.0092	0.0101	0.3760	0.4248
TSHR	rs2268458	14	81462895	C	0.265	-0.0102	0.0085	0.2422	0.6158	0.0080	0.0100	0.4321	0.3644
TSHR	rs2268459	14	81463041	G	0.418	0.0088	0.0077	0.2626	0.5808	-0.0088	0.0090	0.3364	0.4731
TSHR	rs1990597	14	81463132	C	0.252	-0.0118	0.0089	0.1923	0.7160	0.0078	0.0104	0.4631	0.3343
TSHR	rs2268460	14	81463536	C	0.249	-0.0113	0.0089	0.2150	0.6675	0.0071	0.0104	0.5046	0.2970
TSHR	rs12437005	14	81463745	G	0.245	-0.0130	0.0089	0.1542	0.8119	0.0052	0.0104	0.6253	0.2039
TSHR	rs67391693	14	81463834	G	0.077	0.0112	0.0126	0.3841	0.4155	0.0225	0.0146	0.1337	0.8739
TSHR	rs12323356	14	81464279	G	0.449	-0.0043	0.0076	0.5800	0.2366	0.0094	0.0088	0.2994	0.5237
TSHR	rs12323699	14	81464286	C	0.081	0.0084	0.0119	0.4880	0.3116	0.0226	0.0138	0.1112	0.9540

Supplementary Table 1. Continued.

Gene	SNP	Chrom	Position	Minor allele	MAF	Femoral neck BMD				Lumbar spine BMD			
						Beta	SE	P-value	-log (P-value)	Beta	SE	P-value	-log (P-value)
TSHR	rs2122253	14	81127338	G	0.149	-0.0133	0.0108	0.2290	0.6401	-0.0057	0.0126	0.6620	0.1792
TSHR	rs56169819	14	81464471	C	0.081	0.0079	0.0120	0.5182	0.2855	0.0203	0.0139	0.1545	0.8111
TSHR	rs4903962	14	81464486	G	0.449	-0.0044	0.0076	0.5716	0.2429	0.0087	0.0088	0.3374	0.4718
TSHR	rs2284729	14	81464854	G	0.074	0.0101	0.0127	0.4346	0.3619	0.0185	0.0148	0.2203	0.6569
TSHR	rs2284730	14	81464901	C	0.462	-0.0032	0.0075	0.6763	0.1699	0.0095	0.0088	0.2910	0.5362
TSHR	rs2284731	14	81464955	C	0.074	0.0110	0.0127	0.3954	0.4029	0.0214	0.0147	0.1555	0.8081
TSHR	rs2284732	14	81465597	C	0.074	0.0114	0.0127	0.3796	0.4207	0.0218	0.0148	0.1488	0.8274
TSHR	rs7149900	14	81465845	T	0.074	0.0115	0.0127	0.3746	0.4265	0.0224	0.0147	0.1375	0.8617
TSHR	rs7154106	14	81465984	G	0.074	0.0110	0.0126	0.3935	0.4050	0.0220	0.0147	0.1435	0.8433
TSHR	rs58729405	14	81466368	C	0.074	0.0114	0.0127	0.3788	0.4216	0.0234	0.0147	0.1216	0.9151
TSHR	rs113850939	14	81466495	T	0.074	0.0111	0.0126	0.3907	0.4082	0.0223	0.0147	0.1393	0.8561
TSHR	rs11159480	14	81466662	G	0.140	0.0003	0.0111	0.9816	0.0081	0.0012	0.0125	0.9281	0.0324
TSHR	rs68104217	14	81466897	G	0.074	0.0105	0.0127	0.4162	0.3807	0.0221	0.0147	0.1434	0.8433
TSHR	rs67130762	14	81466980	A	0.074	0.0110	0.0126	0.3931	0.4055	0.0222	0.0147	0.1401	0.8534
TSHR	rs12896769	14	81467052	G	0.368	-0.0086	0.0079	0.2896	0.5382	0.0006	0.0093	0.9481	0.0232
TSHR	rs2268462	14	81467335	G	0.074	0.0109	0.0126	0.3992	0.3988	0.0220	0.0147	0.1437	0.8425
TSHR	rs2268463	14	81467450	G	0.074	0.0109	0.0126	0.4004	0.3975	0.0219	0.0147	0.1461	0.8353
TSHR	rs929630	14	81467458	T	0.368	-0.0085	0.0079	0.2922	0.5343	0.0008	0.0092	0.9297	0.0316
TSHR	rs2268464	14	81467594	A	0.074	0.0171	0.0156	0.2854	0.5445	0.0254	0.0173	0.1529	0.8155
TSHR	rs2268465	14	81467614	G	0.074	0.0110	0.0126	0.3947	0.4037	0.0222	0.0147	0.1394	0.8557
TSHR	rs8007809	14	81468235	T	0.072	0.0105	0.0127	0.4192	0.3776	0.0218	0.0148	0.1493	0.8259
TSHR	rs35472552	14	81468250	G	0.369	-0.0096	0.0079	0.2329	0.6329	0.0036	0.0092	0.7016	0.1539
TSHR	rs17111365	14	81468264	C	0.405	0.0083	0.0080	0.3075	0.5122	-0.0106	0.0090	0.2501	0.6019
TSHR	rs58417382	14	81468446	C	0.323	-0.0071	0.0079	0.3803	0.4199	0.0147	0.0092	0.1191	0.9241

Supplementary Table 1. Continued.

Gene	SNP	Chrom	Position	Minor allele	MAF	Femoral neck BMD				Lumbar spine BMD			
						Beta	SE	P-value	-log (P-value)	Beta	SE	P-value	-log (P-value)
TSHR	rs2122253	14	81127338	G	0.149	-0.0133	0.0108	0.2290	0.6401	-0.0057	0.0126	0.6620	0.1792
TSHR	rs35336887	14	81468463	T	0.119	0.0047	0.0118	0.6995	0.1552	-0.0080	0.0136	0.5644	0.2484
TSHR	rs58266067	14	81468476	C	0.324	-0.0074	0.0079	0.3573	0.4469	0.0145	0.0092	0.1242	0.9060
TSHR	rs58241131	14	81468579	A	0.324	-0.0072	0.0079	0.3756	0.4253	0.0149	0.0092	0.1152	0.9386
TSHR	rs60462373	14	81468655	C	0.323	-0.0072	0.0079	0.3729	0.4284	0.0153	0.0092	0.1058	0.9755
TSHR	rs4903964	14	81468954	A	0.325	-0.0075	0.0089	0.4135	0.3835	0.0132	0.0101	0.2040	0.6903
TSHR	rs7152373	14	81469592	C	0.324	-0.0098	0.0084	0.2570	0.5901	0.0151	0.0092	0.1091	0.9622
TSHR	rs55960644	14	81470024	G	0.325	-0.0076	0.0079	0.3489	0.4573	0.0154	0.0092	0.1028	0.9879
TSHR	rs55957493	14	81470054	A	0.325	-0.0073	0.0079	0.3672	0.4351	0.0155	0.0092	0.1011	0.9954
TSHR	rs917985	14	81470299	G	0.249	-0.0132	0.0087	0.1400	0.8538	0.0083	0.0102	0.4282	0.3684
TSHR	rs917986	14	81470655	C	0.226	0.0072	0.0090	0.4333	0.3632	0.0114	0.0101	0.2712	0.5667
TSHR	rs28440011	14	81470718	T	0.086	0.0127	0.0121	0.3035	0.5179	0.0223	0.0141	0.1213	0.9163
TSHR	rs75453896	14	81471085	G	0.131	0.0066	0.0122	0.5970	0.2240	-0.0074	0.0132	0.5860	0.2321
TSHR	rs7545310	14	81471139	C	0.376	-0.0068	0.0079	0.3990	0.3990	0.0037	0.0092	0.6944	0.1584
TSHR	rs71416856	14	81471235	T	0.119	0.0045	0.0118	0.7115	0.1478	-0.0082	0.0136	0.5572	0.2540
TSHR	rs3783944	14	81471277	G	0.074	0.0100	0.0127	0.4401	0.3564	0.0218	0.0147	0.1486	0.8279
TSHR	rs3783943	14	81471446	G	0.404	0.0065	0.0077	0.4048	0.3927	-0.0109	0.0090	0.2369	0.6255
TSHR	rs7154878	14	81471582	T	0.361	0.0169	0.0089	0.0621	1.2068	0.0031	0.0096	0.7495	0.1253
TSHR	rs10136511	14	81471589	A	0.075	0.0025	0.0166	0.8809	0.0551	0.0064	0.0180	0.7288	0.1374
TSHR	rs7155069	14	81471706	T	0.140	-0.0053	0.0108	0.6328	0.1988	-0.0021	0.0125	0.8723	0.0593
TSHR	rs72695026	14	81472030	T	0.178	-0.0156	0.0100	0.1279	0.8931	-0.0019	0.0117	0.8710	0.0600
TSHR	rs12897126	14	81472456	T	0.141	-0.0073	0.0115	0.5322	0.2739	0.0059	0.0138	0.6735	0.1717
TSHR	rs35554250	14	81472563	G	0.119	0.0039	0.0118	0.7443	0.1282	-0.0079	0.0136	0.5697	0.2444
TSHR	rs34146411	14	81472807	A	0.347	0.0041	0.0078	0.6062	0.2174	0.0048	0.0091	0.6038	0.2191

Supplementary Table 1. Continued.

Gene	SNP	Chrom	Position	Minor allele	MAF	Femoral neck BMD				Lumbar spine BMD			
						Beta	SE	P-value	-log (P-value)	Beta	SE	P-value	-log (P-value)
TSHR	rs2122253	14	81127338	G	0.149	-0.0133	0.0108	0.2290	0.6401	-0.0057	0.0126	0.6620	0.1792
TSHR	rs117917762	14	81472915	C	0.074	0.0095	0.0127	0.4653	0.3322	0.0206	0.0148	0.1736	0.7605
TSHR	rs12185020	14	81473078	G	0.214	0.0004	0.0088	0.9628	0.0165	0.0078	0.0103	0.4560	0.3411
TSHR	rs118186899	14	81473080	G	0.074	0.0087	0.0127	0.5008	0.3003	0.0193	0.0148	0.2022	0.6941
TSHR	rs113137098	14	81474619	A	0.181	-0.0200	0.0105	0.0617	1.2095	-0.0069	0.0123	0.5829	0.2344
TSHR	rs76759837	14	81474796	A	0.074	0.0052	0.0128	0.6898	0.1613	0.0152	0.0149	0.3198	0.4952
TSHR	rs72695035	14	81477015	C	0.067	0.0083	0.0135	0.5450	0.2636	0.0191	0.0157	0.2353	0.6284
TSHR	rs4903965	14	81477437	A	0.312	0.0049	0.0079	0.5420	0.2660	0.0057	0.0092	0.5437	0.2646
TSHR	rs72689904	14	81478278	G	0.286	-0.0131	0.0086	0.1338	0.8734	0.0006	0.0100	0.9549	0.0200
TSHR	rs12883673	14	81480822	G	0.119	0.0073	0.0117	0.5421	0.2659	-0.0045	0.0135	0.7438	0.1286
TSHR	rs2300521	14	81481332	C	0.270	-0.0217	0.0091	0.0189	1.7243	-0.0007	0.0102	0.9468	0.0237
TSHR	rs12890676	14	81481701	C	0.119	0.0066	0.0117	0.5833	0.2341	-0.0062	0.0135	0.6556	0.1833
TSHR	rs10873332	14	81481849	A	0.312	0.0052	0.0080	0.5228	0.2817	0.0070	0.0092	0.4579	0.3392
TSHR	rs72689907	14	81481886	A	0.067	0.0074	0.0135	0.5946	0.2258	0.0184	0.0158	0.2551	0.5934
TSHR	rs12436366	14	81481952	T	0.312	0.0054	0.0079	0.5068	0.2952	0.0064	0.0092	0.4969	0.3037
TSHR	rs59484155	14	81482108	A	0.312	0.0055	0.0079	0.4989	0.3020	0.0065	0.0092	0.4903	0.3096
TSHR	rs72689910	14	81482756	C	0.067	0.0071	0.0135	0.6042	0.2189	0.0185	0.0157	0.2491	0.6036
TSHR	rs12880945	14	81483881	A	0.119	0.0067	0.0117	0.5746	0.2406	-0.0051	0.0135	0.7102	0.1486
TSHR	rs12881268	14	81483897	A	0.119	0.0066	0.0117	0.5802	0.2365	-0.0052	0.0135	0.7091	0.1493
TSHR	rs71486630	14	81484459	C	0.112	-0.0022	0.0115	0.8509	0.0701	0.0037	0.0134	0.7887	0.1031
TSHR	rs4903967	14	81485149	G	0.179	0.0060	0.0096	0.5383	0.2690	0.0112	0.0108	0.3102	0.5083
TSHR	rs4899784	14	81487500	G	0.300	0.0060	0.0081	0.4734	0.3248	0.0070	0.0095	0.4713	0.3267
TSHR	rs34074777	14	81488262	T	0.119	0.0096	0.0120	0.4351	0.3614	-0.0044	0.0139	0.7580	0.1204
TSHR	rs35789100	14	81488416	T	0.119	0.0109	0.0120	0.3742	0.4269	-0.0036	0.0139	0.8023	0.0957

Supplementary Table 1. Continued.

Gene	SNP	Chrom	Position	Minor allele	MAF	Femoral neck BMD				Lumbar spine BMD			
						Beta	SE	P-value	-log (P-value)	Beta	SE	P-value	-log (P-value)
TSHR	rs2122253	14	81127338	G	0.149	-0.0133	0.0108	0.2290	0.6401	-0.0057	0.0126	0.6620	0.1792
TSHR	rs11159481	14	81490742	T	0.269	-0.0181	0.0089	0.0468	1.3293	-0.0030	0.0104	0.7804	0.1077
TSHR	rs11159482	14	81490842	T	0.066	-0.0119	0.0153	0.4478	0.3489	0.0141	0.0179	0.4421	0.3545
TSHR	rs2268467	14	81492606	C	0.192	-0.0002	0.0094	0.9835	0.0072	-0.0039	0.0109	0.7282	0.1377
TSHR	rs2268468	14	81492615	G	0.173	-0.0034	0.0097	0.7313	0.1359	-0.0040	0.0114	0.7321	0.1354
TSHR	rs10143800	14	81496342	G	0.222	-0.0040	0.0086	0.6480	0.1884	0.0063	0.0100	0.5382	0.2691
TSHR	rs2300522	14	81496640	T	0.231	-0.0051	0.0085	0.5546	0.2560	0.0046	0.0099	0.6484	0.1882
TSHR	rs2300523	14	81496820	G	0.231	-0.0052	0.0085	0.5529	0.2573	0.0043	0.0100	0.6754	0.1705
TSHR	rs2300524	14	81496936	G	0.231	-0.0050	0.0085	0.5647	0.2481	0.0047	0.0099	0.6419	0.1925
TSHR	rs2300525	14	81497393	C	0.231	-0.0054	0.0085	0.5383	0.2690	0.0041	0.0099	0.6837	0.1651
TSHR	rs2300526	14	81497467	G	0.222	-0.0040	0.0086	0.6450	0.1904	0.0064	0.0100	0.5328	0.2734
TSHR	rs12147797	14	81497634	T	0.231	-0.0052	0.0085	0.5506	0.2592	0.0045	0.0099	0.6597	0.1807
TSHR	rs17628249	14	81497980	C	0.231	-0.0057	0.0085	0.5125	0.2903	0.0043	0.0099	0.6709	0.1733
TSHR	rs17545722	14	81498002	C	0.231	-0.0052	0.0085	0.5516	0.2584	0.0045	0.0099	0.6600	0.1805
TSHR	rs2110697	14	81498066	T	0.454	0.0075	0.0080	0.3608	0.4427	-0.0012	0.0087	0.8903	0.0505
TSHR	rs61978723	14	81498125	T	0.231	-0.0050	0.0085	0.5640	0.2488	0.0045	0.0100	0.6578	0.1819
TSHR	rs61978724	14	81498303	C	0.231	-0.0053	0.0085	0.5431	0.2651	0.0044	0.0099	0.6627	0.1787
TSHR	rs2268469	14	81498779	T	0.221	-0.0042	0.0086	0.6364	0.1963	0.0058	0.0100	0.5753	0.2401
TSHR	rs2268472	14	81500146	C	0.231	-0.0050	0.0085	0.5642	0.2486	0.0047	0.0099	0.6437	0.1913
TSHR	rs28416942	14	81500309	T	0.231	-0.0051	0.0085	0.5575	0.2537	0.0041	0.0099	0.6850	0.1643
TSHR	rs28478356	14	81500378	T	0.231	-0.0053	0.0085	0.5465	0.2624	0.0040	0.0099	0.6940	0.1587
TSHR	rs2268473	14	81500396	G	0.124	-0.0034	0.0086	0.6979	0.1562	0.0078	0.0101	0.4515	0.3453
TSHR	rs28448639	14	81500397	G	0.121	-0.0036	0.0086	0.6799	0.1675	0.0075	0.0101	0.4684	0.3293
TSHR	rs12323621	14	81500598	T	0.231	-0.0041	0.0085	0.6411	0.1930	0.0032	0.0099	0.7564	0.1213

Supplementary Table 1. Continued.

Gene	SNP	Chrom	Position	Minor allele	MAF	Femoral neck BMD				Lumbar spine BMD			
						Beta	SE	P-value	-log (P-value)	Beta	SE	P-value	-log (P-value)
TSHR	rs2122253	14	81127338	G	0.149	-0.0133	0.0108	0.2290	0.6401	-0.0057	0.0126	0.6620	0.1792
TSHR	rs10873333	14	81500813	C	0.231	-0.0050	0.0085	0.5633	0.2493	0.0051	0.0099	0.6158	0.2106
TSHR	rs726627	14	81500884	T	0.231	-0.0066	0.0129	0.6160	0.2105	0.0124	0.0152	0.4276	0.3690
TSHR	rs726626	14	81501114	T	0.230	-0.0054	0.0085	0.5367	0.2703	0.0039	0.0099	0.7012	0.1542
TSHR	rs726625	14	81501149	T	0.231	-0.0053	0.0085	0.5392	0.2682	0.0039	0.0099	0.6997	0.1551
TSHR	rs726628	14	81501199	A	0.231	-0.0052	0.0085	0.5526	0.2576	0.0044	0.0099	0.6634	0.1782
TSHR	rs11159483	14	81501311	T	0.231	-0.0053	0.0085	0.5390	0.2685	0.0041	0.0099	0.6883	0.1622
TSHR	rs61978725	14	81501722	G	0.231	-0.0052	0.0085	0.5503	0.2594	0.0041	0.0099	0.6864	0.1634
TSHR	rs1005292	14	81501957	T	0.191	-0.0033	0.0098	0.7414	0.1300	-0.0027	0.0110	0.8138	0.0895
TSHR	rs12323481	14	81502046	C	0.231	-0.0055	0.0085	0.5266	0.2785	0.0040	0.0099	0.6932	0.1591
TSHR	rs12323785	14	81502070	G	0.231	-0.0052	0.0085	0.5509	0.2589	0.0041	0.0099	0.6899	0.1612
TSHR	rs12323790	14	81502277	T	0.231	-0.0052	0.0085	0.5491	0.2603	0.0041	0.0099	0.6886	0.1621
TSHR	rs12323799	14	81502527	T	0.231	-0.0049	0.0085	0.5719	0.2426	0.0041	0.0099	0.6893	0.1616
TSHR	rs12323491	14	81502677	C	0.231	-0.0052	0.0085	0.5485	0.2608	0.0035	0.0099	0.7273	0.1383
TSHR	rs59741121	14	81502778	C	0.183	-0.0116	0.0101	0.2612	0.5831	-0.0028	0.0110	0.8023	0.0957
TSHR	rs11159484	14	81503553	T	0.231	-0.0060	0.0085	0.4892	0.3105	0.0036	0.0100	0.7234	0.1406
TSHR	rs12323893	14	81503653	A	0.231	-0.0052	0.0086	0.5546	0.2560	0.0039	0.0100	0.7010	0.1543
TSHR	rs72689920	14	81503708	T	0.094	-0.0100	0.0098	0.3180	0.4975	0.0001	0.0115	0.9913	0.0038
TSHR	rs12323890	14	81503752	A	0.214	-0.0110	0.0100	0.2808	0.5516	0.0026	0.0110	0.8169	0.0878
TSHR	rs2888047	14	81504097	C	0.192	0.0006	0.0094	0.9527	0.0210	-0.0020	0.0110	0.8556	0.0677
TSHR	rs11159485	14	81504119	G	0.230	-0.0114	0.0100	0.2660	0.5751	0.0005	0.0110	0.9624	0.0167
TSHR	rs11159486	14	81504124	T	0.230	-0.0102	0.0100	0.3184	0.4970	0.0014	0.0110	0.9003	0.0456
TSHR	rs11159487	14	81504156	G	0.231	-0.0101	0.0097	0.3098	0.5089	0.0018	0.0107	0.8694	0.0608
TSHR	rs11159488	14	81504159	A	0.231	-0.0101	0.0097	0.3102	0.5084	0.0011	0.0107	0.9235	0.0346

Supplementary Table 1. Continued.

Gene	SNP	Chrom	Position	Minor allele	MAF	Femoral neck BMD				Lumbar spine BMD			
						Beta	SE	P-value	-log (P-value)	Beta	SE	P-value	-log (P-value)
TSHR	rs2122253	14	81127338	G	0.149	-0.0133	0.0108	0.2290	0.6401	-0.0057	0.0126	0.6620	0.1792
TSHR	rs13353102	14	81504267	A	0.222	-0.0033	0.0088	0.7128	0.1470	0.0032	0.0103	0.7643	0.1167
TSHR	rs13353103	14	81504375	T	0.231	-0.0046	0.0085	0.5981	0.2233	0.0041	0.0100	0.6897	0.1614
TSHR	rs13353125	14	81504423	C	0.231	-0.0046	0.0085	0.5947	0.2257	0.0040	0.0099	0.6916	0.1602
TSHR	rs13353104	14	81504551	T	0.232	-0.0049	0.0085	0.5720	0.2426	0.0038	0.0100	0.7062	0.1511
TSHR	rs12323355	14	81504860	A	0.231	-0.0041	0.0086	0.6425	0.1921	0.0044	0.0100	0.6633	0.1783
TSHR	rs12323350	14	81504932	A	0.231	-0.0048	0.0086	0.5843	0.2334	0.0046	0.0101	0.6576	0.1821
TSHR	rs12323693	14	81504957	G	0.231	-0.0055	0.0087	0.5378	0.2694	0.0053	0.0101	0.6067	0.2170
TSHR	rs12323422	14	81504973	T	0.222	-0.0042	0.0087	0.6353	0.1971	0.0063	0.0102	0.5488	0.2606
TSHR	rs12323762	14	81505089	C	0.231	-0.0051	0.0086	0.5596	0.2521	0.0042	0.0100	0.6783	0.1686
TSHR	rs61978742	14	81505367	A	0.231	-0.0055	0.0086	0.5347	0.2719	0.0034	0.0100	0.7396	0.1310
TSHR	rs61978743	14	81505391	A	0.231	-0.0054	0.0086	0.5390	0.2684	0.0039	0.0100	0.7047	0.1520
TSHR	rs12184961	14	81505551	G	0.231	-0.0043	0.0085	0.6221	0.2062	0.0049	0.0099	0.6311	0.1999
TSHR	rs12185035	14	81505679	C	0.231	-0.0045	0.0085	0.6028	0.2198	0.0035	0.0100	0.7311	0.1360
TSHR	rs12184963	14	81505737	T	0.231	-0.0044	0.0085	0.6123	0.2131	0.0033	0.0100	0.7470	0.1267
TSHR	rs12100732	14	81506485	C	0.174	-0.0096	0.0102	0.3576	0.4467	-0.0007	0.0112	0.9505	0.0220
TSHR	rs4903968	14	81507724	A	0.191	0.0008	0.0095	0.9361	0.0287	-0.0028	0.0110	0.8062	0.0936
TSHR	rs2110698	14	81510740	T	0.191	0.0001	0.0095	0.9919	0.0035	-0.0036	0.0110	0.7489	0.1256
TSHR	rs112548722	14	81510856	G	0.231	-0.0047	0.0085	0.5881	0.2306	0.0038	0.0100	0.7130	0.1469
TSHR	rs10147243	14	81511178	A	0.263	-0.0052	0.0083	0.5413	0.2665	0.0044	0.0097	0.6597	0.1806
TSHR	rs61978744	14	81511484	C	0.183	-0.0108	0.0101	0.2965	0.5279	-0.0035	0.0111	0.7589	0.1198
TSHR	rs61978745	14	81515284	A	0.173	-0.0096	0.0103	0.3608	0.4427	-0.0029	0.0112	0.7981	0.0980
TSHR	rs2024422	14	81516937	T	0.219	-0.0043	0.0087	0.6262	0.2033	0.0051	0.0101	0.6222	0.2060
TSHR	rs10131847	14	81517478	T	0.219	-0.0046	0.0087	0.6026	0.2200	0.0050	0.0101	0.6290	0.2014

Supplementary Table 1. Continued.

Gene	SNP	Chrom	Position	Minor allele	MAF	Femoral neck BMD				Lumbar spine BMD			
						Beta	SE	P-value	-log (P-value)	Beta	SE	P-value	-log (P-value)
TSHR	rs2122253	14	81127338	G	0.149	-0.0133	0.0108	0.2290	0.6401	-0.0057	0.0126	0.6620	0.1792
TSHR	rs2689928	14	81518033	C	0.219	-0.0048	0.0087	0.5891	0.2298	0.0050	0.0101	0.6293	0.2011
TSHR	rs2888048	14	81518074	A	0.195	0.0019	0.0094	0.8423	0.0745	-0.0022	0.0109	0.8412	0.0751
TSHR	rs55947533	14	81518826	T	0.173	-0.0095	0.0103	0.3647	0.4381	-0.0017	0.0112	0.8844	0.0534
TSHR	rs7158224	14	81519207	C	0.260	-0.0071	0.0084	0.4040	0.3936	0.0037	0.0097	0.7091	0.1493
TSHR	rs10138781	14	81519862	T	0.260	-0.0071	0.0084	0.4087	0.3886	0.0034	0.0098	0.7365	0.1328
TSHR	rs4619328	14	81519920	A	0.219	-0.0045	0.0087	0.6098	0.2148	0.0052	0.0101	0.6175	0.2094
TSHR	rs2024423	14	81520260	T	0.195	0.0016	0.0094	0.8695	0.0607	-0.0025	0.0109	0.8215	0.0854
TSHR	rs2024424	14	81520379	C	0.222	-0.0046	0.0087	0.6012	0.2210	0.0052	0.0101	0.6175	0.2093
TSHR	rs28855991	14	81520562	C	0.219	-0.0046	0.0087	0.6058	0.2177	0.0054	0.0101	0.6046	0.2185
TSHR	rs10150381	14	81521033	A	0.219	-0.0047	0.0087	0.5957	0.2249	0.0052	0.0101	0.6154	0.2108
TSHR	rs28750397	14	81521399	C	0.219	-0.0037	0.0087	0.6725	0.1723	0.0059	0.0101	0.5666	0.2467
TSHR	rs2371467	14	81521432	T	0.173	-0.0095	0.0103	0.3683	0.4338	-0.0018	0.0112	0.8781	0.0564
TSHR	rs10150860	14	81521552	C	0.219	-0.0045	0.0087	0.6075	0.2164	0.0055	0.0101	0.5969	0.2241
TSHR	rs2215982	14	81522110	T	0.174	-0.0096	0.0103	0.3621	0.4412	-0.0016	0.0112	0.8864	0.0523
TSHR	rs61978747	14	81523051	A	0.173	-0.0097	0.0103	0.3556	0.4490	-0.0021	0.0112	0.8550	0.0680
TSHR	rs4424851	14	81523082	A	0.192	0.0008	0.0095	0.9365	0.0285	-0.0022	0.0111	0.8436	0.0739
TSHR	rs71711394	14	81523128	C	0.174	-0.0091	0.0103	0.3837	0.4160	-0.0014	0.0112	0.9013	0.0451
TSHR	rs28675993	14	81523742	G	0.222	-0.0038	0.0086	0.6641	0.1778	0.0055	0.0101	0.5935	0.2266
TSHR	rs10134487	14	81523886	C	0.262	-0.0061	0.0084	0.4758	0.3225	0.0044	0.0098	0.6628	0.1786
TSHR	rs17111396	14	81524054	A	0.219	-0.0046	0.0087	0.6050	0.2183	0.0058	0.0101	0.5762	0.2394
TSHR	rs17111398	14	81524255	C	0.228	-0.0046	0.0086	0.6021	0.2203	0.0057	0.0101	0.5826	0.2347
TSHR	rs2268474	14	81524407	C	0.228	-0.0046	0.0086	0.6003	0.2216	0.0056	0.0100	0.5886	0.2302
TSHR	rs7147357	14	81526452	C	0.262	-0.0062	0.0084	0.4687	0.3291	0.0051	0.0098	0.6147	0.2113

Supplementary Table 1. Continued.

Gene	SNP	Chrom	Position	Minor allele	MAF	Femoral neck BMD				Lumbar spine BMD			
						Beta	SE	P-value	-log (P-value)	Beta	SE	P-value	-log (P-value)
TSHR	rs2122253	14	81127338	G	0.149	-0.0133	0.0108	0.2290	0.6401	-0.0057	0.0126	0.6620	0.1792
TSHR	rs72689940	14	81528119	A	0.093	0.0017	0.0131	0.8989	0.0463	0.0178	0.0152	0.2551	0.5933
TSHR	rs17546166	14	81530202	C	0.069	-0.0072	0.0150	0.6390	0.1945	0.0107	0.0176	0.5538	0.2567
TSHR	rs12372891	14	81531392	C	0.184	-0.0021	0.0097	0.8338	0.0789	-0.0064	0.0112	0.5786	0.2376
TSHR	rs11845164	14	81531754	C	0.122	-0.0210	0.0111	0.0625	1.2039	-0.0056	0.0128	0.6683	0.1750
TSHR	rs80043258	14	81533379	G	0.052	-0.0370	0.0187	0.0532	1.2742	-0.0068	0.0219	0.7618	0.1182
TSHR	rs11159490	14	81534440	T	0.376	-0.0108	0.0077	0.1723	0.7637	-0.0026	0.0090	0.7793	0.1083
TSHR	rs61978750	14	81536262	C	0.115	-0.0184	0.0113	0.1125	0.9489	-0.0011	0.0132	0.9361	0.0287
TSHR	rs77425375	14	81536698	T	0.098	-0.0165	0.0124	0.1945	0.7110	-0.0132	0.0145	0.3728	0.4285
TSHR	rs10148749	14	81536799	C	0.122	-0.0202	0.0110	0.0731	1.1361	-0.0037	0.0128	0.7805	0.1076
TSHR	rs10151660	14	81537484	C	0.122	-0.0205	0.0110	0.0683	1.1654	-0.0043	0.0128	0.7423	0.1294
TSHR	rs7161244	14	81537685	G	0.122	-0.0207	0.0110	0.0660	1.1802	-0.0044	0.0128	0.7384	0.1317
TSHR	rs12147099	14	81538927	C	0.071	0.0001	0.0168	0.9955	0.0020	0.0116	0.0187	0.5433	0.2650
TSHR	rs12887103	14	81539214	A	0.378	-0.0014	0.0137	0.9222	0.0352	-0.0125	0.0160	0.4448	0.3518
TSHR	rs2160213	14	81540075	T	0.184	-0.0030	0.0096	0.7642	0.1168	-0.0053	0.0112	0.6434	0.1915
TSHR	rs7145101	14	81540332	T	0.121	-0.0208	0.0111	0.0664	1.1780	-0.0047	0.0129	0.7199	0.1427
TSHR	rs7145290	14	81540422	T	0.122	-0.0183	0.0118	0.1275	0.8943	-0.0023	0.0128	0.8595	0.0657
TSHR	rs117641695	14	81542726	A	0.058	0.0162	0.0186	0.3922	0.4065	-0.0014	0.0217	0.9483	0.0231
TSHR	rs6574622	14	81546486	T	0.122	-0.0199	0.0110	0.0775	1.1109	-0.0042	0.0128	0.7504	0.1247
TSHR	rs75235317	14	81547827	A	0.071	-0.0035	0.0163	0.8346	0.0785	0.0114	0.0188	0.5537	0.2567
TSHR	rs7151543	14	81548766	G	0.120	-0.0194	0.0111	0.0886	1.0527	-0.0033	0.0130	0.8035	0.0950
TSHR	rs6574625	14	81549533	A	0.122	-0.0218	0.0110	0.0538	1.2692	-0.0072	0.0128	0.5855	0.2325
TSHR	rs8019570	14	81549595	A	0.122	-0.0217	0.0111	0.0548	1.2610	-0.0071	0.0129	0.5921	0.2276
TSHR	rs2300527	14	81550888	G	0.115	-0.0188	0.0113	0.1053	0.9777	-0.0012	0.0132	0.9306	0.0312

Supplementary Table 1. Continued.

Gene	SNP	Chrom	Position	Minor allele	MAF	Femoral neck BMD				Lumbar spine BMD			
						Beta	SE	P-value	-log (P-value)	Beta	SE	P-value	-log (P-value)
TSHR	rs2122253	14	81127338	G	0.149	-0.0133	0.0108	0.2290	0.6401	-0.0057	0.0126	0.6620	0.1792
TSHR	rs2300528	14	81551260	A	0.122	-0.0209	0.0111	0.0639	1.1948	-0.0062	0.0129	0.6377	0.1954
TSHR	rs2284733	14	81552204	C	0.120	-0.0197	0.0111	0.0842	1.0746	-0.0041	0.0130	0.7563	0.1213
TSHR	rs2284734	14	81553733	G	0.307	-0.0141	0.0083	0.0964	1.0159	-0.0053	0.0093	0.5793	0.2371
TSHR	rs2284735	14	81553786	A	0.378	-0.0132	0.0080	0.1067	0.9718	-0.0022	0.0090	0.8149	0.0889
TSHR	rs2075173	14	81554617	G	0.115	-0.0185	0.0114	0.1115	0.9528	-0.0017	0.0132	0.9012	0.0452
TSHR	rs28654442	14	81556979	A	0.121	-0.0185	0.0112	0.1051	0.9785	-0.0037	0.0130	0.7831	0.1062
TSHR	rs10083503	14	81557084	G	0.184	-0.0048	0.0100	0.6376	0.1955	-0.0043	0.0112	0.7047	0.1520
TSHR	rs28701517	14	81557109	A	0.121	-0.0184	0.0112	0.1080	0.9664	-0.0016	0.0130	0.9037	0.0440
TSHR	rs2075174	14	81558505	A	0.120	-0.0189	0.0111	0.0972	1.0124	-0.0041	0.0130	0.7559	0.1215
TSHR	rs2075177	14	81559221	A	0.121	-0.0195	0.0112	0.0877	1.0570	-0.0029	0.0130	0.8246	0.0837
TSHR	rs2241120	14	81559431	A	0.122	-0.0209	0.0110	0.0636	1.1967	-0.0041	0.0128	0.7549	0.1221
TSHR	rs60447919	14	81559600	T	0.122	-0.0209	0.0110	0.0635	1.1974	-0.0041	0.0128	0.7580	0.1203
TSHR	rs7158747	14	81560007	G	0.123	-0.0207	0.0111	0.0672	1.1728	-0.0041	0.0129	0.7563	0.1213
TSHR	rs17111431	14	81560134	G	0.122	-0.0218	0.0111	0.0545	1.2639	-0.0065	0.0129	0.6228	0.2057
TSHR	rs28417017	14	81560286	A	0.121	-0.0188	0.0111	0.0984	1.0069	-0.0024	0.0130	0.8552	0.0679
TSHR	rs7159477	14	81560292	T	0.122	-0.0194	0.0118	0.1065	0.9729	-0.0044	0.0128	0.7373	0.1324
TSHR	rs7160204	14	81560504	A	0.122	-0.0213	0.0110	0.0589	1.2297	-0.0042	0.0129	0.7475	0.1264
TSHR	rs7143259	14	81560994	G	0.123	-0.0213	0.0111	0.0608	1.2161	-0.0039	0.0129	0.7709	0.1130
TSHR	rs7143593	14	81561116	T	0.122	-0.0211	0.0110	0.0616	1.2107	-0.0039	0.0128	0.7688	0.1142
TSHR	rs7145351	14	81561389	A	0.122	-0.0212	0.0111	0.0605	1.2183	-0.0047	0.0129	0.7232	0.1407
TSHR	rs10142999	14	81561425	A	0.122	-0.0210	0.0111	0.0635	1.1972	-0.0047	0.0129	0.7210	0.1421
TSHR	rs10143087	14	81561557	C	0.122	-0.0215	0.0110	0.0567	1.2463	-0.0047	0.0128	0.7207	0.1422
TSHR	rs10142959	14	81561643	G	0.122	-0.0217	0.0110	0.0548	1.2612	-0.0055	0.0128	0.6731	0.1719

Supplementary Table 1. Continued.

Gene	SNP	Chrom	Position	Minor allele	MAF	Femoral neck BMD				Lumbar spine BMD			
						Beta	SE	P-value	-log (P-value)	Beta	SE	P-value	-log (P-value)
TSHR	rs2122253	14	81127338	G	0.149	-0.0133	0.0108	0.2290	0.6401	-0.0057	0.0126	0.6620	0.1792
TSHR	rs28599787	14	81561681	A	0.121	-0.0205	0.0112	0.0722	1.1413	-0.0034	0.0130	0.7960	0.0991
TSHR	rs7150193	14	81562442	G	0.122	-0.0209	0.0111	0.0643	1.1915	-0.0044	0.0129	0.7405	0.1305
TSHR	rs10146192	14	81562581	T	0.122	-0.0209	0.0110	0.0639	1.1944	-0.0040	0.0128	0.7632	0.1173
TSHR	rs10146203	14	81562623	T	0.122	-0.0209	0.0111	0.0640	1.1939	-0.0033	0.0129	0.8025	0.0955
TSHR	rs10149740	14	81563831	T	0.121	-0.0205	0.0113	0.0740	1.1309	-0.0025	0.0131	0.8517	0.0697
TSHR	rs10149807	14	81563860	T	0.121	-0.0205	0.0112	0.0743	1.1288	-0.0027	0.0131	0.8388	0.0763
TSHR	rs10149739	14	81563871	G	0.121	-0.0211	0.0113	0.0668	1.1750	-0.0029	0.0131	0.8273	0.0823
TSHR	rs10149992	14	81563900	A	0.121	-0.0186	0.0112	0.1021	0.9908	-0.0022	0.0130	0.8706	0.0602
TSHR	rs10141582	14	81564185	C	0.121	-0.0195	0.0112	0.0881	1.0550	-0.0025	0.0130	0.8484	0.0714
TSHR	rs10129380	14	81564339	T	0.122	-0.0212	0.0111	0.0606	1.2179	-0.0051	0.0129	0.6973	0.1566
TSHR	rs10141777	14	81564374	C	0.122	-0.0211	0.0111	0.0622	1.2061	-0.0050	0.0129	0.7050	0.1518
TSHR	rs2300529	14	81564505	A	0.122	-0.0210	0.0111	0.0631	1.1998	-0.0051	0.0129	0.6990	0.1555
TSHR	rs2300530	14	81564994	A	0.122	-0.0219	0.0111	0.0527	1.2779	-0.0061	0.0128	0.6410	0.1932
TSHR	rs2300531	14	81565058	A	0.121	-0.0213	0.0111	0.0596	1.2251	-0.0049	0.0129	0.7113	0.1480
TSHR	rs2300532	14	81565125	G	0.122	-0.0209	0.0111	0.0639	1.1944	-0.0048	0.0129	0.7132	0.1468
TSHR	rs17111471	14	81565762	G	0.122	-0.0213	0.0111	0.0599	1.2224	-0.0053	0.0129	0.6846	0.1646
TSHR	rs10133458	14	81565918	A	0.122	-0.0209	0.0111	0.0644	1.1914	-0.0054	0.0129	0.6842	0.1648
TSHR	rs10147921	14	81566059	G	0.122	-0.0214	0.0111	0.0588	1.2305	-0.0060	0.0129	0.6466	0.1893
TSHR	rs10136213	14	81566362	A	0.122	-0.0208	0.0111	0.0657	1.1827	-0.0038	0.0129	0.7735	0.1115
TSHR	rs2300533	14	81566475	T	0.122	-0.0210	0.0111	0.0633	1.1989	-0.0048	0.0129	0.7170	0.1445
TSHR	rs6574626	14	81567060	A	0.181	-0.0060	0.0101	0.5623	0.2501	-0.0051	0.0113	0.6629	0.1786
TSHR	rs2300534	14	81567080	A	0.148	-0.0108	0.0109	0.3293	0.4824	0.0034	0.0122	0.7860	0.1046
TSHR	rs2300535	14	81567248	A	0.129	-0.0139	0.0117	0.2478	0.6060	0.0061	0.0128	0.6433	0.1916

Supplementary Table 1. Continued.

Gene	SNP	Chrom	Position	Minor allele	MAF	Femoral neck BMD				Lumbar spine BMD			
						Beta	SE	P-value	-log (P-value)	Beta	SE	P-value	-log (P-value)
TSHR	rs2122253	14	81127338	G	0.149	-0.0133	0.0108	0.2290	0.6401	-0.0057	0.0126	0.6620	0.1792
TSHR	rs11625902	14	81568441	G	0.361	-0.0064	0.0083	0.4539	0.3431	0.0042	0.0091	0.6472	0.1890
TSHR	rs7143914	14	81568600	G	0.172	0.0001	0.0100	0.9918	0.0036	0.0172	0.0116	0.1498	0.8246
TSHR	rs75382066	14	81569562	C	0.053	-0.0143	0.0200	0.4847	0.3145	-0.0134	0.0236	0.5795	0.2369
TSHR	rs1017141	14	81569678	G	0.181	-0.0063	0.0098	0.5256	0.2793	-0.0073	0.0113	0.5272	0.2780
TSHR	rs1861272	14	81570150	T	0.354	-0.0031	0.0078	0.7010	0.1543	0.0058	0.0091	0.5315	0.2745
TSHR	rs7150670	14	81571088	T	0.495	0.0047	0.0077	0.5487	0.2607	-0.0068	0.0090	0.4578	0.3393
TSHR	rs7151769	14	81571659	C	0.367	-0.0010	0.0078	0.9009	0.0453	0.0080	0.0091	0.3916	0.4072
TSHR	rs2268475	14	81572310	C	0.426	0.0030	0.0076	0.7036	0.1527	-0.0063	0.0089	0.4878	0.3118
TSHR	rs1990595	14	81572386	A	0.348	-0.0014	0.0078	0.8642	0.0634	0.0082	0.0091	0.3777	0.4229
TSHR	rs1990596	14	81572534	T	0.348	-0.0015	0.0078	0.8558	0.0676	0.0077	0.0091	0.4072	0.3902
TSHR	rs2371468	14	81572865	A	0.348	-0.0016	0.0078	0.8397	0.0759	0.0079	0.0091	0.3982	0.3999
TSHR	rs11159491	14	81572939	T	0.349	-0.0010	0.0078	0.9025	0.0446	0.0088	0.0091	0.3454	0.4617
TSHR	rs7141675	14	81573148	C	0.426	0.0033	0.0076	0.6752	0.1706	-0.0064	0.0089	0.4822	0.3168
TSHR	rs2024425	14	81573437	T	0.348	-0.0016	0.0078	0.8402	0.0756	0.0077	0.0091	0.4085	0.3888
TSHR	rs61980862	14	81573539	T	0.171	0.0023	0.0100	0.8217	0.0853	0.0180	0.0117	0.1321	0.8791
TSHR	rs2024426	14	81573616	T	0.348	-0.0015	0.0078	0.8487	0.0712	0.0076	0.0091	0.4148	0.3821
TSHR	rs7147527	14	81574038	G	0.349	-0.0012	0.0078	0.8823	0.0544	0.0076	0.0091	0.4164	0.3805
TSHR	rs12884734	14	81574150	C	0.407	-0.0013	0.0078	0.8707	0.0601	0.0086	0.0091	0.3589	0.4451
TSHR	rs12884578	14	81574151	A	0.414	0.0001	0.0079	0.9858	0.0062	0.0090	0.0092	0.3371	0.4723
TSHR	rs11845715	14	81574283	T	0.177	-0.0045	0.0099	0.6563	0.1829	-0.0054	0.0114	0.6440	0.1911
TSHR	rs12885526	14	81574429	G	0.347	-0.0031	0.0081	0.7139	0.1463	0.0077	0.0091	0.4099	0.3873
TSHR	rs2080305	14	81575419	C	0.424	0.0044	0.0079	0.5886	0.2302	-0.0094	0.0089	0.3023	0.5196
TSHR	rs139622705	14	81575644	G	0.107	0.0180	0.0145	0.2259	0.6461	0.0054	0.0159	0.7411	0.1301

Supplementary Table 1. Continued.

Gene	SNP	Chrom	Position	Minor allele	MAF	Femoral neck BMD				Lumbar spine BMD			
						Beta	SE	P-value	-log (P-value)	Beta	SE	P-value	-log (P-value)
TSHR	rs2122253	14	81127338	G	0.149	-0.0133	0.0108	0.2290	0.6401	-0.0057	0.0126	0.6620	0.1792
TSHR	rs2371469	14	81575653	C	0.352	-0.0034	0.0078	0.6704	0.1736	0.0082	0.0091	0.3756	0.4253
TSHR	rs8017199	14	81575811	G	0.176	-0.0049	0.0099	0.6247	0.2044	-0.0054	0.0115	0.6471	0.1890
TSHR	rs2080306	14	81576139	C	0.345	-0.0023	0.0079	0.7774	0.1094	0.0115	0.0091	0.2176	0.6623
TSHR	rs7160420	14	81576332	G	0.346	-0.0026	0.0079	0.7500	0.1249	0.0096	0.0091	0.3061	0.5142
TSHR	rs7160596	14	81576426	G	0.345	-0.0019	0.0079	0.8153	0.0887	0.0109	0.0091	0.2426	0.6150
TSHR	rs12878605	14	81576888	T	0.344	-0.0018	0.0079	0.8204	0.0860	0.0098	0.0091	0.2934	0.5326
TSHR	rs12881644	14	81577134	A	0.344	-0.0022	0.0079	0.7829	0.1063	0.0099	0.0091	0.2922	0.5343
TSHR	rs12883448	14	81577442	T	0.345	-0.0021	0.0079	0.7911	0.1018	0.0105	0.0091	0.2630	0.5801
TSHR	rs10144209	14	81577831	T	0.345	-0.0018	0.0079	0.8196	0.0864	0.0106	0.0091	0.2576	0.5891
TSHR	rs10146801	14	81578212	T	0.344	-0.0019	0.0079	0.8094	0.0918	0.0114	0.0091	0.2240	0.6498
TSHR	rs10146811	14	81578238	T	0.345	-0.0015	0.0079	0.8511	0.0700	0.0111	0.0091	0.2366	0.6260
TSHR	rs2888049	14	81578305	A	0.345	-0.0027	0.0079	0.7341	0.1342	0.0088	0.0091	0.3478	0.4587
TSHR	rs917983	14	81578543	G	0.347	-0.0012	0.0079	0.8845	0.0533	0.0124	0.0092	0.1860	0.7305
TSHR	rs917984	14	81578702	C	0.349	-0.0028	0.0082	0.7394	0.1311	0.0128	0.0092	0.1722	0.7638
TSHR	rs12888772	14	81578926	A	0.171	-0.0040	0.0102	0.7049	0.1519	-0.0032	0.0119	0.7900	0.1024
TSHR	rs34082357	14	81579289	A	0.071	0.0045	0.0159	0.7795	0.1082	0.0143	0.0186	0.4540	0.3429
TSHR	rs74587938	14	81579335	G	0.059	-0.0224	0.0188	0.2431	0.6142	-0.0164	0.0220	0.4673	0.3304
TSHR	rs2300536	14	81580189	T	0.350	0.0115	0.0078	0.1513	0.8203	-0.0033	0.0091	0.7192	0.1432
TSHR	rs11159492	14	81580501	A	0.215	0.0120	0.0089	0.1898	0.7217	-0.0105	0.0104	0.3213	0.4930
TSHR	rs10131259	14	81580566	G	0.215	0.0055	0.0097	0.5763	0.2393	-0.0143	0.0105	0.1860	0.7305
TSHR	rs4903970	14	81580670	T	0.265	-0.0015	0.0135	0.9121	0.0400	0.0115	0.0157	0.4757	0.3227
TSHR	rs4903971	14	81580710	A	0.079	-0.0096	0.0212	0.6586	0.1814	-0.0077	0.0241	0.7564	0.1212
TSHR	rs12586613	14	81580765	T	0.170	0.0070	0.0099	0.4913	0.3086	-0.0054	0.0115	0.6455	0.1901

Supplementary Table 1. Continued.

Gene	SNP	Chrom	Position	Minor allele	MAF	Femoral neck BMD				Lumbar spine BMD			
						Beta	SE	P-value	-log (P-value)	Beta	SE	P-value	-log (P-value)
TSHR	rs2122253	14	81127338	G	0.149	-0.0133	0.0108	0.2290	0.6401	-0.0057	0.0126	0.6620	0.1792
TSHR	rs35080161	14	81580804	T	0.170	0.0071	0.0099	0.4809	0.3180	-0.0058	0.0115	0.6242	0.2047
TSHR	rs149396989	14	81582351	T	0.214	0.0410	0.0158	0.0111	1.9564	0.0134	0.0170	0.4386	0.3579
TSHR	rs12895445	14	81586172	A	0.254	0.0018	0.0158	0.9107	0.0406	0.0082	0.0171	0.6402	0.1937
TSHR	rs12896284	14	81586182	A	0.207	-0.0067	0.0140	0.6394	0.1942	-0.0040	0.0154	0.7992	0.0974
TSHR	rs8020969	14	81586674	T	0.215	0.0119	0.0089	0.1919	0.7170	-0.0100	0.0103	0.3438	0.4637
TSHR	rs2284736	14	81586721	G	0.352	0.0124	0.0078	0.1179	0.9287	-0.0036	0.0090	0.6940	0.1586
TSHR	rs2284738	14	81586934	C	0.215	0.0116	0.0089	0.2030	0.6926	-0.0114	0.0103	0.2819	0.5499
TSHR	rs2284739	14	81587002	C	0.215	0.0115	0.0089	0.2087	0.6804	-0.0101	0.0104	0.3402	0.4682
TSHR	rs2284740	14	81587006	T	0.215	0.0116	0.0089	0.2045	0.6893	-0.0098	0.0104	0.3550	0.4497
TSHR	rs72691609	14	81587243	A	0.170	0.0071	0.0098	0.4772	0.3213	-0.0039	0.0114	0.7400	0.1308
TSHR	rs7143719	14	81587649	A	0.215	0.0124	0.0089	0.1733	0.7612	-0.0104	0.0103	0.3233	0.4904
TSHR	rs7144198	14	81587866	T	0.214	0.0123	0.0089	0.1790	0.7471	-0.0115	0.0104	0.2787	0.5548
TSHR	rs7144061	14	81587887	C	0.215	0.0120	0.0089	0.1879	0.7260	-0.0104	0.0103	0.3224	0.4916
TSHR	rs28373271	14	81587924	G	0.215	0.0119	0.0089	0.1896	0.7222	-0.0106	0.0103	0.3133	0.5040
TSHR	rs2888050	14	81588015	T	0.214	0.0119	0.0089	0.1938	0.7126	-0.0118	0.0104	0.2654	0.5761
TSHR	rs67428672	14	81588054	G	0.215	0.0112	0.0089	0.2182	0.6611	-0.0101	0.0104	0.3417	0.4664
TSHR	rs67045633	14	81588175	G	0.213	0.0119	0.0089	0.1920	0.7167	-0.0120	0.0104	0.2599	0.5852
TSHR	rs76956096	14	81588245	G	0.215	0.0127	0.0089	0.1635	0.7865	-0.0094	0.0103	0.3750	0.4260
TSHR	rs113866322	14	81588469	C	0.352	0.0115	0.0078	0.1470	0.8326	-0.0034	0.0090	0.7125	0.1472
TSHR	rs56369010	14	81588915	G	0.216	0.0122	0.0089	0.1780	0.7497	-0.0105	0.0103	0.3212	0.4932
TSHR	rs55993259	14	81588958	C	0.215	0.0118	0.0089	0.1954	0.7090	-0.0106	0.0103	0.3135	0.5037
TSHR	rs56306987	14	81588995	C	0.215	0.0116	0.0089	0.1997	0.6995	-0.0109	0.0103	0.3008	0.5217
TSHR	rs8019356	14	81589806	C	0.216	0.0120	0.0089	0.1860	0.7305	-0.0100	0.0103	0.3432	0.4645

Supplementary Table 1. Continued.

Gene	SNP	Chrom	Position	Minor allele	MAF	Femoral neck BMD				Lumbar spine BMD			
						Beta	SE	P-value	-log (P-value)	Beta	SE	P-value	-log (P-value)
TSHR	rs2122253	14	81127338	G	0.149	-0.0133	0.0108	0.2290	0.6401	-0.0057	0.0126	0.6620	0.1792
TSHR	rs8012915	14	81590016	A	0.215	0.0093	0.0092	0.3216	0.4927	-0.0102	0.0103	0.3327	0.4780
TSHR	rs28559861	14	81590918	G	0.215	0.0126	0.0089	0.1663	0.7791	-0.0102	0.0103	0.3347	0.4754
TSHR	rs67915209	14	81591037	T	0.214	0.0115	0.0089	0.2078	0.6825	-0.0108	0.0104	0.3089	0.5102
TSHR	rs72691624	14	81591396	T	0.214	0.0127	0.0089	0.1634	0.7868	-0.0112	0.0104	0.2920	0.5346
TSHR	rs7158881	14	81591754	C	0.296	0.0112	0.0082	0.1782	0.7492	-0.0030	0.0095	0.7530	0.1232
TSHR	rs71416860	14	81592167	T	0.056	0.0065	0.0148	0.6687	0.1748	-0.0011	0.0173	0.9527	0.0211
TSHR	rs2268476	14	81592717	G	0.216	0.0097	0.0092	0.3047	0.5161	-0.0098	0.0103	0.3510	0.4547
TSHR	rs7143580	14	81592857	C	0.079	0.0032	0.0149	0.8318	0.0800	0.0184	0.0172	0.2968	0.5275
TSHR	rs66996522	14	81593018	G	0.215	0.0112	0.0089	0.2204	0.6567	-0.0117	0.0104	0.2688	0.5705
TSHR	rs79305847	14	81593371	C	0.079	0.0051	0.0150	0.7366	0.1328	0.0178	0.0173	0.3141	0.5029
TSHR	rs9652304	14	81593982	C	0.295	0.0113	0.0082	0.1789	0.7473	-0.0030	0.0095	0.7598	0.1193
TSHR	rs7152963	14	81594028	A	0.215	0.0123	0.0089	0.1759	0.7547	-0.0096	0.0103	0.3620	0.4413
TSHR	rs59334515	14	81594143	T	0.214	0.0122	0.0089	0.1807	0.7431	-0.0118	0.0104	0.2687	0.5707
TSHR	rs3783938	14	81594380	T	0.081	0.0044	0.0146	0.7666	0.1155	0.0185	0.0168	0.2819	0.5499
TSHR	rs58884826	14	81594750	A	0.079	0.0032	0.0150	0.8340	0.0788	0.0178	0.0172	0.3120	0.5058
TSHR	rs57632490	14	81595110	T	0.123	0.0117	0.0116	0.3235	0.4901	-0.0049	0.0134	0.7218	0.1416
TSHR	rs8022675	14	81595359	C	0.295	0.0112	0.0082	0.1792	0.7466	-0.0035	0.0095	0.7183	0.1437
TSHR	rs8021423	14	81595369	T	0.294	0.0111	0.0085	0.2016	0.6955	-0.0045	0.0095	0.6441	0.1910
TSHR	rs60832047	14	81595628	T	0.294	0.0121	0.0082	0.1520	0.8183	-0.0043	0.0095	0.6613	0.1796
TSHR	rs59395915	14	81595671	G	0.294	0.0111	0.0082	0.1863	0.7298	-0.0043	0.0095	0.6593	0.1809
TSHR	rs10140322	14	81595928	C	0.352	0.0120	0.0078	0.1330	0.8762	-0.0037	0.0090	0.6891	0.1617
TSHR	rs57548236	14	81596270	T	0.294	0.0120	0.0082	0.1555	0.8083	-0.0042	0.0095	0.6653	0.1770
TSHR	rs11848926	14	81596553	G	0.056	0.0006	0.0153	0.9716	0.0125	-0.0050	0.0171	0.7735	0.1115

Supplementary Table 1. Continued.

Gene	SNP	Chrom	Position	Minor allele	MAF	Femoral neck BMD				Lumbar spine BMD			
						Beta	SE	P-value	-log (P-value)	Beta	SE	P-value	-log (P-value)
TSHR	rs2122253	14	81127338	G	0.149	-0.0133	0.0108	0.2290	0.6401	-0.0057	0.0126	0.6620	0.1792
TSHR	rs59500227	14	81597547	C	0.124	0.0134	0.0115	0.2549	0.5936	-0.0031	0.0133	0.8185	0.0870
TSHR	rs78851612	14	81597714	A	0.079	0.0043	0.0150	0.7800	0.1079	0.0204	0.0173	0.2477	0.6061
TSHR	rs57800782	14	81598055	T	0.175	0.0094	0.0098	0.3498	0.4561	0.0063	0.0114	0.5909	0.2285
TSHR	rs7157307	14	81598096	C	0.063	-0.0022	0.0146	0.8817	0.0547	-0.0083	0.0164	0.6229	0.2056
TSHR	rs34871525	14	81598677	C	0.071	0.0004	0.0132	0.9769	0.0102	-0.0149	0.0155	0.3447	0.4625
TSHR	rs930099	14	81599511	A	0.155	0.0000	0.0101	0.9996	0.0002	-0.0034	0.0117	0.7804	0.1077
TSHR	rs66489957	14	81600204	C	0.255	0.0081	0.0087	0.3657	0.4369	0.0095	0.0101	0.3585	0.4455
TSHR	rs7157900	14	81600351	A	0.227	0.0064	0.0087	0.4758	0.3226	-0.0177	0.0102	0.0882	1.0546
TSHR	rs35933410	14	81601930	T	0.071	0.0021	0.0131	0.8773	0.0568	-0.0112	0.0153	0.4735	0.3247
TSHR	rs12891336	14	81603033	A	0.071	0.0025	0.0131	0.8506	0.0703	-0.0103	0.0153	0.5109	0.2917
TSHR	rs1957547	14	81604138	G	0.371	0.0119	0.0079	0.1424	0.8464	-0.0021	0.0088	0.8135	0.0897
TSHR	rs2300538	14	81607233	G	0.360	0.0070	0.0077	0.3788	0.4216	-0.0022	0.0090	0.8075	0.0929
TSHR	rs2300539	14	81607364	G	0.365	0.0059	0.0077	0.4580	0.3392	-0.0051	0.0090	0.5771	0.2387
TSHR	rs733235	14	81607494	G	0.288	0.0066	0.0083	0.4348	0.3617	0.0007	0.0096	0.9460	0.0241
TSHR	rs733234	14	81607661	C	0.290	0.0062	0.0083	0.4653	0.3323	0.0003	0.0096	0.9739	0.0115
TSHR	rs733236	14	81607707	C	0.290	0.0066	0.0083	0.4349	0.3616	0.0003	0.0096	0.9725	0.0121
TSHR	rs2300540	14	81607731	A	0.290	0.0066	0.0083	0.4380	0.3585	0.0004	0.0096	0.9681	0.0141
TSHR	rs2300541	14	81607830	T	0.177	0.0080	0.0101	0.4364	0.3601	0.0055	0.0113	0.6361	0.1965
TSHR	rs2300542	14	81607930	C	0.290	0.0065	0.0083	0.4416	0.3550	0.0003	0.0096	0.9751	0.0109
TSHR	rs17630128	14	81611128	C	0.301	0.0016	0.0086	0.8549	0.0681	0.0021	0.0097	0.8318	0.0800
TSHR	rs2288493	14	81611606	T	0.193	-0.0003	0.0099	0.9740	0.0114	0.0000	0.0110	0.9986	0.0006
TSHR	rs2288495	14	81611919	C	0.494	-0.0089	0.0075	0.2429	0.6146	0.0041	0.0087	0.6435	0.1914
TSHR	rs2288496	14	81612114	C	0.494	-0.0064	0.0077	0.4161	0.3808	0.0053	0.0087	0.5479	0.2613

Supplementary Table 1. Continued.

Gene	SNP	Chrom	Position	Minor allele	MAF	Femoral neck BMD				Lumbar spine BMD			
						Beta	SE	P-value	-log (P-value)	Beta	SE	P-value	-log (P-value)
TSHR	rs2122253	14	81127338	G	0.149	-0.0133	0.0108	0.2290	0.6401	-0.0057	0.0126	0.6620	0.1792
TSHR	rs8022931	14	81613138	G	0.484	-0.0140	0.0075	0.0662	1.1790	0.0029	0.0087	0.7470	0.1267
TSHR	rs73342247	14	81613418	T	0.075	0.0056	0.0131	0.6763	0.1698	-0.0140	0.0153	0.3733	0.4280
TSHR	rs12883574	14	81614148	C	0.075	0.0036	0.0132	0.7892	0.1028	-0.0142	0.0154	0.3683	0.4338
TSHR	rs12884573	14	81614177	A	0.076	0.0048	0.0132	0.7209	0.1421	-0.0126	0.0154	0.4245	0.3721
TSHR	rs36022732	14	81614486	A	0.075	0.0062	0.0132	0.6478	0.1885	-0.0121	0.0155	0.4447	0.3519
TSHR	rs12883801	14	81614514	G	0.490	-0.0148	0.0074	0.0520	1.2841	0.0024	0.0087	0.7886	0.1032
TSHR	rs11846779	14	81615734	T	0.268	-0.0021	0.0088	0.8143	0.0892	0.0038	0.0098	0.7059	0.1512
TSHR	rs72691643	14	81616471	G	0.111	0.0143	0.0127	0.2693	0.5697	0.0102	0.0147	0.4977	0.3031
TSHR	rs11622435	14	81617996	A	0.055	0.0054	0.0189	0.7814	0.1071	0.0122	0.0218	0.5855	0.2324
TSHR	rs55893992	14	81618618	A	0.055	0.0031	0.0189	0.8732	0.0589	0.0133	0.0218	0.5507	0.2591
TSHR	rs2371562	14	81619474	A	0.232	-0.0001	0.0087	0.9890	0.0048	0.0074	0.0102	0.4801	0.3187
TSHR	rs741651	14	81619569	C	0.055	0.0003	0.0187	0.9881	0.0052	0.0155	0.0215	0.4813	0.3176
TSHR	rs12893151	14	81619945	A	0.204	0.0162	0.0093	0.0884	1.0538	0.0085	0.0108	0.4434	0.3532
TSHR	rs7150551	14	81620453	A	0.264	0.0005	0.0084	0.9580	0.0186	0.0046	0.0098	0.6460	0.1897
TSHR	rs28441485	14	81620883	T	0.475	-0.0110	0.0076	0.1569	0.8043	-0.0109	0.0088	0.2272	0.6436
TSHR	rs12590557	14	81621664	C	0.267	0.0000	0.0084	0.9981	0.0008	0.0048	0.0098	0.6290	0.2013
TSHR	rs11620837	14	81622342	T	0.475	-0.0115	0.0076	0.1394	0.8558	-0.0124	0.0088	0.1703	0.7687
TSHR	rs61980878	14	81622650	A	0.475	-0.0109	0.0076	0.1608	0.7936	-0.0108	0.0088	0.2303	0.6377
TSHR	rs10130445	14	81622935	G	0.267	0.0007	0.0084	0.9354	0.0290	0.0055	0.0097	0.5798	0.2367
TSHR	rs58236569	14	81624520	C	0.055	0.0033	0.0189	0.8657	0.0626	0.0140	0.0218	0.5312	0.2748
TSHR	rs77435835	14	81625045	G	0.055	0.0044	0.0190	0.8222	0.0850	0.0145	0.0218	0.5158	0.2875
TSHR	rs11844076	14	81629000	A	0.475	-0.0122	0.0076	0.1146	0.9410	-0.0110	0.0088	0.2223	0.6530
TSHR	rs1885602	14	81629996	T	0.230	-0.0024	0.0087	0.7834	0.1060	0.0004	0.0101	0.9670	0.0146

Supplementary Table 1. Continued.

Gene	SNP	Chrom	Position	Minor allele	MAF	Femoral neck BMD				Lumbar spine BMD			
						Beta	SE	P-value	-log (P-value)	Beta	SE	P-value	-log (P-value)
TSHR	rs2122253	14	81127338	G	0.149	-0.0133	0.0108	0.2290	0.6401	-0.0057	0.0126	0.6620	0.1792
TSHR	rs12372876	14	81630194	T	0.475	-0.0109	0.0075	0.1568	0.8047	-0.0104	0.0088	0.2460	0.6090
TSHR	rs72695720	14	81635105	C	0.060	0.0059	0.0171	0.7367	0.1327	-0.0276	0.0199	0.1766	0.7529
TSHR	rs10131728	14	81635323	T	0.077	0.0142	0.0125	0.2661	0.5749	-0.0074	0.0146	0.6217	0.2064
TSHR	rs57436191	14	81635329	A	0.112	0.0130	0.0123	0.2999	0.5231	0.0145	0.0142	0.3182	0.4973
TSHR	rs9646167	14	81635888	T	0.450	-0.0105	0.0076	0.1723	0.7637	-0.0088	0.0088	0.3273	0.4850
TSHR	rs12883532	14	81635945	T	0.077	0.0136	0.0125	0.2872	0.5417	-0.0082	0.0146	0.5833	0.2341
TSHR	rs1864167	14	81637074	A	0.445	-0.0082	0.0075	0.2879	0.5408	-0.0067	0.0088	0.4530	0.3439
TSHR	rs11159493	14	81639159	T	0.443	-0.0081	0.0075	0.2954	0.5295	-0.0063	0.0088	0.4839	0.3153
TSHR	rs1957546	14	81639984	C	0.220	-0.0036	0.0090	0.6950	0.1580	0.0016	0.0105	0.8794	0.0558
TSHR	rs7143837	14	81640722	T	0.111	0.0110	0.0122	0.3769	0.4238	0.0120	0.0141	0.4050	0.3925
TSHR	rs1885601	14	81641210	T	0.452	-0.0103	0.0075	0.1809	0.7426	-0.0062	0.0087	0.4914	0.3085
TSHR	rs8012466	14	81641464	A	0.111	0.0126	0.0122	0.3144	0.5025	0.0107	0.0141	0.4596	0.3376
TSHR	rs10483974	14	81642996	C	0.073	-0.0013	0.0151	0.9335	0.0299	0.0159	0.0178	0.3814	0.4186
TSHR	rs12878503	14	81643053	C	0.089	0.0169	0.0117	0.1562	0.8065	-0.0082	0.0136	0.5565	0.2545
TSHR	rs12878339	14	81643692	C	0.089	0.0168	0.0117	0.1594	0.7976	-0.0077	0.0136	0.5802	0.2364
TSHR	rs4899786	14	81643800	T	0.219	-0.0001	0.0095	0.9928	0.0031	0.0031	0.0109	0.7791	0.1084
TSHR	rs67933866	14	81647254	C	0.111	0.0125	0.0122	0.3172	0.4987	0.0116	0.0141	0.4217	0.3750
TSHR	rs8007841	14	81647878	T	0.309	0.0055	0.0079	0.4997	0.3013	-0.0019	0.0092	0.8443	0.0735
TSHR	rs17630746	14	81648560	A	0.089	0.0172	0.0117	0.1502	0.8232	-0.0072	0.0136	0.6082	0.2160
TSHR	rs61980891	14	81652878	T	0.111	0.0127	0.0122	0.3095	0.5093	0.0113	0.0141	0.4326	0.3639
TSHR	rs12147118	14	81652975	C	0.311	0.0052	0.0079	0.5227	0.2818	-0.0021	0.0092	0.8254	0.0833
TSHR	rs61980892	14	81653897	C	0.111	0.0126	0.0122	0.3136	0.5037	0.0125	0.0141	0.3856	0.4139
TSHR	rs61980893	14	81653951	C	0.111	0.0128	0.0122	0.3035	0.5178	0.0121	0.0141	0.4037	0.3940

Supplementary Table 1. Continued.

Gene	SNP	Chrom	Position	Minor allele	MAF	Femoral neck BMD				Lumbar spine BMD			
						Beta	SE	P-value	-log (P-value)	Beta	SE	P-value	-log (P-value)
TSHR	rs2122253	14	81127338	G	0.149	-0.0133	0.0108	0.2290	0.6401	-0.0057	0.0126	0.6620	0.1792
TSHR	rs12891828	14	81654524	C	0.089	0.0173	0.0117	0.1473	0.8317	-0.0085	0.0136	0.5422	0.2659
TSHR	rs8016352	14	81654875	C	0.220	-0.0034	0.0090	0.7120	0.1475	0.0021	0.0105	0.8485	0.0714
TSHR	rs111974404	14	81656011	C	0.111	0.0133	0.0122	0.2841	0.5466	0.0123	0.0141	0.3935	0.4051
TSHR	rs61980894	14	81656376	G	0.111	0.0124	0.0122	0.3192	0.4959	0.0119	0.0141	0.4104	0.3868
TSHR	rs7144005	14	81656437	C	0.219	-0.0039	0.0090	0.6731	0.1719	0.0021	0.0105	0.8456	0.0728
TSHR	rs7149672	14	81657336	A	0.309	0.0053	0.0079	0.5158	0.2875	-0.0027	0.0092	0.7729	0.1119
TSHR	rs8014406	14	81657760	T	0.219	-0.0031	0.0090	0.7317	0.1356	0.0025	0.0105	0.8179	0.0873
TSHR	rs2288497	14	81659731	A	0.454	-0.0102	0.0075	0.1832	0.7370	-0.0050	0.0087	0.5799	0.2366
TSHR	rs2288498	14	81660710	T	0.219	-0.0037	0.0090	0.6836	0.1652	0.0023	0.0105	0.8333	0.0792
TSHR	rs17111574	14	81660837	G	0.111	0.0119	0.0122	0.3391	0.4697	0.0110	0.0141	0.4466	0.3501
TSHR	rs2288499	14	81660997	T	0.453	-0.0096	0.0075	0.2103	0.6771	-0.0050	0.0087	0.5774	0.2386
TSHR	rs12879576	14	81661239	A	0.089	0.0166	0.0117	0.1636	0.7862	-0.0097	0.0136	0.4853	0.3140
TSHR	rs12896266	14	82190689	T	0.230	0.0072	0.0081	0.3823	0.4176	0.0080	0.0094	0.4059	0.3916
TSHR	rs12881438	14	82267267	G	0.205	0.0060	0.0085	0.4860	0.3134	0.0062	0.0099	0.5385	0.2688
TSHR	rs71416876	14	82273645	T	0.205	0.0054	0.0085	0.5364	0.2705	0.0058	0.0099	0.5667	0.2466
TSHR	rs12893820	14	82290191	T	0.322	0.0068	0.0078	0.3908	0.4080	0.0080	0.0090	0.3848	0.4148

Abbreviations: SNP, single nucleotide polymorphism; Chrom, chromosome, MAF, minor allele frequency; BMD, bone mineral density; SE, standard error. Data presented as beta coefficients per minor allele.





# CHAPTER 6

Thyroid Function and Risk of Anemia:  
A Multivariable-Adjusted and Mendelian  
Randomization Analysis in the UK Biobank

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*J Clin Endocrinol Metab.* 2022 Jan 18;107(2):e643-e652.

## ABSTRACT

**Context:** Thyroid dysfunction is associated with higher anemia prevalence, although causality remains unclear.

**Objective:** This study aimed to investigate the association between thyroid function and anemia.

**Methods:** This cross-sectional and Mendelian randomization study included 445 482 European participants from the UK Biobank (mean age 56.77 years (SD 8.0); and 54.2% women). Self-reported clinical diagnosis of hypothyroidism was stated by 21 860 (4.9%); self-reported clinical diagnosis of hyperthyroidism by 3431 (0.8%). Anemia, defined as hemoglobin level of < 13 g/dL in men and < 12 g/dL in women, was present in 18 717 (4.2%) participants.

**Results:** In cross-sectional logistic regression analyses, self-reported clinical diagnoses of hypo- and hyperthyroidism were associated with higher odds of anemia (OR 1.12; 95%CI, 1.05-1.19 and OR 1.09; 95%CI, 0.91-1.30), although with wide confidence intervals for hyperthyroidism. We did not observe an association of higher or lower genetically-influenced thyrotropin (TSH) with anemia (vs middle tertile: OR lowest tertile 0.98 [95% CI, 0.95-1.02]; highest tertile 1.02 [95% CI, 0.98-1.06]), nor of genetically-influenced free thyroxine (fT4) with anemia. Individuals with genetic variants in the *DIO3OS* gene implicated in intracellular regulation of thyroid hormones had a higher anemia risk (OR 1.05; 95% CI, 1.02-1.10); no association was observed with variants in *DIO1* or *DIO2* genes.

**Conclusion:** While self-reported clinical diagnosis of hypothyroidism was associated with higher anemia risk, we did not find evidence supporting a causal association with variation of thyroid function within the euthyroid range. However, intracellular regulation of thyroid hormones might play a role in developing anemia.

## INTRODUCTION

Anemia is a very common condition, affecting 27% of the world population <sup>1</sup>. The definition of anemia is a deficiency of healthy erythrocytes, associated with reduced circulating levels of hemoglobin (Hb); the World Health Organization has set a universal threshold for anemia at a Hb level of <13g/dL in men and <12g/dL in women <sup>2</sup>. Common causes of anemia include deficiency of iron, vitamin B12 and folate; chronic kidney disease; and inflammatory diseases <sup>3,4</sup>. Another possible cause for the development of anemia is an abnormal thyroid status <sup>5</sup>.

Regulation of thyroid hormone availability is complex and occurs both centrally (hypothalamus-pituitary-thyroid [HPT] axis) and locally (by differential expression of thyroid hormone transporters, deiodinases, and nuclear thyroid hormone receptors, as well as transcriptional co-repressors and co-activators) <sup>6</sup>. Under physiological conditions, both circulating thyroid-stimulating hormone (TSH) and free thyroxine (fT4) concentrations are regulated through negative feedback by the HPT axis. In target tissues, deiodinases can activate or deactivate thyroid hormones: deiodinase type 1 and 2 (D1 and D2) can convert the prohormone T4 into the active hormone triiodothyronine (T3), while deiodinase type 3 (D3) can convert T4 into inactive reverse T3 <sup>7</sup>. The central regulation may fail in the presence of thyroid gland pathology, causing either hypothyroidism (biochemically characterized by low fT4 and elevated TSH) <sup>8</sup> or hyperthyroidism (characterized by elevated fT4 and low TSH) <sup>9</sup>.

The production of sufficient numbers of differentiated red blood cells from hematopoietic progenitor cells is dependent on a delicate balance between proliferation and differentiation of progenitor cells. In addition to erythropoietin, interleukin 3, stem cell factor, and insulin-like growth factor 1, thyroid hormone has been implicated in erythropoiesis. Patients with resistance to thyroid hormone  $\alpha$  (RTH $\alpha$ ) have mild anemia. Also, animal studies suggest a role for thyroid hormone and thyroid hormone receptor (TR) $\alpha$  in erythropoiesis. Similarly, mice expressing a mutated TR $\alpha$ 1 display anemia and reduced erythropoiesis. In wild-type mice, thyroid hormone stimulates the differentiation of progenitors to mature erythrocytes. In line with this, a reduction of mature erythrocytes was observed in TR $\alpha$ 1 mutant mice <sup>10</sup>. In addition, data from ex vivo cultures from RTH $\alpha$  patients suggest that thyroid hormone resistance results in a disturbed balance between proliferation and differentiation of erythrocytic progenitors, which may contribute to anemia <sup>11</sup>. Multiple studies have suggested an association between thyroid dysfunction and anemia, including a possible U-shaped relationship between thyroid hormone levels and anemia <sup>12</sup>, but causality of these associations remain to be proven <sup>12-14</sup>. In Mendelian randomization (MR) studies, genetic variants associated with the exposure

are used as instrumental variables free from most confounding and reverse causation<sup>15</sup>. In the present MR study, we used genetic instruments for TSH and fT4 that were obtained in populations with participants within the euthyroid range as exposure, and anemia the outcome. For the weighted candidate gene analysis, we used genetic variants mapped to genes encoding deiodinases which were identified in recent genome-wide association studies (GWAS)<sup>16</sup> as associated with circulating fT4 levels. We therefore hypothesized that these variants may have functional implications in local thyroid hormone homeostasis.

## **METHODS**

To investigate whether the relationship between thyroid function and anemia is causal, we used a multi-approach strategy and performed cross-sectional multivariable-adjusted logistic regression, Mendelian Randomization (MR), and weighted candidate gene analyses. Multivariable-adjusted regression analyses were conducted to replicate previous studies regarding the association of hypo- and hyperthyroidism and anemia.

### **Study population**

The UK Biobank (UKB) cohort is a prospective general population cohort. Baseline assessments took place between 2006 and 2010 in 22 different assessment centers across the United Kingdom<sup>17</sup>. A total of 502 628 participants aged between 40 and 70 years were recruited from the general population. Invitation letters were sent to eligible adults registered to the National Health Services (NHS) and living within a 25 miles distance from one of the study assessment centers. The response rate was 5.5%<sup>18</sup>. At the study assessment center, participants completed touchscreen-based questionnaires that included topics such as sociodemographic characteristics, physical and mental health, lifestyle, and habitual food intake.

For the present study, we selected all individuals with a self-reported European ancestry, available genomics data, and data on Hb levels. As a result, we included a total sample of 445 482 individuals. The UK Biobank has approval from the NHS North West Multi-Centre Research Ethics Committee (ref 11/NW/0382). All participants from the UKB cohort provided written informed consent, and the study was approved by the medical ethics committee. The current project was completed under project number 32743.

## Exposure assessment

### *Self-reported clinical diagnosis of hypo- or hyperthyroidism*

At the assessment centre, UKB participants were asked in a verbal interview by a trained research nurse whether they had either a clinical diagnosis of hypo- or hyperthyroidism, and whether they used any medication for thyroid dysfunction<sup>17</sup>. Within this study, liothyronine (T3 suppletion), levothyroxine (T4 suppletion), antithyroid medication or a combination therapy were considered as thyroid medication (ATC code H03).

### *Selection of the genetic instruments for TSH and fT4 for the Mendelian Randomization*

We selected all independently associated single nucleotide polymorphisms (SNPs) ( $P$  value  $< 5 \times 10^{-8}$ <sup>15</sup>) for circulating TSH and fT4 levels within the reference range as instrumental variables from a previously performed genome-wide association meta-analysis<sup>16</sup>. Study-specific protocols of the studies contributing to the meta-analysis are described previously<sup>16</sup>. This genome-wide association meta-analysis, being the largest conducted in European-ancestry participants on TSH and fT4 concentrations in the euthyroid range, was performed in 71 167 European-ancestry participants from 22 different cohorts<sup>16</sup>. From this effort, we derived a total of 62 SNPs associated with TSH (explaining 9.4% of the total variance in TSH level<sup>19</sup>), and 31 SNPs associated with fT4 (explaining 4.8% of the total variance in fT4 level<sup>19</sup>). Based on the SNP effect sizes, we calculated weighted genetic risk scores for each individual included in the study.

### *Genetic risk scores based on deiodinase activity*

To explore the role of deiodinase activity in anemia implicated in intracellular regulation of thyroid hormones, we calculated separate genetic risk scores based on fT4-associated SNPs mapped to *DIO1*, *DIO2*, and *DIO3OS*. These scores were weighted to the association of each SNP with circulating fT4, as a surrogate for the magnitude of the intracellular effects.

## Anemia

For measuring blood Hb levels, blood was collected into a 4-mL EDTA vacutainer and held in temperature-controlled shipping boxes (4 °C). Complete blood cell counts were conducted using a Coulter counter. The universal definition of anemia in adults is an Hb level of  $<13$  g/dL in men and  $<12$  g/dL in women, as proposed by the World Health Organization<sup>2</sup>.

## Covariates

Low-grade systemic inflammation was considered a possible important confounder for the analyses with self-reported clinical diagnosis of hypo- and

hyperthyroidism, since inflammation affects both thyroid status <sup>20</sup> and risk of anemia <sup>21</sup> via distinct biological pathways. These analyses were therefore adjusted for factors related to low-grade systemic inflammation, notably levels of high-sensitivity C-reactive protein (hs-CRP) and the lifestyle factors smoking and alcohol intake. Smoking status was assessed via a touchscreen questionnaire inquiring whether the participant was currently smoking, past smoker or never smoker; for the present study we dichotomized smoking status into current smoker or non-smoker. Alcohol intake was also assessed by touchscreen questionnaire. All participants were asked how frequently they drank alcoholic beverages. As there is no guideline on frequency of drinking alcoholic beverages, we aimed to divide into two groups of similar size which could be interpreted as drinking more or less frequently than average. For the present population the cutoff point was up to two times per week or more than twice a week.

### **Statistical Analyses**

Participant characteristics were presented in the whole study population as well as in men and women separately as mean (SD; for normally distributed continuous data), as median (interquartile range; for nonnormally distributed data) or as proportion (for categorical variables).

We first performed multivariable-adjusted logistic regression analyses to investigate the associations between self-reported hypo- and hyperthyroidism and anemia. Two models were constructed; a minimally adjusted model comprising age and sex, and a fully adjusted model additionally comprising natural log-transformed hs-CRP, smoking status, and alcohol intake. For sensitivity purposes, the dichotomous outcome of anemia was supplemented with analyses of Hb as a continuous outcome. Similar models were constructed as above, but applied to multivariable-adjusted linear regression to assess the difference in Hb in g/dL.

Subsequently, we assessed the association of genetically influenced TSH and fT4 concentration, as instrumental variables, with anemia in the total study population. Since the previously found association, based on a large multicohort analysis, between thyroid function and anemia by Wopereis *et al.* was U-shaped <sup>12</sup>, the genetic risk scores were divided into equal-sized tertiles. With the middle group as reference, a multivariable-adjusted logistic regression model was constructed adjusted for age, sex and the first 10 principal components to correct for possible population stratification. Additional analyses were performed with the lowest group as reference. Similar models were built for the genetic risk scores for deiodinase activity, again with the middle tertile as a reference group. To assess consistency, similar models were constructed to investigate the difference in Hb in g/dL between the highest and lowest tertile of genetic

risk scores compared to the middle tertile using multivariable-adjusted linear regression.

To assess sex-specific associations, the main analyses were also performed stratified by sex and interaction analyses (on a multiplicative scale) were performed when sex differences were apparent by including an interaction term between sex and the exposure in the logistic regression model. In addition, summary-level data-based methods for MR were employed to assess whether unbalanced directional pleiotropy may have biased our analyses on genetically determined TSH and fT4 with anemia. The inverse variance-weighted (IVW) analysis provides a weighted mean estimate of the association of the individual genetic variants which assumes that none of the instruments were invalid<sup>22</sup>. In addition, the weighted median estimator (WME), MR Egger regression and MR pleiotropy residual sum and outlier (MR-PRESSO) analyses were employed, as they do take into account possible bias caused by directional pleiotropy based on different assumptions on the number of invalid instrumental variables<sup>22, 23</sup>.

The results for the multivariable logistic regression of self-reported hypo- and hyperthyroidism are presented as the estimated odds ratio (OR) of having anemia for those who reported hypo- or hyperthyroidism compared with those without thyroid disease with the accompanying 95% CI. For the multivariable logistic regression of tertiles of genetic risk score, the OR and 95% CI of having anemia are given for the 33% with the highest and the lowest genetically determined TSH and fT4 compared with the middle third. For the multivariable linear regression analyses, we present the estimated difference in Hb concentration (expressed in g/dL) between the groups with accompanying 95% CI. All analyses and data visualization were performed in R version 3.6.1<sup>24</sup> supplemented with the following packages; MRCIEU/TwoSampleMR<sup>25</sup>, rondolab/MR-PRESSO<sup>23</sup>, metafor<sup>26</sup>, ggplot2<sup>27</sup>.

## RESULTS

### Participant Characteristics

Baseline characteristics of the UKB participants are displayed in **Table 1**. Of the 445 482 participants included in this study, 241 337 (54.2%) were women. The mean age  $\pm$  SD was  $56.8 \pm 8.0$  years, which was similar in men ( $57.0 \pm 8.1$ ) and women ( $56.6 \pm 7.9$ ). Men were more frequently current smokers (12.2%) than women (8.9%), and more likely to drink alcoholic beverages more than twice a week (53.1% of men, 38.1% of women). Among women, there was a higher prevalence of both a self-reported clinical diagnosis of hypothyroidism (7.7%) and hyperthyroidism (1.2%) compared with the respective prevalence of 1.6% and 0.3% among men. In agreement with this, a higher use of T4 suppletion was

reported in women (8.8%) than in men (1.9%), as was the reported use of T3 supplementation and antithyroid medication. Mean Hb level  $\pm$  SD was  $15.0 \pm 1.0$  g/dL for men and  $13.5 \pm 1.0$  g/dL for women. A total of 18 717 (4.2%) of participants had anemia; 5907 (2.9%) were men and 12 810 (5.3%) women.

**Table 1.** Participant characteristics at the baseline visit of the UK Biobank

	All (N = 445,482)	Men (N = 204,145)	Women (N = 241,337)
Age in years (Mean $\pm$ SD)	56.8 $\pm$ 8.0	57.0 $\pm$ 8.1	56.6 $\pm$ 7.9
Smoking currently (n (%))	46,412 (10.5%)	24,903 (12.2%)	21,509 (8.9%)
Alcohol intake >2 times/week (n (%))	200,322 (45.0%)	108,334 (53.1%)	91,988 (38.1%)
hs-CRP (Median (IQR))	1.33 (0.66-2.75)	1.28 (0.66-2.55)	1.37 (0.65-2.95)
GRS TSH (Mean $\pm$ SD)	3.11 $\pm$ 0.32	3.11 $\pm$ 0.32	3.11 $\pm$ 0.32
GRS fT4 (Mean $\pm$ SD)	2.22 $\pm$ 0.21	2.22 $\pm$ 0.21	2.22 $\pm$ 0.21
Self-reported clinical diagnosis of thyroid disorder			
Hypothyroidism (n (%))	21,860 (4.9%)	3,236 (1.6%)	18,624 (7.7%)
Hyperthyroidism (n (%))	3,431 (0.8%)	627 (0.3%)	2,804 (1.2%)
Thyroid hormone supplementation			
T3 (n (%))	123 (0.03%)	15 (0.01%)	108 (0.04%)
T4 (n (%))	25,021 (5.6%)	3,914 (1.9%)	21,107 (8.8%)
Anti-thyroid medication (n (%))	383 (0.09%)	79 (0.04%)	304 (0.13%)
Hb (Mean $\pm$ SD)	14.19 $\pm$ 1.23	15.00 $\pm$ 1.02	13.51 $\pm$ 0.95
Anemia (n (%))	18,717 (4.2%)	5,907 (2.9%)	12,810 (5.3%)

Abbreviations: BMI; body mass index, GRS; genetic risk score, TSH; thyroid stimulating hormone, fT4; free thyroxine, T4; thyroxine, T3; triiodothyronine, Hb; hemoglobin. Anemia defined as hemoglobin <13 g/dL for men and hemoglobin < 12 g/dL for women.

### Associations Between Self-Reported Clinical Diagnosis of Hypo- and Hyperthyroidism With Anemia

Associations between self-reported clinical diagnosis of hypo- and hyperthyroidism and anemia are shown in **Table 2**. Self-reported clinical diagnosis of hypothyroidism was associated with higher risk of anemia (OR 1.12 [95% CI 1.05, 1.19];  $P$  value  $6.51 \times 10^{-4}$ ), independent of C-reactive protein, alcohol intake, and smoking. However, the effect estimates of these associations were stronger in men than in women ( $P$  value for interaction  $9.48 \times 10^{-15}$ ). Although analyses for self-reported clinical diagnosis hyperthyroidism showed a similar direction of effect (OR 1.09), this observation was not supported by evidence from statistical testing (95% CI, 0.91; 1.30). Similar to the analyses on self-reported clinical diagnosis of hypothyroidism, the risk estimate was slightly higher in men than in women ( $P$  value for interaction 0.09).

Results with Hb as a continuous outcome were directionally consistent for hypothyroidism (-0.02 g/dL [95% CI, -0.03; -0.01]  $P$  value  $7.09 \times 10^{-3}$ ), with a larger effect estimate in men (-0.13 g/dL [95% CI, -0.17; -0.10]) than in women (-0.02 g/dL [95% CI, -0.04; -0.01]) (**Supplementary table 1**). For hyperthyroidism, results were inconsistent with a self-reported clinical diagnosis of hyperthyroidism being associated with higher Hb in all (0.04 g/dL [95% CI, 0.01; 0.08]  $P$  value 0.03) and in women (0.05 g/dL [95% CI, 0.00; 0.08]), while with lower Hb in men (-0.03 g/dL [95% CI -0.13; 0.06]).

### Genetically Influenced TSH and fT4 in Relation to the Risk of Anemia

For the MR analyses, TSH and fT4 levels were instrumented in weighted genetic risk scores based on effect estimates of individuals SNPs derived from previous genetic meta-analyses conducted in euthyroid European participants<sup>16</sup>. In general, genetically influenced higher TSH was not associated with risk of anemia. Furthermore, neither individuals with lower genetically influenced TSH nor those with higher genetically influenced TSH (expressed as the lower and higher third of the TSH genetic risk score) had an increased risk of having anemia (compared with the middle tertile: OR for lowest tertile 0.98 [95% CI, 0.95-1.02]; highest tertile 1.02 [95% CI, 0.98-1.06]) (**Table 3**). In addition, genetically influenced fT4 above or below the reference group was not associated with anemia risk (compared with the middle tertile: OR for lowest tertile 1.00 [95% CI, 0.96-1.03]; highest tertile 0.99 [95% CI, 0.95-1.03]). Similar results were observed for men and women. When we used the group with the lowest TSH genetic risk scores as reference, individuals in the highest group had a higher risk of anemia (OR 1.04; 95% CI, 1.00-1.08), with particular evidence present in women (OR 1.05; 95% CI, 1.00-1.10). No evidence was found for fT4 and in men (**Supplementary Table 2**).

Sensitivity analyses on genetically influenced TSH or fT4 in relation to Hb did not yield any associations either (**Supplementary table 3**). Although some associations between genetically influenced fT4 and Hb were observed in either men or women only, effect estimates were very small (men with fT4 below average -0.01 g/dL, 95% CI -0.02; 0.00; women with fT4 above average 0.01 g/dL, 95% CI 0.00; 0.02).

**Table 2.** Associations between self-reported clinical diagnosis of hypothyroidism or hyperthyroidism and anemia compared to participants without a self-reported clinical diagnosis of thyroid dysfunction

	All		Men		Women		OR (95% CI)	P-value
	N exposed/ N unexposed	OR (95% CI)	N exposed/ N unexposed	P-value	N exposed/ N unexposed	P-value		
<b>Hypothyroidism</b>								
Model 1	20,980/ 421,071	1.18 (1.11;1.25)	3,103/ 200,415	1.83 (1.56;2.13)	17,877/ 220,656	1.17 (1.09;1.25)	1.17 (1.09;1.25)	4.30x10 <sup>-6</sup>
Model 2	19,959/ 401,260	1.12 (1.05;1.19)	2,942/ 190,877	1.71 (1.45;2.00)	17,017/ 210,383	1.12 (1.05;1.20)	1.12 (1.05;1.20)	1.01x10 <sup>-3</sup>
<b>Hyperthyroidism</b>								
Model 1	2,551/ 421,071	1.14 (0.95;1.35)	494/ 200,415	1.49 (0.94;2.23)	2,057/ 220,656	1.13 (0.93;1.37)	1.13 (0.93;1.37)	0.195
Model 2	2,429/ 401,260	1.09 (0.91;1.30)	466/ 190,877	1.54 (0.97;2.32)	1,963/ 210,383	1.09 (0.89;1.32)	1.09 (0.89;1.32)	0.413

All models were adjusted for age, analyses in men and women combined were additionally adjusted for sex, Model 2 was additionally adjusted for CRP, current smoking and alcohol intake more than twice a week. Abbreviations: OR; odds ratio, 95% CI; 95% confidence interval.

N exposed refers to the number of individuals included in the analysis exposed to thyroid disease, the N unexposed refers to the number of included individuals without thyroid disease. Hypothyroidism and hyperthyroidism were self-reported, anemia was defined as hemoglobin <13 g/dL for men and hemoglobin < 12 g/dL for women.

**Table 3.** Genetically-influenced thyroid status of TSH and FT4 and anemia in the UK Biobank population<sup>a</sup>

	All			Men			Women		
	OR (95% CI)	P-value		OR (95% CI)	P-value		OR (95% CI)	P-value	
<i>Genetically-influenced TSH</i>									
Lowest tertile	0.98 (0.95;1.02)	0.427		1.03 (0.96;1.10)	0.417		0.96 (0.92;1.01)	0.125	
Middle tertile	Reference			Reference			Reference		
Highest tertile	1.02 (0.98;1.06)	0.247		1.04 (0.97;1.11)	0.243		1.01 (0.97;1.06)	0.534	
<i>Genetically-influenced FT4</i>									
Lowest tertile	1.00 (0.96;1.03)	0.835		1.03 (0.96;1.10)	0.418		0.98 (0.94;1.03)	0.434	
Middle tertile	Reference			Reference			Reference		
Highest tertile	0.99 (0.95;1.03)	0.649		0.99 (0.92;1.06)	0.711		0.99 (0.95;1.04)	0.765	

All analyses were adjusted for age and the first 10 principal components to correct for possible population stratification; analyses in men and women combined were additionally adjusted for sex. Abbreviations: OR; odds ratio, 95% CI; 95% confidence interval.

<sup>a</sup>Anemia defined as hemoglobin <13 g/dL for men and hemoglobin < 12 g/dL for women

Sensitivity analyses using summary-level data-based methods for MR showed similar results, and the MR Egger intercepts and MR PRESSO distortion tests did not indicate presence of severe unbalanced directional pleiotropy except possibly for fT4 and anemia (distortion test  $P = 0.04$ ) (**Supplementary table 4, Supplementary figure 1 to 4**).

### Genetic Variation in Deiodinases and Anemia

Individuals who had genetic variants in *DIO3OS* that were associated with fT4 above or below average had a slightly higher risk of anemia than those in the average group (OR lowest tertile 1.04 [95% CI, 1.00-1.08]; highest tertile 1.05 [95% CI, 1.02-1.10]) (**Table 4**). The individual SNPs in *DIO3OS* had similar effect estimates on risk of anemia, though the width of the confidence interval differed (**Supplementary table 5**). No associations were observed with genetic variation in *DIO1* and *DIO2*. In sensitivity analyses, genetic variation in *DIO1*, *DIO2* and *DIO3OS* was not associated with Hb (**Supplementary table 6**).

**Table 4.** Genetic variation in deiodinase genes and anemia in the UK Biobank population<sup>a</sup>

	<i>DIO1</i>		<i>DIO2</i>		<i>DIO3OS</i>	
	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value
Lowest tertile	0.98 (0.95;1.02)	0.395	0.98 (0.95;1.02)	0.365	1.04 (1.00;1.08)	0.070
Middle tertile	Reference		Reference		Reference	
Highest tertile	0.98 (0.94;1.02)	0.260	1.02 (0.97;1.07)	0.459	1.05 (1.02;1.10)	0.006

All analyses were adjusted for age, analyses in men and women combined were additionally adjusted for sex. Abbreviations: OR; odds ratio, 95% CI; 95% confidence interval. <sup>a</sup>Anemia defined as hemoglobin <13 g/dL for men and hemoglobin < 12 g/dL for women

## DISCUSSION

In this study, multivariable-adjusted and Mendelian Randomization analyses were performed in a large population of individuals of European descent to examine a possible relationship between thyroid function and anemia. We found that individuals in our study population with a self-reported clinical diagnosis of hypothyroidism had a higher risk of anemia compared with those without thyroid diseases, especially in men. However, we observed no consistent evidence favoring an association between genetically influenced variation in circulating TSH and fT4 levels and anemia using multiple statistical methodologies and approaches, suggesting the observed association between the self-reported clinical diagnosis of hypo- or hyperthyroidism with anemia cannot be extrapolated to variation in thyroid function within the euthyroid range. However, in explorative

analyses specifically looking at variation in genes encoding deiodinases, a novel significant U-shaped association between variants in the *DIO3OS* gene and risk of anemia was found.

A higher risk of anemia in relation to thyroid diseases has been described previously. In the EPIC-Norfolk cohort, overt hypo- and hyperthyroidism were associated with higher risk of anemia while subclinical thyroid dysfunction was not<sup>13, 14</sup>. In an individual participant meta-analysis of 16 cohorts, hypo- and hyperthyroidism were associated with higher prevalence of anemia, and the risk was higher for those with overt thyroid dysfunction than in subclinical disease<sup>12</sup>. In the present study, we observed similar results through self-reported data on hypothyroidism, and showed that these associations are independent of (low-grade) systemic inflammation and related lifestyle factors. Adjustment for these factors did not explain the observed difference in association between men and women; it is currently unclear which mechanisms/factors contribute to the higher thyroid dysfunction-associated anemia risk in men. Speculatively, the difference in strength of the association between thyroid dysfunction and anemia could be due to differential misclassification bias or competing risk. Since men in the UK consult their GP less frequently than women with similar conditions<sup>28</sup>, it could be that men only consult a clinician with severe thyroid dysfunction while women are more likely to consult with milder thyroid dysfunction leading to a relatively more severely affected population of men with a self-reported diagnosis than for the women. Moreover, women generally have a higher risk of anemia than men, due to other causes of anemia such as blood loss during menstruation and nutritional deficiencies of iron and other crucial micronutrients<sup>1, 29</sup>. However, this is all remains speculation; exploration of these contributing factors for these sex differences was beyond the scope of the present study. Further research is required to address these sex-specific mechanisms.

Although this is the first study using Mendelian randomization to study genetically influenced TSH and fT4 levels in relation to anemia, various studies did assess this association using other research designs. Two previous cross-sectional studies also did not observe an association between circulating TSH and Hb levels in euthyroid populations, although both did find an association between low fT4 and lower Hb<sup>30, 31</sup>. In our analyses, individuals with lower genetically influenced fT4 level also had lower Hb, but the effect estimates were very small. No association was found between genetically influenced fT4 and risk of anemia.

Various explanations are possible for the observed discordance between the results from the multivariable-adjusted analyses and the MR analyses. On the one hand, it is possible that the observed association between hypothyroidism and anemia is due to confounding and selection bias of people having their thyroid

function checked. For instance, fatigue and tiredness are common reasons to have thyroid function checked. On the other hand, individuals with a diagnosis of hypo- or hyperthyroidism are usually treated, although thyroid hormone use in older people has been associated with a high frequency of thyroid hormone over- or under-replacement<sup>32,33</sup>. Consequently, self-reported thyroid dysfunction is not analogous to real (biochemical) deficit or excess of thyroid hormones, and the presence of over- and undertreatment complicate interpretation. It is likely that within the group with a diagnosis of hypo- or hyperthyroidism more individuals have a biochemical thyroid function outside the reference range than among those without such diagnosis, due to either persistent disease or over- or undertreatment. Another possibility is that people with a diagnosis of hypothyroidism who are treated with levothyroxine have a lower fT3/fT4 ratio when TSH is normalized, which may have contributed to or driven the observed association. Unfortunately, the potential causal association of fT3 or fT3/fT4 ratio and anemia could not be assessed by MR as instruments for fT3 and fT3/fT4 ratio were not available. For circulating levels of TSH and fT4 we did have genetic instruments, although only for variation within the reference range. Therefore, the MR analyses address a subtly different research question than the multivariable-adjusted regression analyses, namely the association between variation in circulating TSH and fT4 within the reference range and the risk of anemia. Potentially erythropoiesis is only hampered by true deficit or excess thyroid hormone beyond buffering capacity, which would not arise within the reference range in individuals without defects in thyroid hormone receptors or other signaling pathway constituents. In summary, differences in findings could be due to selection bias and residual confounding in the multivariable-adjusted regression analyses, or due to differences in exposures assessed by the different methods with different potential downstream effects.

The production of sufficient numbers of differentiated red blood cells from hematopoietic progenitor cells is dependent on a delicate balance between proliferation and differentiation of progenitor cells. Results from studies on ex vivo cultures from RTHa patients suggest that reduced intracellular thyroid hormone action results in a disturbed balance between proliferation and differentiation of erythrocytic progenitors, which may contribute to anemia<sup>11</sup>. To the best of our knowledge, this is the first study to report a significant U-shaped association between genetic variants in the *DIO3OS* gene and anemia risk. Although the exact biological function of *DIO3OS* remains thus far unclear, it is hypothesized that it may be involved in *DIO3* expression levels<sup>34</sup>. Temporary induction of D3 induces stem cell proliferation<sup>35</sup>, which is also required in erythropoiesis. However, no literature specifically on the role of D3 in erythrocytes or erythropoiesis was found.

Different from the thyroid hormone levels in blood, thyroid hormone deactivation by D3 might affect the risk of anemia. These findings may appear contradictory at first sight, although we hypothesize that they indicate a crucial role for local regulation of thyroid hormone availability in the development of disease. Thyroid signaling can be customized at cellular level through regulation of thyroid hormone transporters, deiodinases, and nuclear thyroid hormone receptors among others <sup>6</sup>. The intracellular exposure to T3 is thereby to some extent independent of centrally regulated TSH and fT4, which is especially important in orchestrating proliferation and differentiation of stem cells and progenitor cells <sup>7</sup>. Because of this local regulation, processes which are sensitive to thyroid hormone levels might be protected from subtle variations in circulating levels. However, when circulating thyroid hormone levels are extremely high or extremely low, these local compensatory mechanisms might no longer suffice, as illustrated previously by Bassett et al. <sup>36</sup>. In line with this, when the machinery for regulation does not work optimally (ie, because of a polymorphism in *DIO2*), local regulation is impaired and cells become more sensitive to changes in circulating levels of thyroid hormones <sup>37</sup>. Based on our results, we now hypothesize that functional genetic variants in *DIO3OS* result in a higher risk of anemia driven by a suboptimal intracellular regulation of thyroid hormone inactivation, although this hypothesis requires additional work and functional validation.

The current study has a few noteworthy strengths. First of all, the large sample size of the UK Biobank results in precise effect estimation and allows for stratified analyses even with rare exposures such as hyperthyroidism. Secondly, the combination of different approaches shed light on multiple facets of thyroid function in relation to development of anemia. Together, these new insights add to the etiological understanding of the relationship between thyroid function and anemia. There are also some limitations to the present study. There may be a degree of selection bias, as individuals who responded to the UK Biobank invitation and attended the assessments are healthier than the general population <sup>18</sup>. In addition, symptoms of anemia, such as fatigue and tiredness may have been common reasons for people to have the thyroid function checked. Furthermore, the clinical diagnosis of hypo- and hyperthyroidism were based on self-report; although the interview was conducted by a trained research nurse some misclassification cannot be excluded. Moreover, observed associations of self-reported hypo- or hyperthyroidism with anemia may have been suffered from attenuation due to normalization caused by treatment. However, thyroid hormone use in older adults has been associated with a high frequency of thyroid hormone over- or under-replacement <sup>32,33</sup>. Consequently, individuals with self-reported thyroid dysfunction are more likely than those without such diagnosis to be exposed to (biochemical) deficit or excess of thyroid hormones. Unfortunately, we were unable to prove this in the present study due to lack of data, so this

therefore remains a hypothesis. Anemia was measured objectively in blood samples taken at the visit; however, variation in blood count caused by laboratory drift cannot be ruled out as hematological assays were performed throughout the recruitment period<sup>38</sup>. However, this has likely not played a role in the analyses we present in our studies given that this increased variation is most likely caused independently from thyroid function. Finally, current analyses were restricted to participants of European ancestry, limiting the generalizability of results to other ancestral groups.

In summary, among individuals of European ancestry participating in UK Biobank, hypothyroidism was associated with a higher risk of anemia independent of inflammation and lifestyle. Genetically determined variation in circulating levels of TSH and fT4 within the reference range is not associated with anemia, although intracellular regulation of thyroid hormones via *DIO3OS* might play a role in development of anemia. Further studies are required to unravel the molecular mechanisms underlying the complex relationship between thyroid hormones and anemia risk.

## **ACKNOWLEDGEMENTS**

This research has been conducted using the UK Biobank Resource under application number 32743. We thank the study participants of the UKB for their participation.

*Financial Support:* This work was supported by the European Commission project THYRAGE (Horizon 2020 research and innovation program, 666869).

*Disclosures:* All authors declare to have no conflict of interest.

*Data Availability:* The data from UK Biobank is open source and available to researchers after acceptance of a research proposal and payment of an access fee.

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## SUPPLEMENTARY MATERIAL

**Supplementary table 1.** Associations between hypothyroidism or hyperthyroidism and hemoglobin compared to euthyroid individuals

	All		Men		Women			
	N exposed/ N unexposed	Beta (95% CI)	N exposed/ N unexposed	Beta (95% CI)	N exposed/ N unexposed	Beta (95% CI)	P-value	
Hypothyroidism								
Model 1	20,980/421,071	-0.02(-0.03;-0.01)	3,103 / 200,415	-0.14(-0.17;-0.10)	1.81x10 <sup>-13</sup>	17,877 / 220,656	-0.02(-0.04;-0.01)	2.16x10 <sup>-3</sup>
Model 2	19,959/401,260	-0.02(-0.03;-0.01)	2,942/190,877	-0.13(-0.17;-0.10)	2.20x10 <sup>-12</sup>	17,017/210,383	-0.02(-0.04;-0.01)	4.02x10 <sup>-3</sup>
Hyperthyroidism								
Model 1	2,551/421,071	0.05(0.01;0.09)	494 /200,415	-0.02(-0.11;0.06)	0.587	2,057 /220,656	0.05(0.01;0.09)	0.018
Model 2	2,429/401,260	0.04(0.01;0.08)	466/190,877	-0.03(-0.13;0.06)	0.474	1,963/210,383	0.04(0.00;0.08)	0.048

All models were adjusted for age, analyses in men and women combined were additionally adjusted for sex.

Model 2 was additionally adjusted for CRP, current smoking and alcohol intake more than twice a week. Abbreviations: 95% CI; 95% confidence interval.

N exposed refers to the number of individuals included in the analysis exposed to thyroid disease, the N unexposed refers to the number of included individuals without thyroid disease. Hypothyroidism and hyperthyroidism were self-reported, beta represents difference in Hb in g/dL.

**Supplementary table 2.** Genetically determined thyroid status of TSH and fT4 and haemoglobin levels

	All		Men		Women	
	Beta (95% CI)	P-value	Beta (95% CI)	P-value	Beta (95% CI)	P-value
TSH						
Lowest tertile	0.000 (-0.007; 0.008)	0.991	-0.003 (-0.014; 0.008)	0.573	0.003 (-0.007; 0.013)	0.559
Middle tertile	Reference		Reference		Reference	
Highest tertile	-0.004 (-0.012; 0.003)	0.290	-0.002 (-0.014; 0.009)	0.682	-0.006 (-0.015; 0.004)	0.264
fT4						
Lowest tertile	-0.007 (-0.014; 0.001)	0.07	-0.011 (-0.023; 0.000)	0.049	-0.003 (-0.013; 0.006)	0.500
Middle tertile	Reference		Reference		Reference	
Highest tertile	0.007 (0.001; 0.014)	0.08	0.003 (-0.009; 0.014)	0.640	0.010 (0.001; 0.020)	0.038

All analyses were adjusted for age, analyses in men and women combined were additionally adjusted for sex. Abbreviations: 95% CI; 95% confidence interval.

**Supplementary table 3.** Sensitivity analyses for genetically determined thyroid status and anemia and haemoglobin levels

	Anemia		Haemoglobin	
	logOdds (SE)	P-value	Beta (SE)	P-value
<i>TSH</i>				
IVW	0.017 (0.042)	0.68	-0.006 (0.019)	0.76
WME	-0.049 (0.049)	0.33	0.011 (0.010)	0.28
MR Egger				
Estimate	0.086 (0.103)	0.41	-0.047 (0.046)	0.32
Intercept	-0.005 (0.007)	0.47	0.003 (0.003)	0.34
MR-PRESSO				
Global test	.	0.008	.	<0.001
Distortion test	.	0.31	.	0.27
<i>fT4</i>				
IVW	0.009 (0.076)	0.91	0.018 (0.042)	0.66
WME	-0.009 (0.065)	0.89	0.020 (0.014)	0.14
MR Egger				
Estimate	0.175 (0.180)	0.34	-0.055 (0.099)	0.59
Intercept	-0.013 (0.013)	0.32	0.006 (0.007)	0.43
MR-PRESSO				
Global test	.	<0.001	.	<0.001
Distortion test	.	0.04	.	0.22

Abbreviations: IVW; Inverse-variance weighted, WME; Weighted Median Estimator, MR-PRESSO; Mendelian randomization pleiotropy residual sum and outlier, TSH; thyroid stimulating hormone, fT4; free thyroxine.

**Supplementary table 4.** Variation in deiodinase genes and hemoglobin

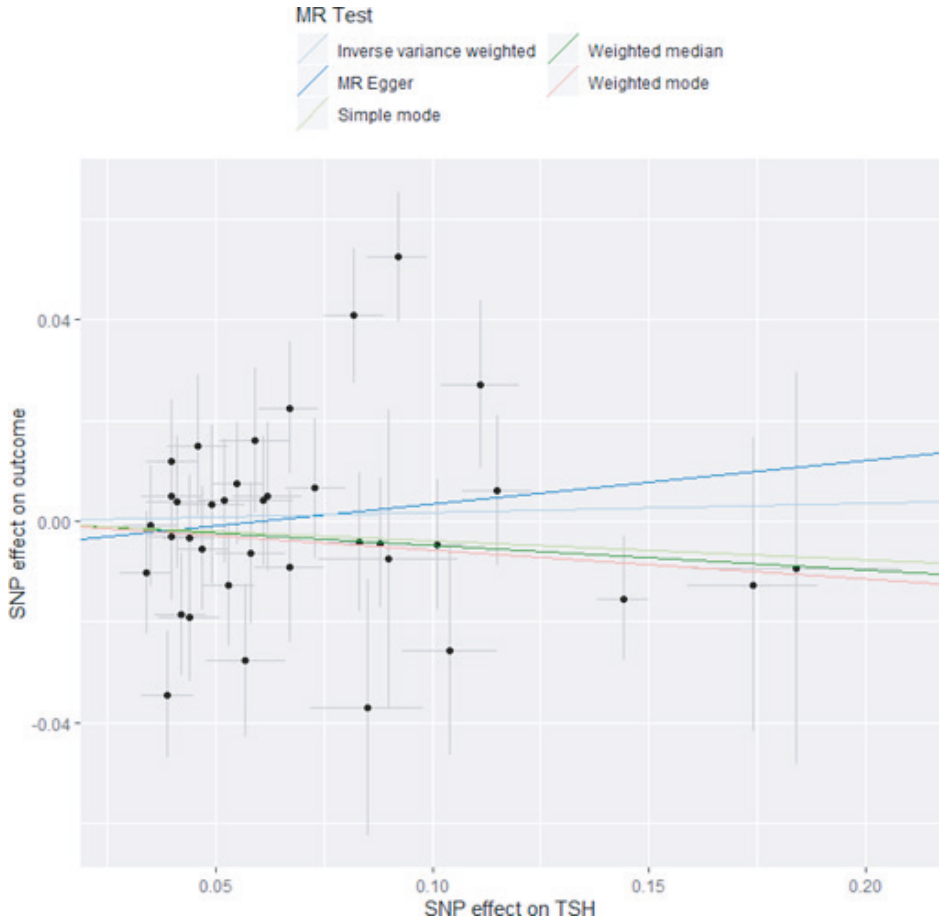
	<i>DIO1</i>			<i>DIO2</i>			<i>DIO3OS</i>		
	Beta (95% CI)	P-value	Beta (95% CI)	Beta (95% CI)	P-value	Beta (95% CI)	Beta (95% CI)	P-value	
Lowest tertile	-0.001 (-0.010; 0.006)	0.785	-0.004 (-0.003; 0.010)	0.252	-0.003 (-0.010; 0.005)	0.504			
Middle tertile	Reference		Reference		Reference				
Highest tertile	0.003 (-0.005; 0.011)	0.474	-0.009 (-0.002; 0.019)	0.098	-0.001 (-0.009; 0.006)	0.741			

All analyses were adjusted for age, analyses in men and women combined were additionally adjusted for sex. Abbreviations: OR; odds ratio, 95% CI; 95% confidence interval.

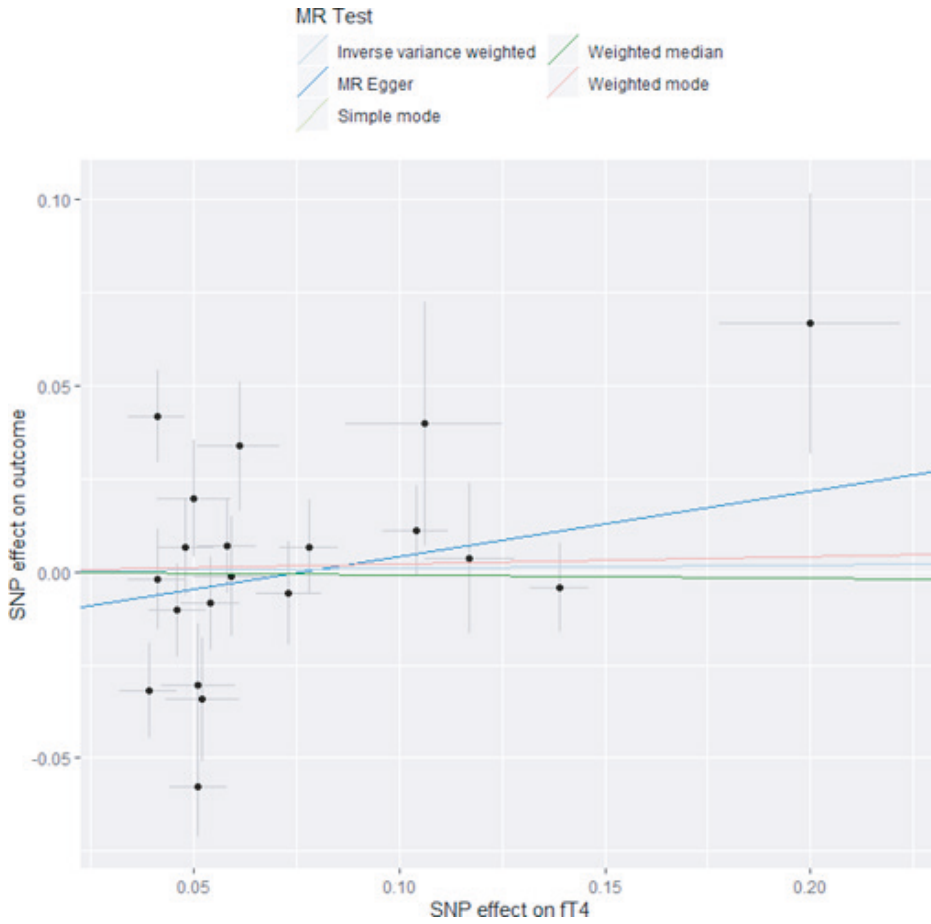
**Supplementary Table 5.** Genetically determined thyroid status of separate *DIO3OS* SNPs and anemia

	<b>rs11626434</b>			<b>rs12323871</b>		
	OR (95% CI)	P-value	OR (95% CI)	OR (95% CI)	P-value	P-value
Lowest tertile	1.03 (1.00-1.06)	0.075	1.03 (0.97-1.11)	0.345		
Middle tertile	Reference		Reference			
Highest tertile	1.04 (1.00-1.09)	0.064	1.03 (0.96-1.10)	0.403		

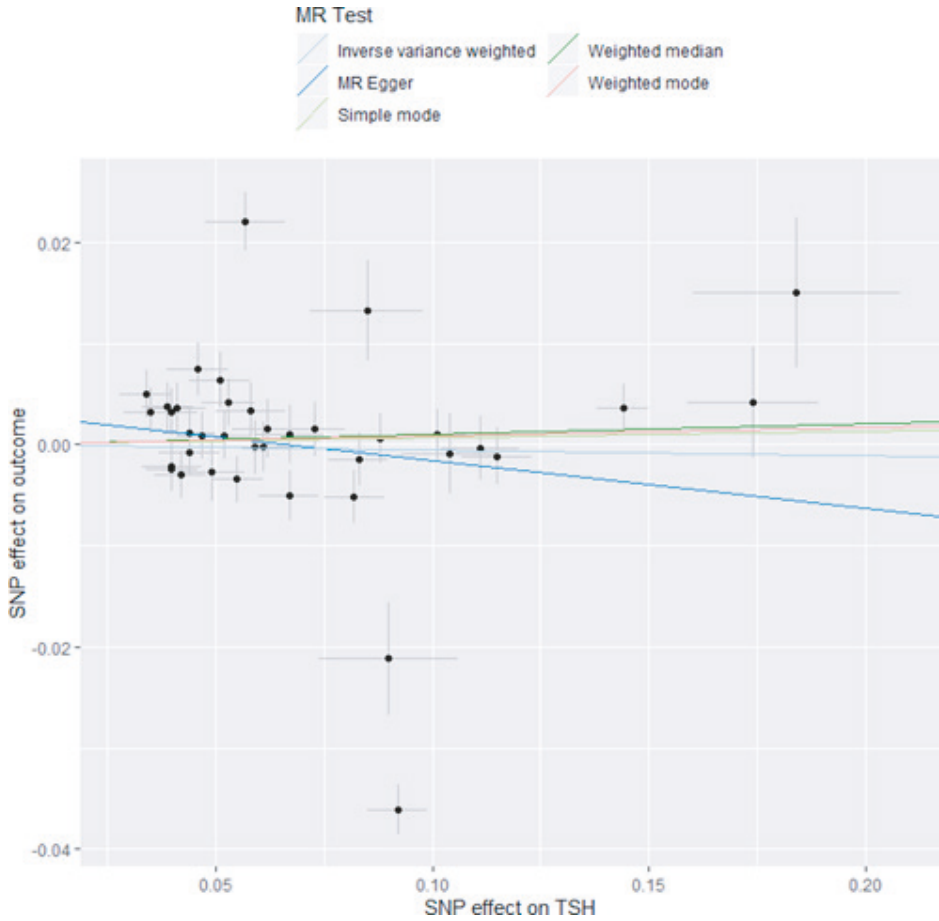
All analyses were adjusted for age and sex. Abbreviations: OR; Odds Ratio, 95% CI; 95% confidence interval.



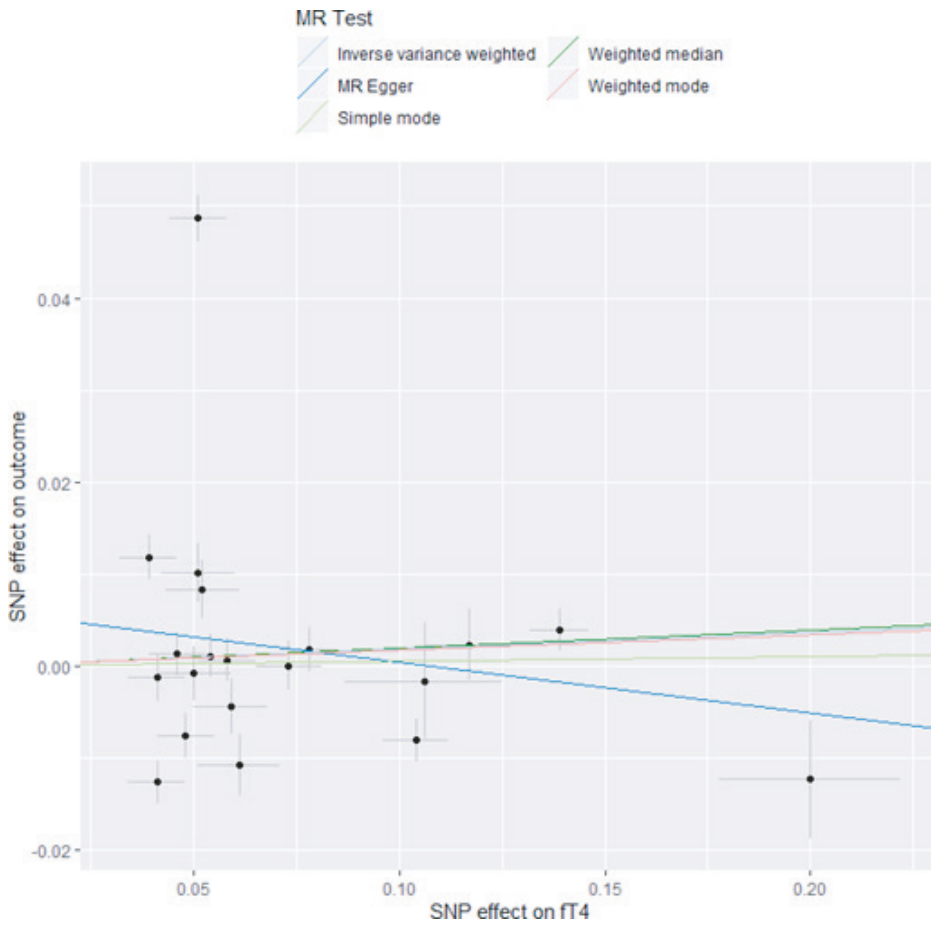
Supplementary figure 1. SNP effect on TSH and anemia



**Supplementary figure 2.** SNP effect on FT4 and anemia



Supplementary figure 3. SNP effect on TSH and Hb



**Supplementary figure 4.** SNP effect on fT4 and Hb





# CHAPTER 7

Genetically Determined Higher TSH Is  
Associated With a Lower Risk of Diabetes  
Mellitus in Individuals With Low BMI

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*J Clin Endocrinol Metab.* 2021 Jun 16;106(7):e2502-e2511.  
doi: 10.1210/clinem/dgab277.

## ABSTRACT

**Context:** Thyroid status is hypothesized to be causally related with the risk of diabetes mellitus (DM), but previous results were conflicting possibly because of a complex interaction between thyrotropin (TSH), body mass index (BMI) and DM.

**Objective:** This work aims to investigate the causal association between thyroid status with DM and glucose homeostasis and to what extent this association is dependent on BMI.

**Methods:** A mendelian randomization study was conducted of European-ancestry participants from the UK Biobank population. The present study involved 408 895 individuals (mean age 57.4 years [SD 8.0], 45.9% men), of whom 19 773 had DM. Genetic variants for circulatory TSH, free thyroxine (fT4) concentrations and BMI to calculate weighted genetic risk scores. The main outcome measures included self-reported DM-stratified analyses by BMI. Analyses were repeated for nonfasting glucose and glycated hemoglobin A<sub>1c</sub> (HbA<sub>1c</sub>) among individuals without DM.

**Results:** Genetically determined TSH and fT4 levels were not associated with risk of DM in the total UK Biobank population. However, in analyses stratified on genetically determined BMI, genetically determined higher TSH, and not fT4, was associated with a lower risk for DM only in the low BMI group (odds ratio 0.91; 95% CI, 0.85-0.98 in low BMI; *P* value for interaction = .06). Similar results were observed for glucose and HbA<sub>1c</sub> among individuals without DM.

**Conclusions:** TSH, but not fT4, is a potential causal risk factor for DM in individuals with genetically determined low BMI highlighting potential protective effects of TSH only in low-risk populations.

## INTRODUCTION

Diabetes mellitus (DM) is a major public health challenge, mainly due to increased prevalence of obesity<sup>1, 2</sup>. DM is a heterogeneous disease that is caused by different mechanisms, notably, insulin resistance in muscle, adipose tissue, and liver, and impaired pancreatic insulin secretion<sup>2</sup>.

Obesity is a major risk factor of DM development<sup>3</sup>. However, another potential risk factor for DM development is thyroid status, assessed by circulating concentrations of thyrotropin (TSH) and free thyroxine (fT4)<sup>4</sup>. Thyroid diseases, characterized by circulating TSH and/or fT4 outside the reference range, frequently coincide with DM<sup>5, 6</sup>. Moreover, associations between variation in circulating levels of TSH and fT4 and DM have been reported in observational studies, though results have been inconsistent<sup>7-9</sup>.

Studies on causal inference regarding thyroid status and DM are complicated by the complex interplay with obesity. While the association between obesity and DM is evident, associations between obesity and thyroid status are not fully understood. Observationally, obesity is associated with higher levels of TSH (and lower levels of fT4), though the direction of causation is still unclear<sup>10, 11</sup>. Contrastingly, previous Mendelian randomization studies found an association between higher genetically determined BMI and higher free 3,5,3'-triiodothyronine (fT3) concentrations, but no association with TSH or fT4 concentrations<sup>12</sup>. Furthermore, the effects of TSH on fat deposits identified in basic research (i.e., increased lipolysis and lower rates of adipogenesis) indicate that higher levels of TSH might be protective against adiposity<sup>13, 14</sup>.

Since not all confounding and causal factors are known and/or measured in observational studies, it remains unclear whether and to what extent thyroid status affects the risk of developing DM and how obesity modifies this effect. In a previous study, we used genetic instruments to ascertain causality for associations of circulating levels of TSH and fT4 with DM but did not find evidence for causality<sup>15</sup>. However, our previous study suffered from some limitations including a limited sample size and lack of sufficiently strong genetic instruments, especially for fT4.

Currently, novel genome-wide association studies (GWAS) have been performed that doubled the number of genetic instruments for TSH and more than quadrupled the number of genetic variants for fT4<sup>16</sup>. Combined with the recent availability of a large sample size of individual participant data in the UK Biobank, we can now readdress this research question in a more rigorous manner. Because the reported relationship between thyroid status and obesity

is paradoxical, the possible effect modification by BMI should be considered to study the effect of thyroid status on DM. We hypothesize that obesity has such a strong effect on the development of DM, especially type 2 DM, that it could overshadow other, more subtle potentially causal pathways, such as thyroid status. By stratification on genetically determined BMI, differential effects of thyroid status on DM can be assessed. The present study aimed to investigate the association between thyroid status and glucose homeostasis and the risk of DM in UK Biobank participants of European ancestry. In addition, we stratified our analyses based on genetically determined BMI to test our hypothesis of a possible effect modification by obesity on the association between thyroid status and DM and glucose homeostasis.

## **MATERIALS AND METHODS**

### **Study Population**

For the present study, we included all European-ancestry participants from the UK Biobank with imputed genotype data and self-reported data on DM diagnosis. Between 2006 and 2011, men and women aged 40 till 69 years living within a reasonable travelling distance of one of the 22 assessment centers in the United Kingdom were invited to participate in the UK Biobank via a population-based register<sup>17</sup>. During their visits to the assessment centers, participants completed questionnaires using a touchscreen device regarding current health and medical history. BMI was established by dividing the weight in kilograms by the height in meters squared. Participants were asked to remove their shoes and heavy clothing before weighing<sup>18</sup>. Medication use was assessed by a trained research nurse; for the present study we used reported use of levothyroxine (thyroid hormone supplementation). The UK Biobank operates within the terms of an ethics and governance framework and all participants provided signed written informed consent<sup>18,19</sup>.

### **Design**

MR uses genetic variants as instrumental variables to investigate associations free from most confounding<sup>20</sup>. Thus, the exposure is approximated based on genetic predisposition instead of an observed value. In the present study genetically determined TSH and fT4 are used as determinants, but also genetically determined BMI for stratification and effect modification. This design was described previously as a factorial MR<sup>21</sup>. Through this factorial design, the association of one risk factor (e.g., TSH) can be investigated in the absence or presence of another risk factor (e.g., high BMI). Though the genetically determined categories are not directly translatable to clinical categories, they are appropriate for etiological research such as the present study.

### Genotyping and Genetic Imputations

UK Biobank genotyping was conducted by Affymetrix using a bespoke BiLEVE Axiom array for approximately 50 000 participants; the remaining participants were genotyped using the Affymetrix UK Biobank Axiom array. Quality control was centrally executed by UK Biobank. More information on the genotyping processes can be found online (<https://www.ukbiobank.ac.uk>). Based on the genotyped single-nucleotide variations (SNVs; formerly single-nucleotide polymorphisms [SNPs]), UK Biobank resources performed centralized imputations on the autosomal SNVs using the UK10K haplotype<sup>22</sup>, 1000 Genomes Phase 3<sup>23</sup>, and Haplotype Reference Consortium (HRC) reference panels<sup>24</sup>. Autosomal SNVs were pre-phased using SHAPEIT3 and imputed using IMPUTE4. In total, approximately 96 million SNVs were imputed. Related individuals were identified by estimating kinship coefficients for all pairs of samples using only markers weakly informative of ancestral background.

### Selection of Single-Nucleotide Variations Associated With Thyrotropin, Free Thyroxine, and Body Mass Index

For this study, we selected genetic instruments from published GWASs in which the UK Biobank did not contribute. For thyroid status, we selected the lead SNVs for all genetic loci that have been shown to be independently associated with the circulating levels of TSH (42 loci) or fT4 (21 loci) ( $P < 5 \times 10^{-8}$ ) as genetic instrumental variables for TSH and fT4 levels, respectively<sup>25</sup>. To investigate the combined effect of the thyroid hormone-associated risk variants, we calculated a weighted genetic risk score (GRS) for circulating TSH or fT4. For the TSH GRS, we excluded rs13100823 mapped to *IGF2BP2*, because this locus has been associated with type 2 DM<sup>25</sup>. For the fT4 GRS, we excluded rs11039355 mapped to *FNBP4* because of its previous association with body height, BMI, and proinsulin<sup>25</sup>. In addition, we calculated a weighted GRS for BMI, for which we selected the lead SNVs for 97 BMI-associated loci<sup>26</sup>. We excluded the rs7903146 polymorphism mapped to *TCF7L2* given its pleiotropic effect on DM risk. For the present study, we considered a low and high genetically determined BMI that were defined based on the median value in the study population.

### Outcome Definition

To define cases with DM, the baseline self-reported interview data collected in the full UK Biobank population was used. All participants reporting to have DM were considered cases. Moreover, individuals were asked about their age of diagnosis and whether they used insulin or insulin analogues within the first year after diagnosis. Given the acknowledged heterogeneity among DM patients, we performed additional exploratory analyses in which we subdivided the DM cases based on the age of diagnosis (given the assumed changing pathophysiology of DM with increasing age) and insulin dependency<sup>27, 28</sup>. Using

this strategy, we homogenized the case population. These subdivisions were based on the median age of diagnosis (low/high) and the self-reported use of insulin or insulin analogues within the first year after diagnosis (yes/no). Blood samples were drawn in nonfasted state at the assessment center and stored at  $-80\text{ }^{\circ}\text{C}$ . Measurements were later performed in random batches. Glucose was measured by hexokinase analysis on a Beckman Coulter AU5800; valid baseline measurements were available for 429 557 participants. Glycated hemoglobin  $A_{1c}$  ( $\text{HbA}_{1c}$ ) was measured by high-performance liquid chromatography analysis on a Bio-Rad VARIANT II Turbo; valid baseline measurements were available for 466 492 participants. To identify occult cases of DM for sensitivity analyses, participants who did not report having DM but had either  $\text{HbA}_{1c}$  greater than or equal to 48 mmol/ml or glucose greater than 11 mmol/L were also marked as having DM. Participants who did not report having DM and had both  $\text{HbA}_{1c}$  and glucose below these thresholds were used as controls for these sensitivity analyses.

### **Statistical Analysis**

Characteristics of the study population were expressed as mean with SD for normally distributed measures, and proportions for categorical variables.

We performed multivariable logistic regression analyses to assess the association between the GRSs and DM (subtypes), and linear regression analyses were performed for the associations between the GRSs and the continuous variables glucose and  $\text{HbA}_{1c}$  adjusted for age, sex, and 4 principal components. The resulting estimate is a weighted mean estimate and reflects a SD increase of genetically determined TSH or fT4 on an odds ratio (OR) or unit (mmol/L for glucose and mmol/mL for  $\text{HbA}_{1c}$ , respectively) increase of our study outcome. To investigate possible effect modification by BMI, our analyses were additionally stratified based on the median of the GRS for BMI. Effect modification by sex was assessed by performing the main analyses stratified for men and women. To investigate whether the presence of thyroid disease could explain sex-specific association, participants using levothyroxine were omitted in sensitivity analyses. To formally test for interaction of the GRSs with BMI, we added an interaction term between the thyroid GRS and BMI GRS in their association with DM to the regression models.

All statistical analyses were performed using R statistical software version 3.5.3<sup>29</sup>. Results were reported as ORs (for dichotomous outcomes) or  $\beta$  estimates (for glucose and  $\text{HbA}_{1c}$ ) with 95% CI.

## RESULTS

### Population Characteristics

After excluding individuals lacking genetic information or those who were of non-European ancestry, this study comprised 408 895 participants with a mean age of  $57.5 \pm 8.0$  years, of which 45.9% were men. A total of 19 773 individuals (4.8%) reported a diagnosis of DM. The population characteristics of the study population are shown in **Table 1** for controls and DM cases. As compared to controls, DM cases had a higher mean age at center visit ( $60.6 \pm 6.9$  years vs  $57.3 \pm 8.0$ ), were more frequently men (61.8% vs 45.1%), and had a higher mean BMI ( $31.5 \pm 5.8$  vs  $27.2 \pm 4.6$ ). Levothyroxine use was reported by 27 084 (5.6%) participants, more commonly by those with DM than without (8.7% vs 5.4%) and more by women than men (8.6% vs 1.9%). Among the participants included in the present study, nonfasting glucose and HbA<sub>1c</sub> measurements were available for 356 598 and 389 773 participants, respectively. DM cases had a higher mean nonfasting glucose ( $7.6 \pm 3.4$  vs  $5.0 \pm 0.8$  mmol/L) and a higher mean HbA<sub>1c</sub> ( $52.4 \pm 13.7$  mmol/mL vs  $35.1 \pm 4.5$  mmol/mL).

**Table 1.** Characteristics of study population

	Controls (n=389 122)	DM (n=19 773)
Age at study visit, y	$57.3 \pm 8.0$	$60.6 \pm 6.9$
Age at diagnosis, y	-	$50.3 \pm 14.7$
Sex, No., % male	175 670 (45.1)	12 215 (61.8)
BMI	$27.2 \pm 4.6$	$31.5 \pm 5.8$
Levothyroxine use, No., %	21 176 (5.4)	1730 (8.7)
Nonfasting glucose, mmol/L	$5.0 \pm 0.8$	$7.6 \pm 3.4$
HbA <sub>1c</sub> , mmol/mL	$35.1 \pm 4.5$	$52.4 \pm 13.7$

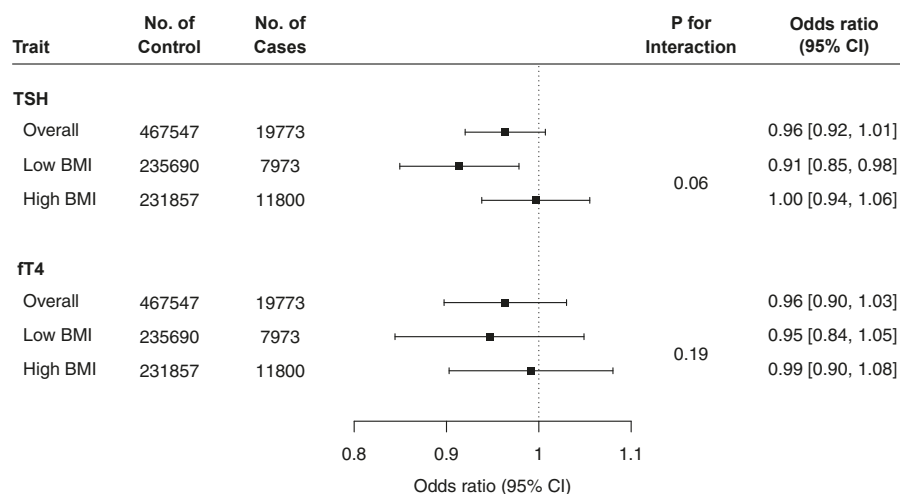
Data presented as mean with SD or as stated otherwise.

Abbreviations: BMI, body mass index; DM, diabetes mellitus; HbA<sub>1c</sub>, glycated hemoglobin A<sub>1c</sub>.

### Genetically Determined Thyrotropin and Free Thyroxine With Diabetes Mellitus

A genetically determined higher TSH was not associated with DM in the overall group (OR = 0.96 per SD increase of TSH; 95% CI, 0.92-1.01) (**Figure 1**). In the subgroup with low genetically determined BMI, a higher GRS for TSH was associated with a lower risk for DM (OR = 0.91 per SD increase of TSH; 95% CI, 0.85-0.98) (**Figure 1**). We did find suggestive evidence for an interaction between genetically determined TSH and genetically determined BMI on DM (*P* value for interaction = .06). No associations between genetically determined fT4 and DM were observed in the main group (OR = 0.96 per SD increase of fT4; 95%CI, 0.90-

1.03) and in the group with low (OR = 0.95 per SD increase of fT4; 95% CI, 0.84-1.05) and high genetically determined BMI (OR = 0.99 per SD increase of fT4; 95% CI, 0.90-1.08). Also, no formal interaction was present between genetically determined fT4 and genetically determined BMI on DM ( $P$  value for interaction = .19). When stratified by sex, higher genetically determined TSH was associated with a lower risk of DM in men with low genetically determined BMI (OR = 0.88 per SD increase of TSH; 95% CI, 0.81-0.97), but not in women (OR = 0.97 per SD increase of TSH; 95% CI, 0.86-1.08) (**Table 2**). Although the effect estimates were different, no formal interaction between genetically determined TSH and sex was observed ( $P$  value for interaction = .27). Among women, higher genetically determined fT4 was associated with a lower risk of DM (OR = 0.88 per SD increase of fT4; 95% CI, 0.79-0.98), mainly in the women with low genetically determined BMI (OR = 0.83 per SD increase of fT4; 95% CI, 0.70-0.99). Genetically determined fT4 was not associated with risk of DM in men (OR = 1.02 per SD increase of fT4; 95% CI, 0.93-1.11). Thus, the effect estimates for the associations between genetically determined fT4 and DM differed considerably between men and women, which was reflected by a low  $P$  value for interaction (.05). Omitting participants using levothyroxine did not materially change the results (data not shown). The results also did not change when 2649 occult DM cases were added to those who reported having DM (data not shown).



**Figure 1.** Associations between genetic risk score for thyrotropin (TSH) and free thyroxine (fT4) with diabetes mellitus in the overall population and stratified by genetically determined body mass index (BMI).

Odds ratio per genetically determined SD increase of TSH and fT4. Low BMI defined as genetic risk score (GRS) for BMI below the median, high BMI as GRS for BMI above the median.

**Table 2.** Associations between the genetic risk score for thyrotropin and free thyroxine with diabetes mellitus in men and women and stratified by genetically determined body mass index

	Men			Women					
	No. of controls	No. of cases	OR (95% CI)	P for interaction BMI	No. of controls	No. of cases	OR (95% CI)	P for interaction BMI	P for interaction Sex
<b>TSH</b>									
Overall	175 610	12 191	0.95 (0.89-1.00)		213 487	7580	0.99 (0.92-1.06)		.27
Low BMI	88 683	4933	0.88 (0.81-0.97)	.35	108 040	3038	0.97 (0.86-1.08)		.07
High BMI	86 927	7258	0.99 (0.92-1.07)		105 447	4542	1.00 (0.91-1.10)		
<b>ft4</b>									
Overall	175 610	12 191	1.02 (0.93-1.11)		213 487	7580	0.88 (0.79-0.98)		.05
Low BMI	88 683	4933	1.02 (0.89-1.18)	.42	108 040	3038	0.83 (0.70-0.99)		.24
High BMI	86 927	7258	1.03 (0.92-1.16)		105 447	4542	0.93 (0.81-1.07)		

OR per genetically determined SD increase of TSH and ft4. Low BMI defined as genetic risk score (GRS) for BMI below the median, high BMI as GRS for BMI above the median.

Abbreviations: BMI; body mass index, ft4; free thyroxine, OR; Odds ratio, TSH; thyrotropin.

**Table 3.** Associations between the genetic risk score for thyrotropin and free thyroxine with nonfasting glucose and glycated hemoglobin A<sub>1c</sub> stratified by genetically determined body mass index

	Overall			Low BMI			High BMI		
	TSH	ft4	TSH	ft4	TSH	ft4	TSH	ft4	
Nonfasting glucose	-0.02 (-0.03 to -0.01)	-0.00 (-0.01 to 0.01)	-0.02 (-0.03 to -0.01)	0.00 (-0.01 to 0.01)	-0.02 (-0.03 to -0.01)	0.00 (-0.01 to 0.02)	-0.02 (-0.03 to -0.01)	-0.00 (-0.02 to 0.01)	
HbA <sub>1c</sub>	-0.03 (-0.07 to 0.02)	0.02 (-0.05 to 0.09)	-0.01 (-0.07 to 0.06)	-0.00 (-0.10 to 0.10)	-0.05 (-0.11 to 0.01)	0.05 (-0.04 to 0.15)			

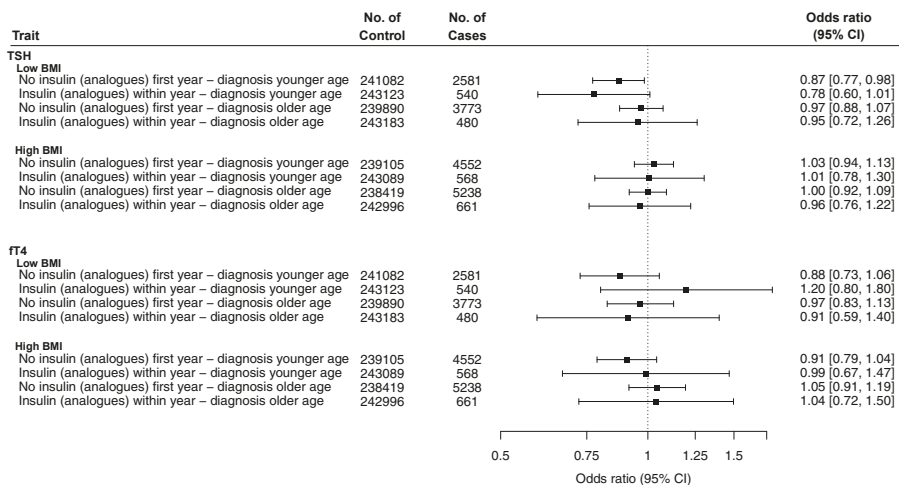
Data presented as SD increase in GRS per SD difference in outcome with accompanying 95% confidence interval. For the analyses on non-fasting glucose 339,398 individuals are included in the overall analysis, 171,513 in the low BMI group and 167,885 in the high BMI group. A total of 370,859 individuals are included in the overall analysis on HbA<sub>1c</sub>, 187,503 in the low BMI group and 183,356 in the high BMI group.

## Genetically Determined Thyrotropin and Free Thyroxine With Nonfasting Glucose and Glycated Hemoglobin A1c

In individuals without DM, a higher genetically determined TSH was associated with a lower level of nonfasting glucose ( $\beta = -0.02$  mmol/L per SD increase of TSH; 95% CI, -0.03 to -0.01) (**Table 3**). Moreover, a SD higher genetically determined TSH was associated with lower non-fasting glucose levels, in the group of individuals with a low BMI ( $\beta = -0.02$  mmol/L per SD increase of TSH; 95% CI, -0.03 to -0.01) as well as in the group of individuals with a high BMI ( $\beta = -0.02$  mmol/L per SD increase of TSH; 95% CI, -0.00 to -0.03)(**Table 3**). The results for the GRS of TSH on HbA<sub>1c</sub> showed a similar trend, although with wider CIs. Genetically determined fT4 was not associated with nonfasting glucose or HbA<sub>1c</sub>.

## Exploratory Analyses in Diabetes Mellitus Subtypes

In additional subanalyses of this study, we also explored the associations in DM subgroups based on initiation of treatment with insulin (analogues) within the first year after diagnosis and on the age at diagnosis to assess whether thyroid status was associated with the risk of particular subtypes of DM. Here we did observe an association between a genetically determined higher TSH and a lower risk for DM in patients with a genetically determined low BMI who were diagnosed at a younger age and did not require insulin (analogues) within the first year (OR = 0.87; 95% CI, 0.77- 0.98) (**Figure 2**). No other subtypes of DM were identified as associated with either genetically determined TSH or fT4.



**Figure 2.** Associations between genetic risk score for thyrotropin (TSH) and free thyroxine (fT4) with subgroups of diabetes mellitus stratified by genetically determined body mass index (BMI).

Odds ratio per genetically determined SD increase of TSH and fT4. Low BMI defined as genetic risk score (GRS) for BMI below the median, high BMI as GRS for BMI above the median. Diabetes mellitus was specified by whether insulin (analogues) were started within a year after diagnosis of diabetes mellitus and age at diagnosis below or above the median age at diagnosis.

## DISCUSSION

In this study, we examined the associations between GRSs for circulating TSH and fT4 levels with DM and glucose homeostasis. In the total European-ancestry UK Biobank population, we did not find evidence for an association between genetically determined TSH or fT4 with DM. However, when stratified based on genetically determined BMI, higher genetically determined TSH was associated with a lower risk of DM in the group with a low genetically determined BMI. This association was more pronounced in men than in women. In addition, a higher genetically determined fT4 was associated with lower risk of DM, regardless of genetically determined BMI, in women but not in men. Along these lines, higher genetically determined TSH was associated with a lower nonfasting glucose level in the overall group and those stratified based on genetically determined BMI.

A strength of this study is the use of a large sample size with a large number of DM cases, which allowed for stratified analyses based on genetically determined BMI and exploratory analyses on DM subgroups. By stratification on genetically determined BMI, the hypothesized catalyzing role of obesity was taken out of the equation, revealing the more subtle causal pathway of the hypothalamic-pituitary-thyroid (HPT) axis on DM. Certain limitations of this study also need to be addressed. The present study made use of self-reported touchscreen-based data, which might be prone to measurement error. Because measurement error is likely to be unrelated to the genetic factors (e.g., nondifferential misclassification) this likely resulted in a reduced statistical power. Indeed, sensitivity analyses that included participants with suspected DM based on their HbA<sub>1c</sub> and glucose measurements did not yield different results. Furthermore, because the genetic instrumental variables, as well as the outcome data set, originated from populations of European ancestry, our results may not be generalized to populations of non-European ancestry. In addition, the participants of the UK Biobank are a selected population who are healthier and wealthier than the general population<sup>30</sup>, therefore population characteristics are not directly generalizable. Moreover, it was not possible in the present study to account for the heterogeneity of the DM subtypes and type 1 DM (T1DM) because of the lack of a formal diagnosis for T1DM. However, a previous study showed that on average 74% of the diabetes cases diagnosed under the age of 30 and 4% over the age of 31 are likely T1DM cases<sup>31</sup>, which would correspond to an estimated total of 2060 (10.4%) cases in our study. Moreover, the use of insulin (analog) treatment within 1 year after diagnosis also gives an indication for T1DM, which amounted to 2280 (11.5%) individuals. In principle we can state that these are probable T1DM cases and that this heterogeneity in DM cases has reasonably been taken into account by means of stratification based on the use of insulin (analog) treatments. Mediation by autoimmunity could not be formally assessed

because no autoantibodies were available for this cohort. Another limitation of this study is that the role of fT3 could not be investigated. Since fT3 is the active thyroid hormone, it would add to the availability of thyroid hormone in the target tissues; we are less able to say anything about this availability in this study.

The findings of the present study add to previous research regarding the role of low thyroid status and DM onset. Several observational studies in humans observed an association of higher TSH level with a higher risk of DM<sup>6,7</sup>. However, not all observational studies showed an association between higher TSH and DM. De Vries and colleagues and Ittermann et al did not observe a relation between plasma TSH levels within the normal range and incident DM<sup>8,9</sup>. The lack of a causal association with TSH and fT4 as observed in the overall study population of the present study may suggest that previously observed associations of alterations in thyroid status and DM onset might have resulted from reverse causality and/or residual confounding. One of the potential interfering mechanisms could be reduced central sensitivity to thyroid hormones commonly seen coinciding with metabolic syndrome<sup>32</sup>. In addition, many other factors such as autoimmune disorders could cause residual confounding. Furthermore, these findings confirm our previous observations of no association between circulating TSH and fT4 and risk of DM at population level using 2-sample MR analyses with fewer instruments in a smaller study population<sup>15</sup>.

The main novel observation of the present study is the association of higher genetically determined circulating TSH levels with a lower risk of DM in individuals with a lower genetically determined BMI. Since we did not observe any association in those with a high BMI, we hypothesize that the presence of high BMI is such a strong risk factor for DM that more subtle factors of influence are overshadowed. Two main routes of action can be hypothesized; either a direct effect of TSH or an indirect route via a lower HPT axis set point. For TSH to have a direct effect on tissue function, activation of TSH receptors in target tissues is required. Extrathyroidal expression of TSH receptors has been observed in various tissues and cell types, including in orbital fibroblasts, adipose tissue, bone, skeletal muscle, thymus, and kidney<sup>33,34</sup>. TSH could exert its protective effect against DM via adipose tissue. In mice and human adipocytes, the expression of TSH receptors was demonstrated previously<sup>13,35</sup>. Adipocytes were also shown to increase lipolysis in response to stimulation with TSH in vitro and in vivo<sup>13,35</sup>. Furthermore, interaction with the insulin signalling pathway was demonstrated, leading to an inhibition of phosphoinositide 3-kinase resulting in lower rates of adipogenesis<sup>14</sup>. Thus, higher levels of TSH could potentially be protective against accumulation of adipose tissue and thereby reduce the risk of DM. Alternatively, TSH could influence glucose homeostasis through increasing insulin sensitivity and glucose uptake of skeletal muscle. Along

these lines, we describe a causal association between higher TSH levels and lower nonfasting glucose levels in this study. Moon et al have demonstrated a direct stimulatory effect of TSH on insulin receptor substrate-1 expression in muscle tissue and improved glucose tolerance<sup>36</sup>. Another potential etiological pathway could be via immunomodulation. TSH receptors were shown to be present in thymus tissue, and stimulation with TSH increased development and differentiation of T cells both in rodent and human thymal cell lines<sup>37</sup>. Hence, individuals with higher circulating levels of TSH might have a more diverse and effective adaptive immune system. Having a diverse arsenal of T cells prevents autoimmunity and other sources of low-grade inflammation<sup>38</sup>. As low-grade inflammation is a well-established causal risk factor for developing DM, any factor targeting inflammatory pathways could be a potential strategy for prevention of DM<sup>39</sup>. Higher TSH levels could be such an immunomodulating factor protecting against DM. Apart from direct effects of TSH, an indirect effect of higher TSH via a different HPT axis set point could also explain our findings. As expected from the strong inverse relationship between TSH and fT4, virtually all genetic variants for higher TSH are associated with lower circulating levels of fT4 in the original GWAS<sup>25</sup>. Therefore, our observation could be elaborated to an association of higher TSH and lower fT4, that is, a lower HPT axis set point, with a lower risk of DM. Previously, thyroid status has been linked to multiple components of glucose homeostasis. It has long been known that thyroid hormones induce hepatic gluconeogenesis<sup>40</sup>. Furthermore, thyroid hormones could affect insulin production and secretion in the pancreas<sup>41</sup>. Although thyroid hormones are required for maturation of pancreatic  $\beta$  cells, senescence is also accelerated by elevated levels of thyroid hormones in these insulin-producing cells<sup>42</sup>. Counterintuitively, we found an association only with genetically determined TSH and not fT4. Owing to the smaller number of genetic instruments for fT4 than TSH (20 loci [4.8% explained variance] vs 41 loci [9.4% explained variance]), the statistical power was not as strong for the analyses on fT4. Furthermore, although the genetic instruments for fT4 were strongly associated with circulating fT4 levels, their specific effects on intracellular thyroid signaling in different target tissues are likely heterogeneous and largely unknown.

There was some evidence for sex-specific associations. More specifically, the association of higher TSH and lower risk of DM in people with a low BMI was more pronounced in men than in women. In women, we found an association between higher genetically determined fT4 with a lower risk of DM in the whole group and a slightly stronger effect in the group with lower BMI than higher BMI. In men, this association with fT4 is completely absent. Since medication use for thyroid disease did not explain the sex difference, we hypothesize that the observed sex-specific associations are not due to differences in thyroid disease prevalence but rather differences in body composition between men and women

that are not explained by BMI. Men generally have more visceral fat than women, which increases the risk of DM and other cardiometabolic diseases more than overall body fat<sup>43</sup>. A potential mechanism for the protective mechanism of TSH is via increasing lipolysis, which might prevent the accumulation of harmful visceral fat in a more relevant magnitude in men than in women. The protective effect of higher fT4 in women and especially in those with low BMI might be related to their low muscle mass. Skeletal muscle is the largest glucose disposal tissue in the body because it aids in the storage and plays a key role in the consumption of glucose; therefore, low muscle mass may impair glucose homeostasis<sup>44</sup>. Thyroid hormones induce glucose uptake by skeletal muscles and glucose consumption<sup>45</sup>. We hypothesize that thyroid hormone effects on glucose homeostasis of skeletal muscle are only noticeable in those with relatively low muscle mass because the total capacity of larger muscle mass might already suffice. Nevertheless, all of these hypotheses are mere speculation, and more research is required into sex-specific risk factors and disease mechanisms.

Here, we specifically studied the effects of circulatory TSH and fT4 on DM onset and glucose homeostasis. However, target tissues customize intracellular thyroid hormone levels to their current needs independently of circulating levels in the blood<sup>46</sup>. Deiodinases are key players in the modulation of the availability of thyroid hormones in target tissues<sup>47</sup>. In previous research by our group, we demonstrated that genetic variation in *DIO1* may affect glucose metabolism<sup>15</sup>. This may be more reflective of target tissue levels of thyroid hormone than the circulating levels. We therefore propose that future studies should focus on the role of deiodinases and availability of thyroid hormones in target tissues on glucose homeostasis and the risk of DM.

In summary, genetically determined circulating TSH was associated with a lower risk of DM in participants with low genetically determined BMI. In line with this, a higher genetically determined TSH is associated with lower nonfasting glucose levels in participants without DM, regardless of their genetically determined BMI. Although the association with HbA<sub>1c</sub> was not statistically significant, the results were directionally consistent with the effect estimates for glucose. As they both represent parameters of glucose homeostasis and were moderately correlated ( $r = 0.40$ ), we treated them as complementary measures. We did not find evidence for a causal association between higher circulatory fT4 concentrations and any of our study outcomes. These findings may indicate that TSH levels affect glucose homeostasis and that higher TSH levels might protect against DM. Finding these associations only in subgroups with lower BMI highlights a potential protective effect of TSH only in low-risk populations.

## ACKNOWLEDGEMENTS

We express our gratitude to all individuals who participate in the UK Biobank study. The present UK Biobank project was conducted under project numbers 22474 (to R.N.) and 32743 (to D.v.H.).

*Financial Support:* Statistical analyses were performed using the facilities of the Dutch Supercomputer (Surfsara Inc; project No. 38013 to R.N.). This work was supported by the European Commission-funded project THYRAGE (Horizon 2020 Research and Innovation Program under grant agreement No. 666869 to D.v.H.).

*Author contributions:* Concept and study design: M.M.B., N.A.v.V., S.P.M., R.N., and D.v.H.; data analyses: M.M.B. and N.A.v.V.; drafting the initial versions of the manuscript: M.M.B. and N.A.v.V.; data collection: R.N.; supervision: R.N. and D.v.H.; commenting on draft versions of the manuscript: R.N., S.P.M., and D.v.H.; final approval of the manuscript: M.M.B., N.A.v.V., S.P.M., R.N., and D.v.H..

*Disclosures:* The authors have nothing to disclose

*Data availability:* Data of the UK Biobank is open to researchers ([www.ukbiobank.ac.uk](http://www.ukbiobank.ac.uk)) after acceptance of a research proposal submitted to UK Biobank recourses.

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# CHAPTER 8

Correspondence: Thyroid Hormone Therapy for Older Adults with Subclinical Hypothyroidism

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*N Engl J Med* 2017 Oct 5;377(14):e20. doi: 10.1056/NEJMc1709989.

**To the editor:**

On the basis of previous observational findings,<sup>1</sup> the TRUST trial was initiated to provide evidence as to whether subclinical hypothyroidism is causally linked to disease in old age. Despite being the largest trial to date with 737 participants, it was underpowered to address the association between hypothyroidism and cardiovascular disease, as highlighted in the Discussion section.

We conducted a two-sample mendelian randomization study with 20 genetic variants for circulating levels of thyrotropin and 3 genetic variants for circulating levels of free thyroxine<sup>2</sup> on summary data of a genomewide association study consisting of 60,801 case patients with coronary artery disease and 123,504 controls from the CARDIoGRAMplusC4D Consortium.<sup>3</sup> On the basis of statistical analyses that have been described previously,<sup>4</sup> we found no evidence for a causal relationship between circulating levels of thyrotropin or free thyroxine and the risk of coronary artery disease (odds ratio of 0.99 per standard deviation of increase in thyrotropin,  $P=0.83$ ; and odds ratio of 1.02 per standard deviation of increase in free thyroxine,  $P=0.74$ ). Thus, even if the TRUST trial had been considerably larger and had longer follow-up, it is likely that the investigators would have found no beneficial effect of levothyroxine treatment on the incidence of coronary artery disease, similar to their findings with respect to other outcomes.

**Acknowledgments**

No potential conflict of interest relevant to this letter was reported.

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# CHAPTER 9

Higher thyrotropin leads to unfavorable lipid profile and somewhat higher cardiovascular disease risk: evidence from multi-cohort Mendelian randomization and metabolomic profiling

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## ABSTRACT

*Background:* Observational studies suggest interconnections between thyroid status, metabolism, and risk of coronary artery disease (CAD), but causality remains to be proven. The present study aimed to investigate the potential causal relationship between thyroid status and cardiovascular disease and to characterize the metabolomic profile associated with thyroid status.

*Methods:* Multi-cohort two-sample Mendelian randomization (MR) was performed utilizing genome-wide significant variants as instruments for standardized thyrotropin (TSH) and free thyroxine (fT4) within the reference range. Associations between TSH and fT4 and metabolic profile were investigated in a two-stage manner: associations between TSH and fT4 and the full panel of 161 metabolomic markers were first assessed hypothesis-free, then directional consistency was assessed through Mendelian randomization, another metabolic profile platform, and in individuals with biochemically defined thyroid dysfunction.

*Results:* Circulating TSH was associated with 52/161 metabolomic markers, and fT4 levels were associated with 21/161 metabolomic markers among 9432 euthyroid individuals (median age varied from 23.0 to 75.4 years, 54.5% women). Positive associations between circulating TSH levels and concentrations of very low-density lipoprotein subclasses and components, triglycerides, and triglyceride content of lipoproteins were directionally consistent across the multivariable regression, MR, metabolomic platforms, and for individuals with hypo- and hyperthyroidism. Associations with fT4 levels inversely reflected those observed with TSH. Among 91,810 CAD cases and 656,091 controls of European ancestry, per 1-SD increase of genetically determined TSH concentration risk of CAD increased slightly, but not significantly, with an OR of 1.03 (95% CI 0.99-1.07; *p* value 0.16), whereas higher genetically determined fT4 levels were not associated with CAD risk (OR 1.00 per SD increase of fT4; 95% CI 0.96-1.04; *p* value 0.59).

*Conclusions:* Lower thyroid status leads to an unfavorable lipid profile and a somewhat increased cardiovascular disease risk.

## BACKGROUND

Hypothyroidism, defined by high thyroid stimulating hormone (TSH) and low free thyroxine (fT4) levels, and subclinical hypothyroidism, defined by high TSH and fT4 within the reference range, are associated with higher total cholesterol, low-density lipoprotein cholesterol (LDL-c) and triglyceride levels<sup>1,2</sup>, and subclinical hypothyroidism has been associated with higher coronary artery disease (CAD) risk<sup>3</sup>. However, two recent randomized placebo-controlled trials on levothyroxine treatment in older adults with subclinical hypothyroidism did not find a reduction in cardiovascular events<sup>4,5</sup>, possibly due to a lack of statistical power<sup>6</sup>.

Mendelian randomization (MR) studies<sup>7</sup> and studies using metabolomics data can further elaborate on the possible causal role of thyroid status in CAD<sup>8</sup>. Previous MR studies on thyroid status and CAD were performed in multi-ancestry populations<sup>9-12</sup>, while thyroid function<sup>13</sup>, prevalence of thyroid dysfunction<sup>13,14</sup>, and risk of myocardial infarction<sup>15</sup> all vary by ancestry. Moreover, genetic variants for thyroid parameters were discovered in European-ancestry populations only<sup>16</sup>. We hypothesized that performing MR in an exclusively European sample could provide a more accurate effect estimation. In addition, metabolomic profiling can be used as intermediate phenotype, to investigate early subclinical stages of diseases, especially when considering the lipoprotein subclasses and their contents<sup>17,18</sup>. Recently, findings from a Brazilian cohort showed already promising results showing subclinical thyroid function to be related to unfavorable lipid profile using a metabolomics platform<sup>19,20</sup>.

We aimed to investigate the potential causal role of thyroid status in cardiovascular disease by assessment of the association between TSH and fT4 levels and CAD using MR in European-ancestry cohorts. Additionally, we investigated the association between thyroid status and metabolomic profile in two stages. First, associations between TSH and fT4 concentrations within the reference range were tested for the complete panel of 161 metabolomic markers. Next, robustness of associations between TSH and fT4 and the metabolomic markers identified in stage one, was tested with MR and a different NMR-metabolomics platform. Since the multivariable-adjusted regression and MR analyses methods are sensitive to different sources of bias, residual confounding and unbalanced horizontal pleiotropy respectively, triangulation of evidence can contribute to causal inference of observational findings<sup>21</sup>. The consistency of associations with metabolomic markers was also examined in individuals with thyroid dysfunction.

## METHODS

### Study populations for multivariable-adjusted regression analyses on the metabolomic profile

We strived to include as much cohorts as possible with data on exposure and outcome being measured in European-ancestry participants. In the end, data from six European-ancestry cohorts were used for first stage analysis of circulating metabolomic marker concentrations and thyroid status; the 500 Functional Genomics Study (500FG) ( $n = 421$ )<sup>22</sup>, the Genetics, Arthrosis and Progression study (GARP) ( $n = 321$ )<sup>23</sup>, the Leiden Longevity Study (LLS) ( $n = 486$ )<sup>24</sup>, the Netherlands Study of Depression and Anxiety (NESDA) ( $n = 2906$ )<sup>25</sup>, PROSPER ( $n = 5316$ )<sup>26</sup>, and the Rotterdam Study (RS) ( $n = 1690$ )<sup>27</sup> (detailed description in **Supplementary Materials**). We used data from Study of Health in Pomerania (SHIP) as validation ( $n = 983$ ) using different metabolomic profiling methods<sup>28</sup>. Each participating study obtained written informed consent from all participants and approval from the appropriate local institutional review boards.

### Thyroid parameters for multivariable regression analyses

For the multivariable regression analyses, TSH and fT4 were measured according to a standardized protocol (See **Supplementary Materials**). For analyses on TSH and fT4 within the reference range, cohort-specific reference ranges were used after which TSH and fT4 levels were inverse normal transformed to approximate normal distribution and facilitate comparison between cohorts. Biochemical thyroid dysfunction was also based on cohort-specific reference ranges; overt hyperthyroidism was defined by TSH levels below the reference range and fT4 levels above the reference range, overt hypothyroidism was defined by either TSH  $> 20$  mIU/L or TSH below 20 mIU/L but above the reference range and fT4 below the reference range.

### Genetic instruments for TSH and fT4

Genetic instruments for TSH and fT4 concentrations were extracted from the largest genome-wide association studies (GWAS) meta-analysis on thyroid function comprising 72,167 European-ancestry participants<sup>16</sup>. A total of 62 independent single nucleotide polymorphisms (SNPs) were identified for circulating TSH (GWAS-based 9.4% explained variance) and 31 independent SNPs for circulating fT4 (GWAS-based 4.8% explained variance)<sup>16</sup> (**Supplementary Table 1**). Median F-statistics was 54 (range 32 to 576) for the TSH instruments and 43 (range 30 to 394) for the fT4 instruments.

### Outcome sources for metabolomic profile

Data for MR analyses on thyroid status and metabolomic profile were derived from four sources; MAGNETIC consortium ( $n = 24,925$ ; downloaded from:

[http://www.computationalmedicine.fi/data#NMR\\_GWAS](http://www.computationalmedicine.fi/data#NMR_GWAS))<sup>29</sup>, the Oxford Biobank ( $n = 6616$ )<sup>30</sup>, the Netherlands Epidemiology of Obesity Study ( $n = 4734$ )<sup>31</sup>, and PROspective Study of Pravastatin in the Elderly at Risk (PROSPER) ( $n = 2343$ )<sup>26</sup> (**Supplementary Materials**). Data of the MAGNETIC consortium was publicly available. For the other studies, linear regression analyses were performed between the SNPs and standardized metabolomic marker concentrations (mean = 0, SD = 1), adjusted for age, sex and up to ten principal components. Findings were validated in the Airwave Health Monitoring Study (Airwave) ( $n = 2021$ ) that used a different NMR platform<sup>32</sup>.

### **Metabolomic profile measurements for multivariable-adjusted regression and Mendelian randomization analyses**

We used metabolomic profile measurements performed on a high-throughput proton NMR platform (Nightingale Health Ltd., Helsinki, Finland)<sup>33</sup>. This method provides quantification of lipoprotein subclass profiling with lipid concentrations within 14 subclasses, fatty acid composition and other small molecules including glycolysis-related metabolites, amino acids and ketone bodies<sup>33</sup> (total 161 metabolomic markers). Out of the 161 metabolic markers, 116 were included in the GWAS of the MAGNETIC consortium. Metabolomic profiling for SHIP and Airwave was generated by Bruker IVDr Lipoprotein Subclass Analysis (B.I.-LISA; Bruker Biospin, Rheinstetten, Germany)<sup>34-36</sup>. Out of the 105 quantified lipoprotein subclasses, 57 subclasses overlapped with Nightingale. Methodological details are described in the **Supplementary Materials**.

### **Outcome sources for CAD**

For MR analyses on thyroid status and CAD, we used data from three studies with European-ancestry participants; CARDIoGRAM consortium (22,233 cases and 64,762 controls; downloaded from: <http://www.cardiogramplusc4d.org/data-downloads/>)<sup>37</sup>, UK Biobank (52,946 cases and 393,549 controls)<sup>38</sup> and FinnGen (16,631 cases and 197,780 controls; freeze 5; downloaded from: [https://www.finnngen.fi/en/access\\_results](https://www.finnngen.fi/en/access_results))<sup>39</sup> to perform MR analyses using maximum sample size and to examine consistency of the MR results across the different cohorts. Case definitions are described in the **Supplementary Materials**.

### **Statistical analyses**

For analyses on circulating metabolomic marker concentrations, values were natural log-transformed and subsequently standardized for analyses.

For the multivariable regression analyses, a prespecified analysis plan and syntax were distributed among cohorts. Population characteristics were derived as number (percentage) for categorical variables, mean and SD for normally distributed variables and median, and interquartile range (IQR) for non-normally

distributed variables. Multivariable linear regression analyses were performed locally, and summarized results were collected centrally for quality control and meta-analysis. The main analysis was adjusted for age, sex, body mass index (BMI) and smoking (current versus former or never), which were considered major confounders. Given potential heterogeneity among cohorts included in the multivariable regression analyses, the meta-analysis comprised a random-effects model according to restricted maximum likelihood (REML). Sensitivity analyses were done with participants excluded who used thyroid therapy or lipid-lowering medication (defined by ATC codes H03 and C10, respectively) or medication for treatment of diabetes mellitus (DM), reported a history of thyroid disease or DM, or had fasting glucose > 7 mmol/L. Results for the multivariable regression analyses are presented as the association between one SD higher TSH and fT4 levels within the reference range and metabolomic marker concentrations in SD with 95% CI. For associations of thyroid dysfunction, regressed differences in circulating metabolomic marker concentrations in SD are shown for overt hyper- and hypothyroid individuals compared to euthyroid individuals. Analyses in this stage were hypothesis-free, and therefore, Bonferroni correction was applied based on 37 uncorrelated metabolomic markers (as previously applied<sup>18</sup>), resulting in a two-sided  $p$  value threshold of less than  $1.34 \times 10^{-3}$  ( $0.05/37$ ). Circos plots were used to summarize and visually compare the fT4 and TSH results. Circos plots were created using EpiViz (version 0.1.0, <https://github.com/mattlee821/EpiViz/>), a Shiny web application and R package built using R (version 3.6.2) and Shiny (version 1.4.0). EpiViz adapts and builds on the Circlize<sup>40</sup> and ComplexHeatmap<sup>41</sup> R packages to create Circos plots compatible with association analysis data.

### **Mendelian randomization analyses**

Two-sample MR analyses were conducted using summary-level data from relatively independent GWAS from exposure and outcomes<sup>42</sup>. We extracted the association of each genetic variant for TSH and fT4 from summary data of GWAS for circulating metabolomic markers and CAD. All palindromic SNPs, which are SNPs with an effect allele frequency close to 0.5 in combination with alleles that correspond to nucleotides that pair with each other, were excluded prior to analyses, as being default in the TwoSampleMR package. Furthermore, to prevent overestimation of the precision of the causal effects, we excluded all SNPs in linkage disequilibrium at  $R^2 > 0.001$  from analyses as well. From each of the remaining SNPs, we calculated the explained variance (as  $(\beta \times \sqrt{(2 \times \text{minor allele frequency} \times (1 - \text{minor allele frequency}))})^2 \times 100$ ) and F-statistics (as  $(\beta / \text{standard error})^2$ ).

Our main analyses were inverse variance-weighted (IVW) analyses, which provide a weighted mean estimate of the association of the genetically determined

exposure and the outcome assuming none of the instruments were invalid using additive random effects<sup>43</sup>. We performed weighted median estimator (WME), MR Egger regression and MR pleiotropy residual sum and outlier (MR-PRESSO) analyses as sensitivity analyses to take into account possible bias caused by directional pleiotropy<sup>43, 44</sup>. MR-Egger is similar to IVW but does not force the regression line (i.e., of the SNP-thyroid status trait association on the SNP-metabolomic measure association) through the intercept. MR-Egger is statistically less efficient (providing wider confidence intervals) but provides a causal estimate (i.e., the regression slope) that is corrected for directional horizontal pleiotropy, and a non-zero intercept which is an indication of the existence of directional pleiotropy. The weighted-median estimator is valid if more than 50% of the weight of the genetic instrument is from valid variants (i.e., if one single SNP or several SNPs jointly contributing 50% or more of the weight in the MR analysis exhibit horizontal pleiotropy the calculated effect estimate may be biased). We first performed MR analyses on each dataset separately and subsequently meta-analysed the summary estimates using fixed-effects models. Effect estimates for MR analyses with metabolomic profile represent the mean difference in metabolomic marker concentration in SD per 1-SD increase in TSH and fT4 levels with 95% CI. For MR analyses on CAD, results are presented as odds ratio (OR) per 1-SD genetically determined increase in circulating TSH and fT4 levels with 95% confidence interval (CI). As all MR analyses were hypothesis-driven, a conventional two-sided *p* value of less than 0.05 was considered statistically significant.

All analyses and data visualization were performed in R version 3.6.1<sup>45</sup> supplemented with the following packages: MRCIEU/TwoSampleMR<sup>46</sup>, rondolab/MR-PRESSO<sup>44</sup>, metafor<sup>47</sup>, ggplot2<sup>48</sup> and ggforestplot<sup>49</sup>.

## RESULTS

### Associations between TSH and fT4 within the reference range and metabolomic profile

*Participant characteristics of the stage-1 cohorts.*

For the multivariable regression analyses, 11,140 adults from six cohorts were included. A total of 9432 (84.7%) were euthyroid, 194 (1.7%) had hypothyroidism, 721 (6.5%) had subclinical hypothyroidism, 263 (2.4%) had subclinical hyperthyroidism, and 54 (0.5%) had hyperthyroidism (**Supplementary Table 2**). Among euthyroid individuals, the median age varied from 23.0 to 75.4 years and 54.5% of these participants were women (**Table 1**). Median TSH levels ranged between 1.73 and 2.13 mIU/L, mean fT4 levels ranged between 15.6 to

16.4 pmol/L, thyroid medication was used by 185 individuals (2.0%) and lipid-modifying medication by 2694 individuals (28.6%).

*Stage 1 analyses.*

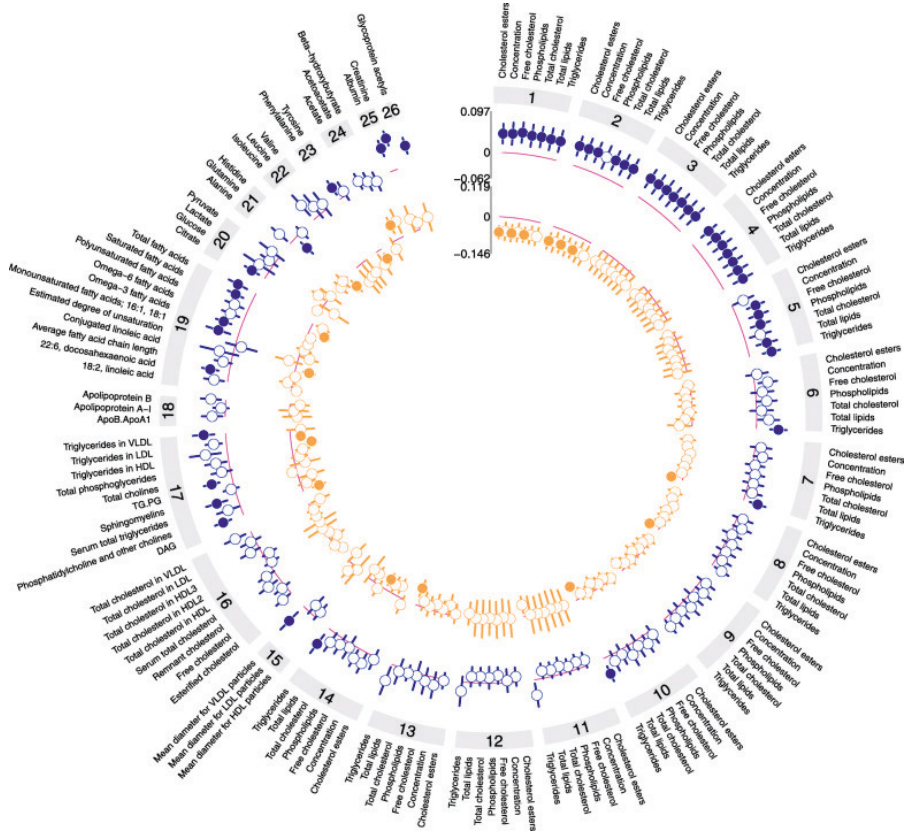
TSH levels were associated with 52/161 metabolomic marker concentrations and fT4 levels associated with 21/161 metabolomic markers (**Figure 1; Supplementary Table 3**). Higher TSH levels were predominantly associated with higher concentrations of very low-density lipoprotein (VLDL) subclasses and components, higher triglyceride concentrations, and higher triglyceride content of lipoproteins. Associations with fT4 were largely an inverse reflection of those observed with TSH. Fluid balance parameters (creatinine and albumin) appeared specific for TSH, while ketone bodies appeared specific for fT4.

**Table 1.** Population characteristics of biochemically euthyroid individuals in included cohorts (n=9432)

	<b>500 FG</b>	<b>GARP</b>	<b>LLS</b>	<b>NESDA</b>	<b>PROSPER</b>	<b>RS</b>
	<b>N = 362</b>	<b>N = 230</b>	<b>N = 419</b>	<b>N = 2467</b>	<b>N = 4513</b>	<b>N = 1441</b>
Age in years (median(IQR))	23.0 (21.0-26.0)	59.8 (55.1-65.5)	65.7 (61.8-70.4)	43.0 (30.0 – 53.0)	75.4 (72.9 – 78.3)	68.9 (65.2-73.3)
Women	200 (55.2)	181 (78.7)	202 (48.2)	1,594 (64.6)	2,195 (48.6)	773 (53.6)
Current smoker	47 (13.1) <sup>a</sup>	38 (16.5)	51 (12.2) <sup>c</sup>	984 (39.9)	1,231 (27.3) <sup>g</sup>	204 (14.2) <sup>h</sup>
BMI (median(IQR))	22.3 (20.8-24.2) <sup>b</sup>	26.0 (24.0-29.0)	26.3 (24.2 – 28.6) <sup>d</sup>	24.6 (22.0 – 28.0) <sup>f</sup>	26.2 (23.8 – 28.9) <sup>g</sup>	26.4 (24.2-29.0)
TSH (median(IQR))	2.09 (1.59-2.79)	1.76 (1.27-2.34)	2.13 (1.54 – 2.89)	2.07 (1.47 – 2.80)	1.73 (1.22 – 2.44)	1.76 (1.27-2.51)
fT4 (mean(SD))	16.4 (2.1)	15.8 (1.8)	15.6 (1.9)	15.6 (2.0)	15.6 (1.9)	15.7 (1.8)
History of diabetes mellitus	0 (0.0)	3 (1.3)	20 (6.0) <sup>e</sup>	103 (4.2)	471 (10.4)	149 (10.4) <sup>i</sup>
Lipid-lowering medication use	0 (0.0)	8 (3.5)	55 (16.6) <sup>e</sup>	184 (7.5)	2,248 (49.8)	199 (14.7) <sup>j</sup>
History of thyroid disease	0 (0.0)	N.A.	N.A.	62 (2.5)	N.A.	111 (7.7)
Thyroid medication use	0 (0.0)	3 (1.3)	7 (2.1) <sup>e</sup>	34 (1.4)	113 (2.5)	28 (1.9)
Medication use influencing the thyroid gland	0 (0.0)	N.A.	1 (0.3) <sup>e</sup>	3 (0.1)	12 (0.3)	N.A.

Results are shown as n (%) unless indicated otherwise. Abbreviations: 500 FG; 500 Functional Genomics Study, GARP; the Genetics, Arthritis and Progression study, LLS; the Leiden Longevity Study, NESDA; the Netherlands Study of Depression and Anxiety, PROSPER; PROSpective Study of Pravastatin in the Elderly at Risk, RS; the Rotterdam Study, BMI; body mass index, TSH; thyroid stimulating hormone, fT4; free thyroxin, N.A.; not available.

<sup>a</sup> Information on 360 individuals, <sup>b</sup> Information on 356 individuals, <sup>c</sup> Information on 410 individuals, <sup>d</sup> Information on 407 individuals, <sup>e</sup> Information on 331 individuals, <sup>f</sup> Information on 2,465 individuals, <sup>g</sup> Information on 4,511 individuals, <sup>h</sup> Information on 1,435 individuals, <sup>i</sup> Information on 1,438 individuals, <sup>j</sup> Information on 1,352 individuals.



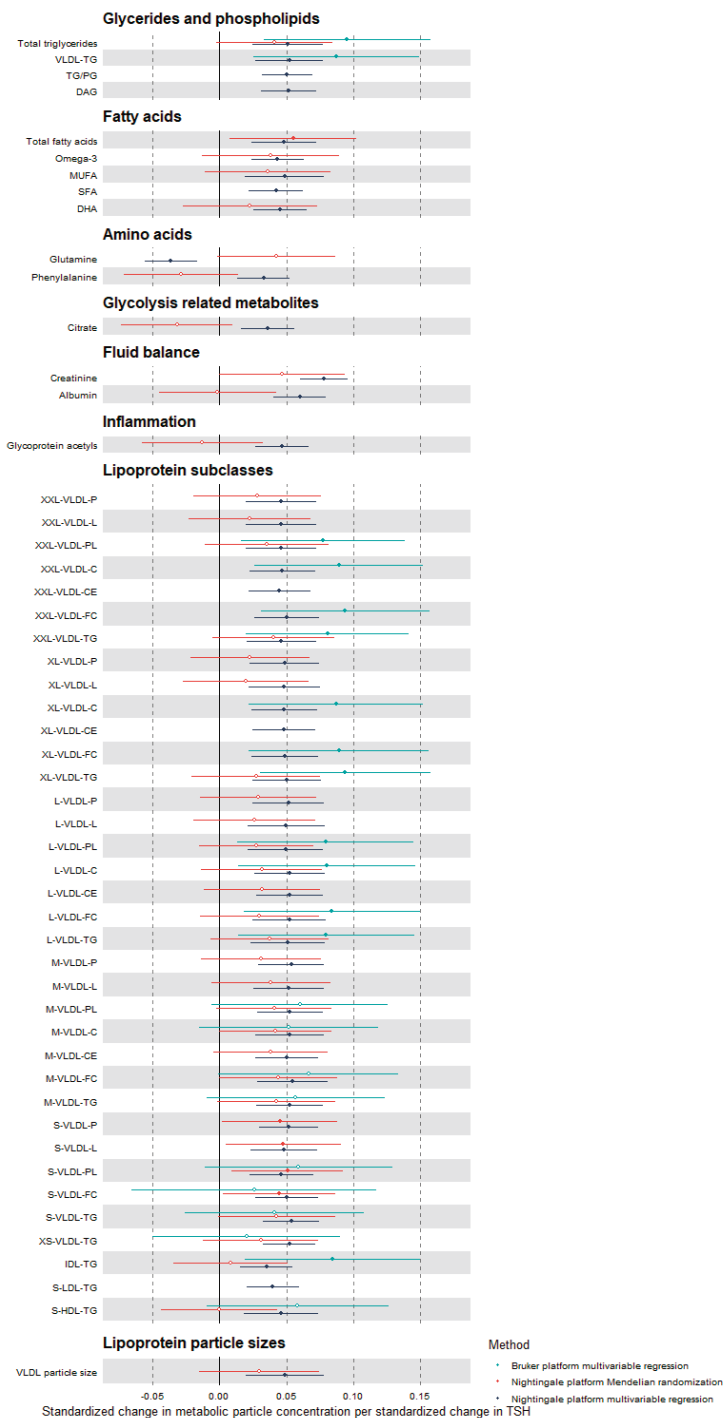
**Figure 1.** First stage associations between standardized TSH and ft4 within the reference range and 161 Nightingale platform metabolomic markers (N=9,353). Point estimates represent the standardized change in metabolomic marker concentration per standard deviation change in TSH, adjusted for age, sex, body mass index and smoking. Red bars indicate positive associations, blue bars indicate negative associations. Hollow effect estimates were not statistically significant after correction for multiple testing ( $p\text{-value} < 1.34 \times 10^{-3}$ ). 1) Extreme large VLDL. 2) Very large VLDL. 3) Large VLDL. 4) Medium VLDL. 5) Small VLDL. 6) Very small VLDL. 7) IDL. 8) Large LDL. 9) Medium LDL. 10) Small LDL. 11) Very large HDL. 12) Large HDL. 13) Medium HDL. 14) Small HDL. 15) Lipoprotein particle size. 16) Cholesterol. 17) Glycerides and phospholipids. 18) Apolipoproteins. 19) Fatty acids. 20) Glycolysis related metabolites. 21) Amino acids. 22) Branched-chain amino acids. 23) Aromatic amino acids. 24) Ketone bodies. 25) Fluid balance. 26) Inflammation. Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very-low density lipoprotein.

*Stage 2 analyses.*

For the metabolomic markers associated with TSH in the first stage, second-stage analyses with MR and/or Bruker platform were performed to assess directional consistency of the results (**Figure 2; Supplementary Table 4**). For the MR meta-analysis of TSH and metabolomic markers, 41/52 metabolomic markers identified in the first stage were available. For the majority of these (34/41), associations observed with MR and multivariable regression were directionally consistent. These markers included various subclasses of VLDL cholesterol, fatty acids and triglyceride subclasses. Inconsistent associations between MR and multivariable regression comprised associations of TSH with triglyceride content of IDL- and small HDL-cholesterol particles, albumin, various amino acids, glycolysis related metabolites and inflammatory markers. Overlapping coverage between Nightingale and Bruker was found for 23/52 of the metabolomic markers identified in the first stage that all showed comparable associations in multivariable regression analyses across both platforms.

For the metabolomic markers associated with fT4 in the first stage, second-stage analyses were performed to assess robustness (**Supplementary Figure 1; Supplementary Table 4**). Of the only 4/21 metabolomic markers from the first stage present in available genetics data, the association with acetoacetate was directionally consistent in MR, but the observations with the amino acids and triglyceride content of IDL-cholesterol were not. A total of 9/21 markers were present on both the Nightingale and Bruker platforms (e.g., VLDL cholesterol subclass, HDL and LDL triglyceride content); all showed directional consistency with similar effect estimates.

Restricting the study sample to those without thyroid or lipid-lowering medication use or metabolic disease, produced similar results as observed in our main analyses (**Supplementary Table 5**). Sensitivity analyses for MR and the MR findings on the Bruker platform were consistent with the main findings (**Supplementary Table 6**).



**Figure 2.** Second stage associations between TSH and 52 metabolomic markers using multivari-

able and Mendelian randomization analyses on Nightingale platform and multivariable analysis on Bruker platform.

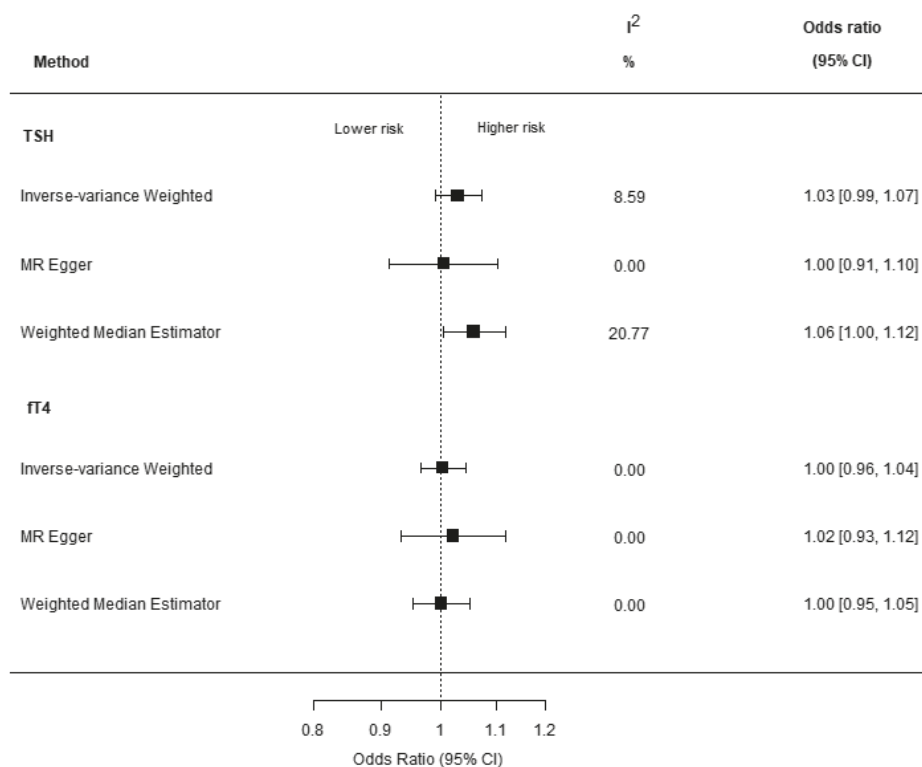
Point estimates represent the standardized change in metabolomic marker concentration per standard deviation change in TSH, error bars indicate 95% confidence intervals. Multivariable analyses were adjusted for age, sex, body mass index and smoking, Mendelian randomization analyses are inverse variance-weighted (IVW) estimate. Hollow effect estimates refer to associations with  $p$ -value  $>0.05$ .

### **Association between biochemical thyroid dysfunction and metabolomic markers identified in relation to TSH and fT4**

Consistency of the observed metabolomic profile was additionally explored in individuals with hyperthyroidism ( $n=54$ ) and hypothyroidism ( $n=194$ ). Virtually all metabolomic markers identified in the first stage analyses with TSH and fT4 were directionally consistent with hypo- and hyperthyroidism (**Supplementary Figure 2**). For TSH, 44/52 and 5/52 and for fT4, 14/21 and 2/31 metabolomic markers reached nominal significant associations ( $p < 0.05$ ) with respectively hypothyroidism and hyperthyroidism (**Supplementary Table 7**). Overall, the VLDL subclasses and components associated with TSH and fT4 within the reference range appeared to associate stronger with hypothyroidism than with hyperthyroidism.

### **Associations between genetically determined TSH and fT4 and coronary artery disease**

Within the multi-cohort MR study comprising 91,810 cases with CAD and 656,091 controls, per 1-SD increase of genetically determined TSH concentration CAD risk increased with an OR of 1.03 (95% CI 0.99-1.07;  $p$  value 0.16) (**Figure 3**). Genetically determined fT4 concentrations were not associated with CAD (OR 1.00 per 1-SD increase of genetically determined fT4; 95% CI 0.96-1.04;  $p$  value 0.89). Heterogeneity between cohorts was low; all study-level effect estimates were congruent and  $I^2 < 21.00\%$ . The MR Egger and WME were consistent with the IVW estimates (**Figure 3**), although some evidence was observed in the meta-analysis that higher TSH was associated with higher CAD risk (OR 1.06 per 1-SD increase of genetically determined TSH; 95% CI 1.00-1.10). The MR Egger intercepts did not deviate from zero and MR-PRESSO did not indicate distortion by outliers (**Supplementary Table 8**).



**Figure 3.** Associations between genetically determined standardized TSH and ft4 within the reference range and coronary artery disease (91,810 CAD cases and 656,091 controls of European ancestry). Odds Ratios (ORs) shown (per 1 s.d. increase in TSH/ft4 concentration) are inverse variance-weighted (IVW) estimate, MR Egger and weighted-median estimator, error bars indicate 95% confidence intervals.

## DISCUSSION

Using a mixed-methods approach of multi-cohort multivariable regression analysis and MR, we identified a robust metabolomic profile associated with lower thyroid status within the reference range, comprising higher concentrations of VLDL subclasses and components, higher triglyceride concentrations, and higher triglyceride content of lipoproteins. These associations were directionally consistent in patients with thyroid disorders. In addition, in this multi-cohort MR study on thyroid status and CAD in an exclusively European-ancestry population, genetically determined higher TSH concentrations were associated with a slightly higher CAD risk (particularly in the WME analysis).

As far as we are aware, this is the first multi-cohort study investigating the association between thyroid status and metabolomic profile. Although various smaller studies were performed previously, results were difficult to compare due to the variety of platforms, techniques and sample types (i.e., plasma, serum, urine)<sup>50</sup>. Nevertheless, in line with our results, most studies indicated a role of thyroid hormones in lipid metabolism. No MR studies on thyroid status parameters and detailed metabolomic profile have been published to date.

Previous multi-ancestry MR studies found no evidence for an association between thyroid status and CAD<sup>9-11</sup>. While one MR study found evidence for an association between hypothyroidism and CAD, no associations were observed between genetically determined TSH or fT4 concentrations and CAD<sup>12</sup>. We hypothesize that the difference between our and previous studies might be because we restricted our analyses to European-ancestry individuals to decrease the risk of population stratification bias. Future research should assess the extent of confounding by population stratification in MR studies on thyroid status.

Multiple mechanisms may underly the observed associations between thyroid status, CAD and metabolomic profile. An important function of thyroid hormone is to stimulate the mobilization and breakdown of cholesterol and bile acids as well as the de-novo synthesis of fatty acids and their uptake by peripheral tissues, especially oxidative tissues such as skeletal muscle, heart and liver<sup>51</sup>. Thyroid status could alter hepatic clearance of lipoproteins and reverse transport of cholesterol<sup>52</sup>. Consequently, disturbances in thyroid hormone availability and action may result in disturbances in the balance between lipid mobilization/synthesis on the one hand and uptake/clearance on the other hand (reviewed by Duntas et al. <sup>53</sup>). In case of higher TSH/lower thyroid hormone, the rate of cholesterol mobilization will be higher than the rate of its degradation, resulting in higher circulating cholesterol levels, which form a substrate for lipid peroxidation and may enhance oxidative stress as well as low grade chronic inflammation. In parallel, higher TSH/lower fT4 may also result in a decreased clearance of TG-rich lipoproteins, which may further aggravate the adverse lipid profile. Lower thyroid status could therefore result in accumulation of fatty acids in VLDL particles and free triglycerides in the circulation, resulting in the observed metabolomic profile. Interestingly, the metabolomic profile that we observed for lower thyroid status resembles that identified previously for myocardial infarction<sup>54</sup>. Therefore, a plausible pathway would be from low thyroid status via unfavorable lipid profile which could provide a substrate for oxidative stress and inflammatory processes to CAD. Although several other potential mediating factors should be considered, including endothelial dysfunction, hypertension and alterations in coagulation<sup>55</sup>.

The stronger effect of TSH compared to fT4 on CAD risk in our study should be interpreted with caution. Though the genetic variants for fT4 were all strongly associated with higher circulating fT4 levels, some of these genes do not result in higher intracellular thyroid hormone signaling<sup>56</sup>. These shortcomings of the fT4 genetic risk score were also demonstrated previously in context of thyroid status and atrial fibrillation<sup>57</sup>. Furthermore, interpretation of the association for genetically determined higher TSH with CAD cannot be specified to either variation within the reference range or including (sub)clinical hypothyroidism, as many of the genetic variants associated with higher TSH within the reference range also associated with TSH levels above the reference range<sup>16</sup>.

The present study has a number of strengths. Owing to the multi-cohort setting, we could compile large study populations for our analyses. Beside the statistical benefits of large sample sizes, multi-cohort studies allow for surpassing cohort-specific effects and therefore contribute to identifying robust and generalizable associations. Apart from assessing consistency of associations between study populations and the possibility of neglecting some important confounders not present in all contributing cohorts (e.g., specific drug use), we made efforts to triangulate our findings, using different epidemiological research methods, on the metabolomic profile. To assess (biological) consistency and robustness, the metabolomic markers associated with variation in TSH and fT4 within the reference range were tested in individuals with thyroid disorders and in studies using another NMR metabolomic profiling platform. The directional consistency among these different approaches indicates robust results.

Our study also has certain limitations. The MR study on the association of TSH and fT4 with CAD was performed in European-ancestry individuals only and is therefore not directly extrapolatable to other ethnicities. Furthermore, although we attempted to include as much cohorts as possible in our study, cohorts with both exposure and outcome were scarce and therefore the power of some of the analyses, in particular the validation analyses, is limited. Despite claims for causal inference in MR studies, caution is warranted for bias due to horizontal pleiotropy, selection bias and latent structure<sup>58-60</sup>. The study population used for first stage analyses of associations between TSH and fT4 and metabolomic profile included a considerable proportion of individuals using lipid-lowering medication (30%) or with a history of DM (8%). Nevertheless, results from our second stage and sensitivity analyses excluding participants with thyroid or lipid-lowering medication and those with a history of thyroid disease or DM were in line with our first stage results. However, not all metabolomic markers could be tested in the second stage analysis due to low overlap of markers in available data and platforms. Furthermore, both the MR study on genetically determined TSH and fT4 with CAD and with circulating metabolites suffered from some

sample overlap between exposure and outcome study populations, which might cause bias, though the extent appears limited<sup>61</sup>. Moreover, multivariable MR to formally assess mediation of the association between TSH and CAD by metabolomic profile was not possible, as specific genetic instruments for separate metabolomic markers are currently unavailable due to the high (genetic) correlation between the different components and subclasses.

## CONCLUSIONS

We found indications for potentially causal elevated risks of unfavorable lipid profile and a somewhat increased risk cardiovascular disease in individuals with TSH on the upper limits of the reference range. However, the effect sizes were small, and therefore do not justify widespread treatment with levothyroxine for prevention of cardiovascular disease. Nevertheless, the present study adds novel insights in the cardiovascular risk profile of those with altered thyroid hormone levels.

## ACKNOWLEDGEMENTS

We thank all study participants for their generous cooperation. We are grateful to all supporting staff and the participating general practitioners and pharmacists. Special thanks go to drs. N. Riyazi, J. Bijsterbosch, H.M. Kroon, I. Watt.

## AUTHORS' CONTRIBUTIONS

Conception: NAvV, MMB, RN, DvH; design of the work: NAvV, MMB, RN, DvH; acquisition, analysis: NAvV, MMB, CST, LC, MP, EH, MJN, RLG, ST, RM, FA, MB1, MB2, KB, CC, AD, CD, PE, ME, HG, MG, AEvH, MAI, MJ, JWJ, IK, FK, MK, JMTAM, IM, YM, SPM, DOM-K, MGN, RTN-M, RPP, MWJHP, NS, PES, HEDS, HV, KWvD, RN, DvH; interpretation of data: NAvV, RN, DvH; drafted the work or substantively revised it: NAvV, MMB, RN, DvH; approved the submitted version: NAvV, MMB, CST, LC, MP, EH, MJN, RLG, ST, RM, FA, MB1, MB2, KB, CC, AD, CD, PE, ME, HG, MG, AEvH, MAI, MJ, JWJ, IK, FK, MK, JMTAM, IM, YM, SPM, DOM-K, MGN, RTN-M, RPP, MWJHP, NS, PES, HEDS, HV, KWvD, RN, DvH; agreed accountability for own contribution to the work: NAvV, MMB, CST, LC, MP, EH, MJN, RLG, ST, RM, FA, MB1, MB2, KB, CC, AD, CD, PE, ME, HG, MG, AEvH, MAI, MJ, JWJ, IK, FK, MK, JMTAM, IM, YM, SPM, DOM-K, MGN, RTN-M, RPP, MWJHP, NS, PES, HEDS, HV, KWvD, RN, DvH. All authors read and approved the final manuscript.

## Funding

This work was supported by the European Commission project THYRAGE (Horizon 2020 research and innovation program, 666869) and BBMRI-NL,

a research infrastructure financed by the Dutch Research Council (NWO, 184.021.007 and 184.033.111).

The PROSPER study was supported by an investigator-initiated grant obtained from Bristol-Myers Squibb. JWJ is an Established Clinical Investigator of the Netherlands Heart Foundation (grant 2001 D 032). Support for genotyping was provided by the seventh framework program of the European commission (grant 223004) and by the Netherlands Genomics Initiative (Netherlands Consortium for Healthy Aging grant 050-060-810).

The infrastructure for the NESDA study ([www.nesda.nl](http://www.nesda.nl)) is funded through the Geestkracht program of the Netherlands Organisation for Health Research and Development (ZonMw, grant number 10-000-1002) and financial contributions by participating universities and mental health care organizations (VU University Medical Center, GGZ inGeest, Leiden University Medical Center, Leiden University, GGZ Rivierduinen, University Medical Center Groningen, University of Groningen, Lentis, GGZ Friesland, GGZ Drenthe, Rob Giel Onderzoekscentrum).

The Rotterdam Study is funded by Erasmus Medical Center and Erasmus University, Rotterdam, Netherlands Organization for the Health Research and Development (ZonMw), the Research Institute for Diseases in the Elderly (RIDE), the Ministry of Education, Culture and Science, the Ministry for Health, Welfare and Sports, the European Commission (DG XII), and the Municipality of Rotterdam.

This work in SHIP was funded by grants from the German Federal Ministry of Education and Research (BMBF, grants 01ZZ0403, 01ZZ0103, 01GI0883, AtheroSysMed 03IS2061B), the Ministry for Education, Research and Cultural Affairs, as well as the Ministry of Social Affairs of the Federal State of Mecklenburg-West Pomerania. This work is also part of the research project Greifswald Approach to Individualized Medicine (GANI\_MED). The GANI\_MED consortium is funded by the Federal Ministry of Education and Research and the Ministry of Cultural Affairs of the Federal State of Mecklenburg-West Pomerania (03IS2061A).

The Leiden Longevity Study has received funding from the European Union's Seventh Framework Programme (FP7/2007-2011) under grant agreement number 259679, the Innovation-Oriented Research Program on Genomics (SenterNovem IGE05007), the Centre for Medical Systems Biology and the Netherlands Consortium for Healthy Ageing (grant 050-060-810), all in the framework of the Netherlands Genomics Initiative, Netherlands Organization for Scientific Research

(NWO), and BBMRI-NL, a Research Infrastructure financed by the Dutch Research Council (NWO 184.021.007 and 184.033.111).

The 500FG was supported by an ERC Consolidator Grant (310372) and an IN-CONTROL CVON grant from the Dutch Heart Foundation (CVON2012-03 and CVON2018-27). MGN was supported by an ERC Advanced Grant (833247) and a Spinoza Grant of the Netherlands Organization for Scientific Research (94-212).

The GARP study is supported by Leiden University Medical Centre, Pfizer Groton, Connecticut, USA and the Dutch Arthritis Society. Furthermore, the research leading to these results has received funding from the Biobanking and BioMolecular resources Research Infrastructure the Netherlands (BBMRI-NL) (complementation project CP2013-84), the Dutch Scientific Research council NWO/ZonMW VICI scheme (nr. 91816631/528) and the Dutch Arthritis Society.

The OBB and Oxford BioResource are funded by the NIHR Oxford Biomedical Research Centre (BRC). CC is supported by an Intermediate Clinical Research Fellowship to (FS/16/45/32359) from the British Heart Foundation and FK from a BHF programme grant (RG/17/1/32663).

The NEO study is supported by the participating Departments, the Division and the Board of Directors of the Leiden University Medical Centre, and by the Leiden University, Research Profile Area ‘Vascular and Regenerative Medicine’.

The Airwave Health Monitoring Study was funded by the UK Home Office (780- TETRA, 2003-2018) and is currently funded by the MRC and ESRC (MR/R023484/1) with additional support from the NIHR Imperial College Biomedical Research Centre in collaboration with Imperial College NHS Healthcare Trust. PE acknowledges support from the MRC and PHE (MR/L01341X/1, 2014-2019) and currently from the MRC for the MRC Centre for Environment and Health (MR/S019669/1). PE is supported by the UK Dementia Research Institute which receives funding from UK DRI Ltd funded by the UK Medical Research Council, Alzheimer’s Society and Alzheimer’s Research UK. PE is associate director of the Health Data Research UK London funded by a consortium led by the UK Medical Research Council. RM was supported by the President’s PhD Scholarships from Imperial College London.

None of the funders influenced data collection, analyses or interpretation, or the decision to publish this research. The views expressed are those of the authors and not necessarily those of the sponsors.

### ***Availability of data and materials***

The data used for the analyses on CAD are all publicly available. The summary-level data for CARDIoGRAM (<http://www.cardiogramplusc4d.org/data-downloads/>) and FinnGen (<https://finngen.gitbook.io/documentation/data-download>) are freely obtainable. The data for UK Biobank are accessible after approval (<https://www.ukbiobank.ac.uk/>). All other datasets used and/or analysed during the current study are available from the corresponding authors on reasonable request.

### **DECLARATIONS**

#### *Ethics approval and consent to participate*

The different cohorts contributing to the meta-analyses of the multivariable adjusted and Mendelian Randomization studies were accepted by the medical ethical committees from the Leiden University Medical Center (GARP, LLS, NEO, PROSPER), Erasmus Medical Center (RS), Radboud University Medical Center (500FG), VU University Medical Center (NESDA), Oxfordshire Clinical Research Ethics Committee (OBB), University of Greifswald (SHIP), and the National Health Service Multi-site Research Ethics Committee (AIRWAVE; MREC/

13/NW/0588).

#### *Consent for publication*

Not applicable.

#### *Competing interests*

DOMK is a part-time research consultant at Metabolon, Inc. All others declare no conflicts of interest.

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## SUPPLEMENTARY MATERIAL

### EXTENDED METHODS

#### **Description of GWAS from which genetic instruments were derived**

Genetic instruments for TSH and fT4 concentrations were extracted from the largest genome-wide association studies (GWAS) meta-analysis to date on thyroid function comprising up to 72,167 participants.<sup>1</sup> This GWAS excluded participants with TSH levels outside the cohort-specific reference range, participants of non-European ancestry, and participants using thyroid medication (Anatomical Therapeutic Chemical (ATC) code H03) and/or history of thyroid surgery. Across the cohorts included in the GWAS, the mean TSH approximated 1.81 mIU/L (standard deviation (SD) 1.52 mIU/L) and the mean fT4 was approximately 14.9 pmol/L (SD 2.4 pmol/L). Both TSH and fT4 levels were inverse normal transformed, to allow for comparability across cohorts.

#### **Study populations for multivariable regression analyses**

##### *The 500 Functional Genomics Study (500FG)*

500FG is a cohort study that aims to identify the host and environmental factors that influence heterogeneity in human immune responses.<sup>2</sup> In this context, multiple datasets were collected including deep immunological phenotyping, physiological parameters (among which hormone concentrations), and multi-omics data (including lipidome and metabolome). 500FG is a cohort of 500 healthy volunteers with caucasian genetic background: any chronic disease (including diabetes, hypercholesterolemia, endocrine diseases) were exclusion criteria. Measurements of TSH and fT4 were performed using electrochemiluminescence immunoassay (ECLIA) on the Cobas 8000 (E801) (Roche, Almere, the Netherlands) in a single batch. The reference ranges at our laboratory are 0.27-4.2 mIU/L for TSH and 10.0-23.0 pmol/L for fT4. For TSH, the total coefficient of variation (CV) ranged between 3.5% at 0.17 mIU/L and 2.1% at 2.3 mIU/L (detection range 0.005-100 mIU/L). The total CV for fT4 ranged between 2.6% at 18.4 pmol/L and 2.2% at 49.7 pmol/L (detection range 0.5-100 pmol/L).

##### *The Genetics, Arthrosis and Progression study (GARP)*

The GARP study has been described in detail previously.<sup>3</sup> It aimed at identifying determinants of osteoarthritis and the progression of this disease. The study is based on sibships of white Dutch ancestry with clinical- and radiographically-confirmed osteoarthritis at two or more joint sites of the hand, spine (cervical or lumbar), knee or hip. In the current analyses we included 321 subjects from whom we had Nightingale metabolomics data and thyroid levels available. For

measurements, blood samples were collected and stored at  $-80^{\circ}\text{C}$  until analysis. Measurements of TSH and fT4 were performed on serum using the Modular E170 (Roche, Almere, the Netherlands) at the Department of Clinical Chemistry of the Leiden University Medical Center. The reference ranges are 0.4-4.0 mIU/L for TSH and 13.0-25.0 pmol/L for fT4.

#### *The Leiden Longevity Study (LLS)*

The Leiden Longevity Study was enrolled from 2002 to 2006 and consists of 1671 members of long-lived families (mean age 60 years) and their 744 partners (mean age 60 years) as population controls. Members of long-lived families are very similar to the general populations, although they have favorable glucose tolerance<sup>4</sup>, favorable lipid metabolism<sup>5</sup>, and a lower prevalence for type 2 diabetes and myocardial infarction<sup>6</sup>. For the current study we have used the data of the inclusion period from 2009 and 2010, in which we obtained Nightingale metabolomics data and thyroid measures from 246 members of long-lived families and 240 controls. Blood samples were collected after an overnight fast and stored ( $-80^{\circ}\text{C}$ ) until analysis. Measurements of TSH and fT4 were performed using electrochemiluminescence immunoassay (ECLIA) on the Cobas 8000 (E602) from Roche, Almere, the Netherlands. The reference ranges at our laboratory are 0.3-4.8 mIU/L for TSH and 12.0-22.0 pmol/L for fT4. For TSH, the interassay coefficient of variation (CV) ranged between 2.32% at 0.176 mIU/L and 2.11% at 21.430 mIU/L (detection range 0.005-100 mIU/L). The interassay CV for fT4 ranged between 5.11% at 13.8 pmol/L and 6.23% at 42.1 pmol/L (detection range 0.3-100 pmol/L). The measurements were performed in a single batch at the Department of Clinical Chemistry of the Leiden University Medical Center.

#### *The Netherlands Study of Depression and Anxiety (NESDA)*

The Netherlands Study of Depression and Anxiety (NESDA) is an observational longitudinal cohort study on the long term course and consequences of depressive and anxiety disorders.<sup>7</sup> In total 2,981 participants aged 18 to 65 years were recruited between 2004 and 2007 through different settings: community, primary care and specialized mental health clinics in order to obtain a representative sample of persons with and without depressive and anxiety disorders. After an overnight fast, baseline EDTA plasma samples were collected and stored in aliquots at  $-80^{\circ}\text{C}$  until further analysis. Blood samples were analyzed in 2 batches (April 2014 and December 2014) by Brainshake Ltd./Nightingale Health. Markers of thyroid function were assessed at the day of the baseline interview. Serum TSH was measured by immunoluminometric assay on a random-access analyzer (Roche Diagnostics, Germany). Serum fT4 was measured with a fluorescence immunoassay on a random-access assay system (Roche Diagnostics, Germany).

*PROspective Study of Pravastatin in the Elderly at Risk (PROSPER) study*

All data come from the PROspective Study of Pravastatin in the Elderly at Risk (PROSPER). A detailed description of the study has been published elsewhere.<sup>8,9</sup> PROSPER was a prospective multicenter randomized placebo-controlled trial to assess whether treatment with pravastatin diminishes the risk of major vascular events in elderly. Between December 1997 and May 1999, we screened and enrolled subjects in Scotland (Glasgow), Ireland (Cork), and the Netherlands (Leiden). Men and women aged 70-82 years were recruited if they had pre-existing vascular disease or increased risk of such disease because of smoking, hypertension, or diabetes. A total number of 5,804 subjects were randomly assigned to pravastatin or placebo. A large number of prospective tests were performed including Biobank tests and cognitive function measurements. In stored (-80 °C) plasma drawn from participant at 6 months, we measured TSH and free T4 in all available samples stored at a single center (University of Glasgow), using the same electrochemiluminescence immunodetection method on a Roche Elecsys 2010 (Burgess Hill, UK). The limit of detection of TSH was below 0.005 mIU/liter. The limit of detection of free T4 was 0.3 pmol/liter, with reference ranges of 12-22 pmol/liter.

*The Rotterdam Study*

From 1989, all inhabitants aged 55 and older from a well-defined suburb in the city of Rotterdam, the Netherlands were invited to participate in the Rotterdam Study.<sup>10</sup> The initial cohort comprised 7,983 participants (RS-I) and was extended in 2000 (RS-II: 3,011 participants) and 2006 (RS-III: 3,932 participants, aged 45 years and older). In total, the Rotterdam Study comprises 14,926 participants aged 45 years or over. The overall response rate across the three waves was 72%. Between 2002 and 2014, metabolomics data was measured by Brainshake Ltd./Nightingale Health from blood samples. For this study we analyzed the plasma samples from RS 1 visit 4 and RS 2 visit 2 (2002-2005). The thyroid function measurements were performed in RS 1 visit 3 and RS 2 visit 1 (1997-2001). TSH and fT4 measurements were performed at the same time in both cohorts in serum sample stored at -80° C (the electrochemiluminescence immunoassay for thyroxine and thyrotropin, Roche, Mannheim, Germany). The reference range was 0.4-4.0 mIU/L for TSH and 11-15 pmol/L for fT4.

*The Study of Health in Pomerania (SHIP)*

The Study of Health in Pomerania (SHIP) is a population-based study conducted in West Pomerania, a rural region in north-east Germany and a detailed description of the sampling procedure and the study population can be found elsewhere.<sup>11</sup> In total, 4420 subjects chose to participate (50.1% response). All participants gave written informed consent before taking part in the study. The study was approved by the ethics committee of the University of Greifswald

and conformed to the principles of the declaration of Helsinki. SHIP data are publicly available for scientific and quality control purposes by application at [www.community-medicine.de](http://www.community-medicine.de). For a subsample of 1000 subjects without self-reported diabetes plasma and urine metabolomic profiling data based on NMR were available. Self-reported history of thyroid disease was recorded comprising hyperthyroidism, hypothyroidism, goiter, thyroid nodules or others. Fasting blood samples were taken from the cubital vein of participants in the supine position between 7.00 a.m. and 12.00 p.m. In the same time span spot urine samples were taken. All samples were either analyzed immediately or stored at  $-80^{\circ}\text{C}$  in the Integrated Research Biobank (LiCONiC, Lichtenstein). Serum levels of TSH, FT3 and FT4 were measured using an immunoassay (Dimension VISTA, Siemens Healthcare Diagnostics, Eschborn, Germany) with a functional sensitivity of 0.005 mIU/l for TSH, 0.77 pmol/l for FT3 and 1.3 pmol/l for FT4. Plasma samples were stored frozen at  $-80^{\circ}\text{C}$  until analysed. After thawing, 250  $\mu\text{l}$  of plasma were mixed with 250  $\mu\text{l}$  of phosphate buffer [prepared with D2O and contained sodium 3-trimethylsilyl-(2,2,3,3-D4)-1-propionate (TSP) as reference, (pH 7.4)]. For total, HDL- and LDL cholesterol as well as total triglycerides comparison with laboratory measurements yielded excellent correlations (all above 0.91). However, six subjects revealed as outlier in PCA were excluded from the data set. Similar to the data above, all values were log<sub>2</sub>-transformed prior statistical analyses.

### **Study populations for Mendelian Randomization analyses**

#### *Coronary ARtery Disease Genome wide Replication and Meta-analysis (CARDIoGRAM) consortium*

Summary-level data was made publicly available of a meta-analysis of 14 GWAS of coronary artery disease (CAD) comprising 22,233 cases and 64,762 controls, all of European ancestry. CAD was defined as an inclusive diagnosis of myocardial infarction, angina pectoris, coronary revascularization or coronary stenosis >50%.<sup>12</sup>

#### *UK Biobank*

The UK Biobank cohort is a prospective general population cohort with 502,628 participants between the age of 40 and 70 years recruited from the general population between 2006 and 2010<sup>13</sup>, more details can be found on the website (<https://www.ukbiobank.ac.uk>). We restricted the analyses to participants of European ancestry, who were in the full released imputed genomics databases (UK10K + HRC). CAD diagnoses were retrieved via linkage with the NHS database, reported according to the International Classification of Diseases 10th Revision (ICD-10) coding.<sup>14</sup> CAD was defined as a composite outcome of angina pectoris (I20), myocardial infarction (I21 and I22), and acute and chronic ischemic heart disease (I24 and I25). In total, 52,946 cases and 393,549 controls were identified.

We performed logistic regression analyses to assess the associations between genetic instruments and CAD, adjusted for age, sex and 10 principal components, and corrected for familial relationships using BOLT\_LMM (v2.3.2).

#### *FinnGen*

FinnGen study is an ongoing cohort study launched in 2017, bringing together Finnish universities, hospitals and hospital districts, national Institute for Health and Welfare, Blood Service, biobanks and international pharmaceutical companies and hundreds of thousands of Finns.<sup>15</sup> For the present study we used “major coronary heart disease (CHD)” as outcome, which comprised hospital data ICD-10 codes angina pectoris (I20), myocardial infarction (I21-I23), ischemic heart diseases (I24 and I25), cardiac arrest (I46) and other unattended or cause unknown sudden death (R96 and R98). More detailed information is provided online (<https://finngen.gitbook.io/documentation/>). The analyses were based on the FinnGen data freeze 5, which consists of 16,631 cases of major CHD and 197,780 controls with complete instruments-CHD associations.

#### *MAGNETIC consortium*

We used publicly-available summary statistics from the MAGNETIC NMR GWAS dataset, which comprises the additive (per-allele) beta coefficients with accompanying standard errors of the associations between genome-wide SNPs and 123 metabolomic measures. In this GWAS meta-analysis data of ~20,000 individuals from 14 cohorts of European ancestry. The 123 metabolomic measures were quantified by an earlier version of the same high-throughput proton NMR metabolomics platform (Nightingale Health Ltd., Helsinki, Finland).

#### *Netherlands Epidemiology of Obesity (NEO) Study*

The present study is a cross-sectional analysis of baseline measurements of the Netherlands Epidemiology of Obesity (NEO) study, a population-based cohort study. The NEO study started in 2008 and included 6,671 individuals aged 45–65 years, with an oversampling of individuals with overweight or obesity. The study design and population are described in more detail elsewhere.<sup>16</sup> Men and women living in the greater area of Leiden (in the West of the Netherlands) were invited to participate if they were aged between 45 and 65 years and had a self-reported BMI of 27 kg/m<sup>2</sup> or higher. In addition, all inhabitants aged between 45 and 65 years from one municipality (Leiderdorp) were invited to participate irrespective of their BMI, allowing for a reference group with a normal BMI distribution. The Medical Ethical Committee of the Leiden University Medical Center (LUMC) approved the design of the study. All participants gave their written informed consent. Genotyping was performed in participants from European ancestry, using the Illumina HumanCoreExome-24 BeadChip (Illumina Inc., San Diego,

California, United States of America). Subsequently, genotypes were imputed to the 1000 Genome Project reference panel (v3 2011).

#### *Oxford Biobank*

The Oxford Biobank (OBB) is a population-based cohort study of randomly selected healthy men and women living in Oxfordshire, UK. The study includes 7185 individuals aged 30 to 50 years old. The exclusion criteria for the OBB were history of myocardial infarction, diabetes mellitus type 1 or 2, heart failure, untreated malignancy, other ongoing systemic diseases, or ongoing pregnancy. Study recruitment criteria and population characteristics are described in detail elsewhere.<sup>17</sup> The OBB protocol is approved by the Oxfordshire Clinical Research Ethics Committee and all participants have provided informed consent. Genotyping was performed using the Affymetrix Axiom chip, which has been similarly used in the UK Biobank.

#### *PROspective Study of Pravastatin in the Elderly at Risk (PROSPER) study*

Prospective multicenter randomized placebo-controlled trial to assess whether treatment with pravastatin diminishes the risk of major vascular events in elderly. Between December 1997 and May 1999, potential participants were screened and enrolled in Scotland (Glasgow), Ireland (Cork), and the Netherlands (Leiden). Men and women aged 70-82 years were recruited if they had pre-existing vascular disease or increased risk of such disease because of smoking, hypertension, or diabetes.<sup>9,18</sup> A total number of 5,804 subjects were randomly assigned to pravastatin or placebo. Participants were followed for an average 3.5 years. A whole genome wide screening has been performed in the sequential PHASE project.<sup>19</sup> Of 5,763 subjects DNA was available for genotyping. Genotyping was performed with the Illumina 660K beadchip, after QC (call rate <95%) 5,244 subjects and 557,192 SNPs were left for analysis. These SNPs were imputed to 2.5 million SNPs based on the HAPMAP built 36 with MACH imputation software. Subsequent imputation of the genotyped data was executed with the HRC Michigan imputation. For the present study only the 2,343 participants from the placebo arm were included.

#### *Airwave Health Monitoring Study*

The Airwave Health Monitoring Study was first established in 2004 as a large-scale cohort of police officers in Great Britain. A total of 53,114 participants were enrolled by end of baseline recruitment in March 2015. Initially, it was aimed to investigate health outcomes related to the use of Terrestrial Trunked Radio (TETRA). This cohort has been expanded to also investigate the health of workforces in more general. The rationale, design, and methods of this study can be found elsewhere.<sup>20</sup> Genotyping and metabolomics analysis have been carried out on part of sample collection. Genotyping was conducted on the Illumina

Infinium HumanCoreExome-12v1-1 BeadChip. Samples with genotype call rate <97% and heterozygosity rate >3SD from the mean were removed during quality control process. Duplicated and second-degree relatives were also excluded. Markers with missing rate >2%, significant deviation from Hardy-Weinberg equilibrium ( $P < 1E-5$ ) or minor allele frequency less than 1% were removed. Genotype imputation was performed with reference to the 1000G reference panel. Metabolomic profiling was performed in 2,021 heparin plasma samples of the Airwave Health Monitoring Study.

## **Metabolomic profile measurements**

### *Nightingale platform*

For the main analyses we used a high-throughput proton NMR metabolomic profiling platform <sup>21</sup> (Nightingale Health Ltd., Helsinki, Finland) to quantify a maximum of 148 metabolomic particle concentrations in fasting serum samples. This method provides quantification of lipoprotein subclass profiling with lipid concentrations within 14 lipoprotein subclasses. The 14 subclass sizes were defined as follows: extremely large VLDL with particle diameters from 75 nm upwards and a possible contribution of chylomicrons, five VLDL subclasses (average particle diameters of 64.0 nm, 53.6 nm, 44.5 nm, 36.8 nm, and 31.3 nm), IDL (28.6 nm), three LDL subclasses (25.5 nm, 23.0 nm, and 18.7 nm), and four HDL subclasses (14.3 nm, 12.1 nm, 10.9 nm, and 8.7 nm). Within the lipoprotein subclasses, the following components were quantified: total cholesterol, total lipids, phospholipids, free cholesterol, cholesteryl esters, and triglycerides. The mean size for VLDL, LDL and HDL particles were calculated by weighting the corresponding subclass diameters with their particle concentrations. An additional 58 small molecules, including those involved in glycolysis, ketone bodies and several amino acids are also quantified. These belonged to classes of apolipoproteins, cholesterol, fatty acids, glycerides, phospholipids, amino acids, fluid balance, glycolysis-related metabolites, inflammation, and ketone bodies. Detailed experimentation and applications of the NMR metabolomics platform have been described previously <sup>21</sup>, as well as representative coefficients of variations (CVs) for the metabolomic markers <sup>22</sup>.

### *Bruker Platform*

For additional analyses on a different NMR platform, metabolomic profiles were generated by Bruker (Bruker Biospin, Rheinstetten, Germany). Spectra were recorded on a Bruker AVANCE-II 600 NMR spectrometer operated by TOPSPIN 3.2 software (both Bruker Biospin, Rheinstetten, Germany), equipped with 5-mm z-gradient probe (Bruker Biospin, Rheinstetten, Germany) and automated tuning and matching (ATMA) unit (Bruker Biospin, Rheinstetten, Germany). Specimens were automatically delivered to the spectrometer via SampleJet

(Bruker Biospin, Rheinstetten, Germany) into standard 5 mm NMR tubes. The acquisition temperature was set to 310°K. A standard one-dimensional  $^1\text{H}$ -NMR pulse sequence with suppression of the water peak (NOESYPRESAT) was used. The sequence has the form -RD-gz,1-90°-t-90°-tm-gz,2-ACQ, where RD is the relaxation delay (4 s) t is a short delay ( $\sim 3 \mu\text{s}$ ), 90° represents the 90° RF hard pulse, tm is the mixing time (10 ms), gz,1 and gz,2 are the magnetic field z gradients both applied for 1 ms and ACQ is the acquisition period (2.7 s) collecting 98304 data points at a sweep width of 30ppm. The receiver gain is set at 90.5 for all experiments. For pre-processing, a line-broadening of 0.3 Hz, a zero-filling to produce 128k data points and a first-order phase correction of 0.0 was applied. Spectral processing included zero-filling, line-broadening, Fourier transform and referencing of the chemical shift and determination of the spectral intensity per 1mmol protons for quantitative referencing. Chemical shifts of plasma spectra were referenced to the CH<sub>3</sub>-group signal of alanine adjusting it to 1.48 ppm. Spectra were segmented into N = 450 consecutive integrated spectral regions (buckets) of fixed width covering the region from 0.3 ppm to 1.4 ppm. Two subregions, i.e. 1.31-1.35 ppm and 1.16-1.21 ppm were excluded from binning to avoid unwanted influences coming from lactate and ethanol CH<sub>3</sub>-group signals in later application of the method in typical population cohorts. Finally, the spectrum is submitted to data analysis for lipoprotein subclass analysis B.I.LISATM (Bruker BioSpin GmbH Germany).



## EXTENDED RESULTS

Supplementary Table 1. Associations of individual genetic instruments for TSH and fT4 with CAD

SNP	Gene	Chr.	Position	A1	A2	AF1	Exposure		
							beta	se	p-value
<b>TSH</b>									
rs12089835	CAPZB	1	19771438	T	C	0.35	0.073	0.007	9.18E-26
rs10917469	CAPZB	1	19843576	A	G	0.84	0.111	0.009	3.00E-35
rs74804879	CAPZB	1	19862320	T	C	0.65	0.05	0.007	1.20E-14
rs334725	NFIA	1	61610049	A	G	0.95	0.174	0.015	2.06E-31
rs17020122	VAV3	1	108357391	T	C	0.09	0.104	0.011	1.62E-21
rs13015993	IGFBP5	2	217625523	A	G	0.73	0.082	0.007	5.38E-32
rs6724073	DIRC3	2	218236786	T	C	0.74	0.051	0.008	9.15E-11
rs1663070	SYN2	3	12239852	T	C	0.74	-0.046	0.007	2.49E-11
rs28502438	TM4SF4	3	149220109	T	C	0.57	0.034	0.006	7.28E-09
rs13100823	IGF2BP2	3	185514088	T	C	0.31	-0.041	0.007	2.35E-09
rs59381142	HES1	3	193916181	A	G	0.24	-0.058	0.008	2.08E-13
rs11732089	NR3C2	4	149665602	T	C	0.80	0.115	0.008	3.71E-47
rs139424329	PDE8B	5	76495539	A	G	0.01	-0.200	0.032	2.05E-10
rs2127387	PDE8B	5	76532571	A	G	0.41	0.144	0.006	1.39E-127
rs1265091	PSORS1C1	6	31108129	T	C	0.20	0.057	0.009	1.20E-10
rs744103	VEGFA/ LOC100132354	6	43805362	A	T	0.69	0.092	0.007	9.35E-40
rs9381266	VEGFA/ LOC100132354	6	43905037	T	C	0.74	0.073	0.007	9.18E-26
rs9497965	SASH1	6	148521292	T	C	0.40	0.044	0.006	1.12E-13
rs1079418	PDE10A	6	166047034	A	G	0.69	0.101	0.007	1.71E-47
rs56009477	SLC25A37	8	23356964	A	G	0.84	0.052	0.008	4.02E-11
rs2439301	NRG1	8	32433013	A	G	0.23	-0.059	0.008	8.22E-14
rs10957494	SULF1	8	70365025	A	G	0.69	-0.040	0.007	5.51E-09
rs118039499	TG	8	133771635	A	C	0.98	0.184	0.024	8.83E-15
rs2739067	TG	8	133951991	A	G	0.60	-0.042	0.006	1.28E-12
rs10814915	GLIS3	9	4290544	T	C	0.44	0.042	0.006	1.28E-12
rs9298749	C9orf92	9	16214340	A	C	0.59	-0.039	0.006	4.02E-11
rs494242	ABO	9	136145118	C	T	0.40	0.052	0.006	2.22E-18
rs11255790	GATA3	10	8682180	T	C	0.30	-0.041	0.007	2.35E-09
rs4933466	PTEN	10	89849519	A	G	0.61	0.040	0.006	1.31E-11
rs12284404	PRDM11	11	45228686	A	G	0.27	-0.067	0.007	5.27E-22
rs4445669	CADM1	11	115045237	T	C	0.46	-0.040	0.006	1.31E-11
rs7329958	SPATA13	13	24782080	T	C	0.35	-0.044	0.007	1.63E-10
rs398745	MBIP	14	36536181	A	C	0.59	-0.052	0.006	2.22E-18
rs11159482	TSHR	14	81490842	T	C	0.09	0.085	0.013	3.11E-11
rs12893151	TSHR	14	81619945	A	C	0.22	-0.062	0.008	4.59E-15

	% explained	F-statistics	CARDIoGRAM		UK Biobank		FinnGen	
			Log Odds	se	Log Odds	se	Log Odds	se
	0.242	109	0.029	0.015	0.010	0.007	-0.018	0.014
	0.331	152	0.039	0.019	0.013	0.009	0.005	0.020
	0.114	51	NA	NA	-0.003	0.007	NA	NA
	0.288	135	-0.001	0.031	0.014	0.015	-0.055	0.046
	0.177	89	-0.043	0.023	-0.019	0.011	0.005	0.020
	0.265	137	0.011	0.015	-0.002	0.007	-0.023	0.013
	0.100	41	-0.026	0.019	-0.013	0.007	0.011	0.013
	0.081	43	0.031	0.017	-0.009	0.007	-0.003	0.015
	0.057	32	0.026	0.016	0.011	0.006	0.006	0.014
	0.072	34	0.017	0.015	0.030	0.007	0.012	0.014
	0.123	53	-0.007	0.020	0.008	0.007	0.004	0.017
	0.423	207	-0.005	0.017	-0.003	0.008	0.025	0.020
	0.079	39	NA	NA	NA	NA	-0.002	0.049
	1.003	576	0.006	0.014	-0.011	0.007	0.024	0.013
	0.104	40	0.030	0.021	0.008	0.008	-0.002	0.020
	0.362	173	0.010	0.018	0.006	0.007	0.019	0.014
	0.205	109	0.028	0.016	0.003	0.007	0.009	0.016
	0.093	54	0.014	0.015	-0.002	0.006	0.007	0.014
	0.436	208	-0.013	0.017	0.006	0.007	0.011	0.015
	0.073	42	NA	NA	-0.005	0.009	0.014	0.019
	0.123	54	0.001	0.016	0.006	0.008	-0.010	0.015
	0.068	33	NA	NA	-0.005	0.007	-0.005	0.014
	0.133	59	NA	NA	0.044	0.021	NA	NA
	0.085	49	-0.014	0.014	0.002	0.007	-0.041	0.014
	0.087	49	0.007	0.014	-0.008	0.006	0.001	0.013
	0.074	42	-0.036	0.015	0.014	0.007	-0.003	0.013
	0.130	75	-0.069	0.015	-0.024	0.007	NA	NA
	0.071	34	0.015	0.015	-0.003	0.007	-0.011	0.017
	0.076	44	0.009	0.020	0.007	0.007	0.009	0.013
	0.177	92	-0.036	0.015	-0.002	0.007	-0.002	0.014
	0.079	44	0.000	0.014	-0.020	0.006	-0.007	0.013
	0.088	40	-0.024	0.017	-0.005	0.007	0.013	0.014
	0.131	75	-0.023	0.014	0.002	0.006	0.009	0.013
	0.118	43	NA	NA	0.001	0.014	-0.050	0.027
	0.132	60	NA	NA	0.009	0.008	-0.015	0.015

**Supplementary Table 1.** Continued.

SNP	Gene	Chr.	Position	A1	A2	AF1	Exposure		
							beta	se	p-value
rs8015085	<i>ITPK1</i>	14	93585331	A	G	0.21	0.067	0.008	2.76E-17
rs17477923	<i>FAM227B/FGF7</i>	15	49711185	T	C	0.74	0.083	0.007	9.88E-33
rs13329353	<i>DET1</i>	15	89113877	T	C	0.68	0.061	0.007	1.46E-18
rs1045476	<i>ADCY9</i>	16	4015313	A	G	0.18	0.049	0.008	4.53E-10
rs30227	<i>MIR365A</i>	16	14405428	T	C	0.61	-0.047	0.006	2.38E-15
rs17767491	<i>MAF</i>	16	79745487	A	G	0.68	0.088	0.007	1.52E-36
rs1157994	<i>BCAS3</i>	17	59338574	A	G	0.05	-0.090	0.016	9.28E-09
rs1042673	<i>SOX9</i>	17	70121339	A	G	0.52	-0.055	0.006	2.44E-20
rs963384	<i>SOX9</i>	17	70369758	T	C	0.46	0.035	0.006	2.72E-09
rs4804413	<i>INSR</i>	19	7222655	T	C	0.44	0.053	0.006	5.08E-19
rs1203944	<i>FOXA2</i>	20	22596879	T	C	0.23	-0.051	0.007	1.60E-13
<b>ft4</b>									
rs145019385	<i>DIO1</i>	1	54252139	T	C	0.98	0.181	0.032	7.74E-09
rs2235544	<i>DIO1</i>	1	54375570	A	C	0.52	0.139	0.007	4.78E-88
rs4954192	<i>ACMSD</i>	2	135632981	T	C	0.44	-0.041	0.007	2.35E-09
rs6785807	<i>SOX2-OT</i>	3	181718601	A	G	0.15	-0.059	0.009	2.77E-11
rs6854291	<i>AADAT</i>	4	170992760	A	G	0.10	0.117	0.011	1.01E-26
rs10946313	<i>ID4</i>	6	19381386	T	C	0.63	0.046	0.007	2.49E-11
rs9356988	<i>SLC17A4</i>	6	25777481	A	G	0.27	-0.051	0.007	1.60E-13
rs17185536	<i>LOC728012</i>	6	100620931	T	C	0.24	0.073	0.008	3.59E-20
rs67583169	<i>CA8</i>	8	61212179	C	G	0.87	0.061	0.010	5.30E-10
rs10119187	<i>GLIS3</i>	9	4223660	T	C	0.81	0.050	0.009	1.38E-08
rs10739496	<i>FOXE1</i>	9	100552559	T	C	0.66	0.078	0.007	3.88E-29
rs10818937	<i>NEK6</i>	9	127015440	T	C	0.31	-0.048	0.007	3.51E-12
rs4842131	<i>LHX3</i>	9	139092679	T	C	0.45	-0.104	0.008	6.12E-39
rs55679545	<i>LHX3</i>	9	139122363	A	G	0.27	0.044	0.008	1.90E-08
rs11039355	<i>FNBP4</i>	11	47737501	T	C	0.34	-0.039	0.007	1.26E-08
rs4149056	<i>SLC01B1</i>	12	21331549	T	C	0.84	-0.051	0.009	7.28E-09
rs225014	<i>DIO2</i>	14	80669580	T	C	0.64	0.054	0.007	6.08E-15
rs12323871	<i>DIO3OS</i>	14	101852075	T	C	0.82	-0.047	0.008	2.11E-09
rs11626434	<i>DIO3OS</i>	14	101998443	C	G	0.36	0.058	0.007	5.87E-17
rs12907106	<i>USP3</i>	15	63873658	C	G	0.27	-0.041	0.007	2.35E-09
rs8063103	<i>SNX29</i>	16	12703395	C	G	0.85	-0.052	0.009	3.78E-09
rs113107469	<i>SLC25A52</i>	18	29306737	T	C	0.03	0.200	0.022	4.91E-20
rs56069042	<i>MC4R</i>	18	57914644	A	G	0.96	0.106	0.019	1.21E-08

Abbreviations: TSH; thyroid stimulating hormone, ft4; free thyroxine, SNP; single nucleotide polymorphism, Chr; chromosome number, A1; effect allele, A2; other allele, AF1; effect allele frequency, logOdds; log odds ratio, se: standard error, N.A.; not available

Estimates, standard errors and p-values were derived directly from summary data of the respective studies and data were harmonized to display estimates for the same allele across studies.

% explained	F-statistics	CARDIoGRAM		UK Biobank		FinnGen	
		Log Odds	se	Log Odds	se	Log Odds	se
0.149	70	NA	NA	0.009	0.008	0.023	0.017
0.265	141	0.018	0.016	0.007	0.007	-0.016	0.014
0.162	76	0.007	0.016	0.002	0.007	0.018	0.014
0.071	38	-0.004	0.023	0.000	0.008	0.012	0.017
0.105	61	-0.028	0.016	-0.011	0.007	-0.005	0.013
0.337	158	-0.018	0.015	0.007	0.007	-0.010	0.014
0.077	32	NA	NA	0.024	0.016	0.096	0.032
0.151	84	-0.019	0.014	-0.004	0.006	0.019	0.013
0.061	34	0.013	0.015	0.000	0.006	0.006	0.013
0.138	78	0.000	0.014	0.016	0.006	0.008	0.013
0.092	53	-0.031	0.017	0.002	0.008	-0.033	0.016
0.128	32	NA	NA	-0.008	0.021	0.099	0.067
0.965	394	0.017	0.016	0.009	0.006	0.009	0.013
0.083	34	0.002	0.015	0.001	0.007	-0.027	0.013
0.089	43	0.012	0.023	0.000	0.009	-0.010	0.015
0.246	113	NA	NA	-0.012	0.011	-0.005	0.029
0.099	43	-0.016	0.015	-0.002	0.007	-0.006	0.013
0.103	53	-0.003	0.016	-0.012	0.007	0.001	0.014
0.194	83	0.011	0.017	-0.007	0.007	-0.017	0.016
0.084	37	0.021	0.021	0.003	0.009	0.001	0.016
0.077	31	NA	NA	-0.003	0.008	0.002	0.018
0.273	124	-0.015	0.015	-0.001	0.007	-0.007	0.014
0.099	47	-0.022	0.015	0.003	0.007	-0.013	0.014
0.535	169	0.039	0.022	0.008	0.006	0.010	0.013
0.076	30	-0.016	0.016	0.010	0.007	-0.002	0.014
0.068	31	0.008	0.015	-0.008	0.007	-0.032	0.014
0.070	32	0.027	0.019	0.004	0.009	0.053	0.016
0.134	60	0.005	0.015	0.005	0.007	0.004	0.015
0.065	35	-0.005	0.022	0.006	0.008	-0.012	0.016
0.155	69	-0.042	0.018	-0.003	0.007	NA	NA
0.066	34	NA	NA	0.001	0.007	-0.001	0.014
0.069	33	0.040	0.023	0.012	0.009	-0.028	0.021
0.233	83	NA	NA	0.039	0.018	-0.049	0.046
0.086	31	NA	NA	-0.001	0.017	-0.025	0.032

**Supplementary Table 2.** Population characteristics of included cohorts (n=11,140)

	<b>500 FG</b> <b>N = 421</b>	<b>GARP</b> <b>N = 321</b>	<b>LLS</b> <b>N = 486</b>	<b>NESDA</b> <b>N = 2,906</b>	<b>PROSPER</b> <b>N = 5,316</b>	<b>RS</b> <b>N = 1,690</b>
<b>Demographics</b>						
Age in years (median(IQR))	23.0 (20.0-26.0)	59.6 (54.3-65.4)	65.9 (61.8-70.5)	43.0 (30.0 – 53.0)	75.5 (72.9 – 78.4)	69.0 (65.3-73.4)
Women	240 (57.0)	261 (81.3)	245 (50.4)	1,929 (66.4)	2,729 (51.3)	938 (55.5)
Current smoker	52 (12.4) <sup>a</sup>	54 (16.8)	60 (12.6) <sup>d</sup>	1,120 (38.5)	1,400 (26.3) <sup>g</sup>	263 (15.6) <sup>i</sup>
BMI (median(IQR))	22.2 (20.7-24.2) <sup>b</sup>	26.0 (24.0-30.0) <sup>c</sup>	26.3 (24.1 – 28.5) <sup>e</sup>	24.7 (22.1 – 28.1)	26.2 (23.9 – 29.0) <sup>h</sup>	26.5 (24.2-29.1)
<b>Thyroid function</b>						
TSH (median(IQR))	2.27 (1.67-3.20)	1.97 (1.32-3.04)	2.28 (1.57 – 3.28)	2.18 (1.51 – 3.06)	1.80 (1.19 – 2.66)	1.83 (1.25-2.74)
fT4 (mean(SD))	16.4 (2.5)	15.2 (2.2)	15.5 (2.5)	15.3 (2.5)	15.5 (2.4)	15.5 (2.3)
Biochemical euthyroidism	362 (86.0)	230 (71.7)	419 (86.2)	2,467 (84.9)	4,513 (84.9)	1,441 (85.3)
Hypothyroidism	55 (13.1)	56 (17.4)	41 (8.4)	239 (8.2)	392 (7.4)	134 (7.9)
Subclinical	54 (12.8)	40 (12.5)	29 (6.0)	197 (67.8)	301 (5.7)	100 (5.9)
Overt	1 (0.2)	16 (5.0)	12 (2.5)	42 (1.4)	89 (1.7)	34 (2.0)
Hyperthyroidism	2 (0.5)	9 (2.8)	6 (1.2)	29 (1.0)	214 (4.0)	64 (3.8)
Subclinical	2 (0.5)	9 (2.8)	2 (0.4)	22 (0.8)	169 (3.2)	59 (3.5)
Overt	0 (0.0)	0 (0.0)	4 (0.8)	7 (0.2)	38 (0.7)	5 (0.3)
<b>Medical history</b>						
History of diabetes	0 (0.0)	5 (1.6)	20 (5.2) <sup>f</sup>	129 (4.4)	569 (10.7)	185 (11.0) <sup>j</sup>
Lipid-lowering medication use	0 (0.0)	13 (4.0)	60 (15.5) <sup>f</sup>	207 (7.1)	2,630 (49.5)	236 (14.9) <sup>k</sup>
History of thyroid disease	0 (0.0)	N.A.	N.A.	102 (3.5)	N.A.	151 (8.9)
Thyroid medication use	0 (0.0)	10 (3.1)	15 (3.9) <sup>f</sup>	61 (2.1)	231 (4.3)	44 (2.6)
Medication use influencing the thyroid gland	0 (0.0)	N.A.	2 (0.5) <sup>f</sup>	7 (0.2)	27 (0.5)	N.A.

Results are shown as n (%) unless indicated otherwise. Abbreviations: 500 FG; 500 Functional Genomics Study, GARP; the Genetics, Arthritis and Progression study, LLS; the Leiden Longevity Study, NESDA; the Netherlands Study of Depression and Anxiety, PROSPER; PROSpective Study of Pravastatin in the Elderly at Risk, RS; the Rotterdam Study, BMI; body mass index, TSH; thyroid stimulating hormone, fT4; free thyroxine, N.A.; not available.

<sup>a</sup> Information on 418 individuals, <sup>b</sup> Information on 412 individuals, <sup>c</sup> Information on 320 individuals, <sup>d</sup> Information on 477 individuals, <sup>e</sup> Information on 474 individuals, <sup>f</sup> Information on 386 individuals, <sup>g</sup> Information on 5,309 individuals, <sup>h</sup> Information on 5,314 individuals, <sup>i</sup> Information on 1681 individuals, <sup>j</sup> Information on 1687 individuals, <sup>k</sup> Information on 1589 individuals.

**Supplementary Table 3.** First stage associations between standardized TSH and fT4 within the reference range and 161 metabolomic markers

Metabolic particle	Description	TSH			fT4				
		Pooled estimate	SE	P-value	I <sup>2</sup>	Pooled estimate	SE	P-value	I <sup>2</sup>
XXL-VLDL-P	Concentration of chylomicrons and extremely large VLDL particles	0.046	0.013	6.05E-04	11.8	-0.056	0.018	1.57E-03	10.0
XXL-VLDL-L	Total lipids in chylomicrons and extremely large VLDL	0.046	0.013	6.04E-04	11.8	-0.057	0.018	1.64E-03	9.9
XXL-VLDL-PL	Phospholipids in chylomicrons and extremely large VLDL	0.046	0.013	6.29E-04	11.7	-0.055	0.018	2.54E-03	9.1
XXL-VLDL-C	Total cholesterol in chylomicrons and extremely large VLDL	0.047	0.013	1.86E-04	14.0	-0.061	0.015	4.38E-05	16.7
XXL-VLDL-CE	Cholesterol esters in chylomicrons and extremely large VLDL	0.045	0.012	1.53E-04	14.3	-0.061	0.012	9.06E-07	24.1
XXL-VLDL-FC	Free cholesterol in chylomicrons and extremely large VLDL	0.051	0.012	4.33E-05	16.7	-0.061	0.017	2.34E-04	13.5
XXL-VLDL-TG	Triglycerides in chylomicrons and extremely large VLDL	0.046	0.013	5.13E-04	12.1	-0.057	0.018	1.62E-03	9.9
XL-VLDL-P	Concentration of very large VLDL particles	0.048	0.013	2.91E-04	13.1	-0.057	0.021	5.49E-03	7.7
XL-VLDL-L	Total lipids in very large VLDL	0.048	0.014	4.18E-04	12.5	-0.056	0.021	9.46E-03	6.7
XL-VLDL-PL	Phospholipids in very large VLDL	0.046	0.015	1.75E-03	9.8	-0.057	0.019	3.15E-03	8.7
XL-VLDL-C	Total cholesterol in very large VLDL	0.048	0.012	1.01E-04	15.1	-0.058	0.017	7.40E-04	11.4
XL-VLDL-CE	Cholesterol esters in very large VLDL	0.048	0.012	6.32E-05	16.0	-0.060	0.017	3.94E-04	12.6
XL-VLDL-FC	Free cholesterol in very large VLDL	0.049	0.013	1.14E-04	14.9	-0.057	0.018	1.17E-03	10.5
XL-VLDL-TG	Triglycerides in very large VLDL	0.050	0.013	1.29E-04	14.7	-0.058	0.021	6.69E-03	7.4
L-VLDL-P	Concentration of large VLDL particles	0.051	0.014	1.62E-04	14.2	-0.050	0.024	3.97E-02	4.2
L-VLDL-L	Total lipids in large VLDL	0.050	0.015	6.65E-04	11.6	-0.046	0.026	7.91E-02	3.1
L-VLDL-PL	Phospholipids in large VLDL	0.049	0.014	5.68E-04	11.9	-0.048	0.024	4.75E-02	3.9
L-VLDL-C	Total cholesterol in large VLDL	0.052	0.014	1.10E-04	15.0	-0.051	0.023	2.97E-02	4.7
L-VLDL-CE	Cholesterol esters in large VLDL	0.052	0.013	3.59E-05	17.1	-0.051	0.022	2.22E-02	5.2
L-VLDL-FC	Free cholesterol in large VLDL	0.052	0.014	2.10E-04	13.7	-0.052	0.023	2.22E-02	5.2

Supplementary Table 3. Continued.

Metabolic particle	Description	TSH				fT4			
		Pooled estimate	Pooled SE	P-value	I <sup>2</sup>	Pooled estimate	Pooled SE	P-value	I <sup>2</sup>
L-VLDL-TG	Triglycerides in large VLDL	0.051	0.014	3.50E-04	12.8	-0.049	0.026	5.65E-02	3.6
M-VLDL-P	Concentration of medium VLDL particles	0.053	0.013	2.02E-05	18.2	-0.039	0.027	1.40E-01	2.2
M-VLDL-L	Total lipids in medium VLDL	0.052	0.013	1.30E-04	14.6	-0.036	0.028	2.00E-01	1.7
M-VLDL-PL	Phospholipids in medium VLDL	0.052	0.013	3.05E-05	17.4	-0.038	0.027	1.66E-01	1.9
M-VLDL-C	Total cholesterol in medium VLDL	0.052	0.013	5.48E-05	16.3	-0.042	0.024	8.47E-02	3.0
M-VLDL-CE	Cholesterol esters in medium VLDL	0.050	0.012	2.82E-05	17.5	-0.045	0.020	2.66E-02	4.9
M-VLDL-FC	Free cholesterol in medium VLDL	0.054	0.013	5.18E-05	16.4	-0.048	0.025	4.88E-02	3.9
M-VLDL-TG	Triglycerides in medium VLDL	0.052	0.013	4.30E-05	16.7	-0.037	0.028	1.77E-01	1.8
S-VLDL-P	Concentration of small VLDL particles	0.052	0.011	3.83E-06	21.4	-0.032	0.028	2.50E-01	1.3
S-VLDL-L	Total lipids in small VLDL	0.048	0.013	1.72E-04	14.1	-0.035	0.027	1.96E-01	1.7
S-VLDL-PL	Phospholipids in small VLDL	0.046	0.012	1.69E-04	14.1	-0.038	0.027	1.57E-01	2.0
S-VLDL-C	Total cholesterol in small VLDL	0.038	0.013	3.56E-03	8.5	-0.032	0.019	9.16E-02	2.9
S-VLDL-CE	Cholesterol esters in small VLDL	0.030	0.013	2.55E-02	5.0	-0.026	0.016	1.16E-01	2.5
S-VLDL-FC	Free cholesterol in small VLDL	0.050	0.012	3.12E-05	17.3	-0.046	0.023	4.16E-02	4.2
S-VLDL-TG	Triglycerides in small VLDL	0.053	0.011	6.22E-07	24.8	-0.038	0.028	1.74E-01	1.9
XS-VLDL-P	Concentration of very small VLDL particles	0.032	0.011	5.14E-03	7.8	-0.022	0.020	2.53E-01	1.3
XS-VLDL-L	Total lipids in very small VLDL	0.029	0.013	2.51E-02	5.0	-0.028	0.017	1.05E-01	2.6
XS-VLDL-PL	Phospholipids in very small VLDL	0.025	0.012	3.94E-02	4.2	-0.021	0.017	2.23E-01	1.5
XS-VLDL-C	Total cholesterol in very small VLDL	0.012	0.014	3.90E-01	0.7	-0.011	0.014	4.13E-01	0.7
XS-VLDL-CE	Cholesterol esters in very small VLDL	0.009	0.013	4.96E-01	0.5	-0.005	0.011	6.52E-01	0.2

Supplementary Table 3. Continued.

Metabolic particle	Description	TSH				fT4			
		Pooled estimate	Pooled SE	P-value	I <sup>2</sup>	Pooled estimate	Pooled SE	P-value	I <sup>2</sup>
XS-VLDL-FC	Free cholesterol in very small VLDL	0.016	0.013	2.26E-01	1.5	-0.018	0.015	2.22E-01	1.5
XS-VLDL-TG	Triglycerides in very small VLDL	0.052	0.010	1.43E-07	27.7	-0.051	0.023	2.65E-02	4.9
IDL-P	Concentration of IDL particles	0.021	0.011	5.47E-02	3.7	-0.012	0.010	2.49E-01	1.3
IDL-L	Total lipids in IDL	0.014	0.012	2.29E-01	1.4	-0.011	0.011	3.13E-01	1.0
IDL-PL	Phospholipids in IDL	0.011	0.011	3.50E-01	0.9	-0.004	0.010	6.82E-01	0.2
IDL-C	Total cholesterol in IDL	0.011	0.012	3.57E-01	0.9	-0.008	0.013	5.61E-01	0.3
IDL-CE	Cholesterol esters in IDL	0.014	0.012	2.53E-01	1.3	-0.012	0.015	3.98E-01	0.7
IDL-FC	Free cholesterol in IDL	0.006	0.011	5.78E-01	0.3	0.005	0.010	6.46E-01	0.2
IDL-TG	Triglycerides in IDL	0.035	0.010	4.36E-04	12.4	-0.046	0.010	5.01E-06	20.8
L-LDL-P	Concentration of large LDL particles	0.021	0.012	6.99E-02	3.3	-0.012	0.012	2.82E-01	1.2
L-LDL-L	Total lipids in large LDL	0.014	0.013	2.72E-01	1.2	-0.010	0.011	3.61E-01	0.8
L-LDL-PL	Phospholipids in large LDL	0.016	0.013	2.04E-01	1.6	-0.012	0.013	3.54E-01	0.9
L-LDL-C	Total cholesterol in large LDL	0.012	0.013	3.57E-01	0.9	-0.008	0.014	5.66E-01	0.3
L-LDL-CE	Cholesterol esters in large LDL	0.014	0.013	2.75E-01	1.2	-0.011	0.014	4.48E-01	0.6
L-LDL-FC	Free cholesterol in large LDL	0.005	0.012	6.68E-01	0.2	0.005	0.010	6.00E-01	0.3
L-LDL-TG	Triglycerides in large LDL	0.027	0.010	6.78E-03	7.3	-0.043	0.010	1.62E-05	18.6
M-LDL-P	Concentration of medium LDL particles	0.023	0.012	4.72E-02	3.9	-0.020	0.015	1.90E-01	1.7
M-LDL-L	Total lipids in medium LDL	0.016	0.013	2.06E-01	1.6	-0.016	0.014	2.56E-01	1.3
M-LDL-PL	Phospholipids in medium LDL	0.023	0.014	8.47E-02	3.0	-0.028	0.017	9.58E-02	2.8
M-LDL-C	Total cholesterol in medium LDL	0.013	0.013	3.16E-01	1.0	-0.010	0.015	4.84E-01	0.5

Supplementary Table 3. Continued.

Metabolic particle	Description	TSH				fT4			
		Pooled estimate	Pooled SE	P-value	I <sup>2</sup>	Pooled estimate	Pooled SE	P-value	I <sup>2</sup>
M-LDL-CE	Cholesterol esters in medium LDL	0.014	0.013	2.88E-01	1.1	-0.009	0.014	5.45E-01	0.4
M-LDL-FC	Free cholesterol in medium LDL	0.012	0.014	4.13E-01	0.7	-0.016	0.016	3.24E-01	1.0
M-LDL-TG	Triglycerides in medium LDL	0.023	0.010	1.82E-02	5.6	-0.037	0.012	1.96E-03	9.6
S-LDL-P	Concentration of small LDL particles	0.022	0.012	5.89E-02	3.6	-0.022	0.014	1.26E-01	2.3
S-LDL-L	Total lipids in small LDL	0.017	0.013	1.86E-01	1.8	-0.017	0.013	2.01E-01	1.6
S-LDL-PL	Phospholipids in small LDL	0.025	0.014	7.69E-02	3.1	-0.032	0.015	2.99E-02	4.7
S-LDL-C	Total cholesterol in small LDL	0.011	0.013	3.91E-01	0.7	-0.007	0.014	6.23E-01	0.2
S-LDL-CE	Cholesterol esters in small LDL	0.011	0.012	3.67E-01	0.8	-0.004	0.013	7.39E-01	0.1
S-LDL-FC	Free cholesterol in small LDL	0.013	0.015	3.88E-01	0.8	-0.015	0.015	3.33E-01	0.9
S-LDL-TG	Triglycerides in small LDL	0.040	0.010	5.38E-05	16.3	-0.059	0.010	4.76E-09	34.3
XL-HDL-P	Concentration of very large HDL particles	-0.017	0.009	8.08E-02	3.1	-0.002	0.038	9.68E-01	0.0
XL-HDL-L	Total lipids in very large HDL	-0.018	0.009	6.15E-02	3.5	-0.002	0.038	9.57E-01	0.0
XL-HDL-PL	Phospholipids in very large HDL	-0.019	0.011	7.50E-02	3.2	0.002	0.039	9.65E-01	0.0
XL-HDL-C	Total cholesterol in very large HDL	-0.015	0.010	1.11E-01	2.5	-0.001	0.035	9.80E-01	0.0
XL-HDL-CE	Cholesterol esters in very large HDL	-0.014	0.010	1.42E-01	2.2	0.000	0.034	9.91E-01	0.0
XL-HDL-FC	Free cholesterol in very large HDL	-0.012	0.010	1.91E-01	1.7	-0.005	0.037	8.90E-01	0.0
XL-HDL-TG	Triglycerides in very large HDL	0.036	0.014	1.02E-02	6.6	-0.047	0.017	5.50E-03	7.7
L-HDL-P	Concentration of large HDL particles	-0.005	0.014	6.96E-01	0.2	-0.005	0.037	8.89E-01	0.0
L-HDL-L	Total lipids in large HDL	-0.014	0.012	2.32E-01	1.4	-0.001	0.036	9.68E-01	0.0
L-HDL-PL	Phospholipids in large HDL	-0.014	0.013	3.08E-01	1.0	-0.004	0.035	9.03E-01	0.0

Supplementary Table 3. Continued.

Metabolic particle	Description	TSH				fT4			
		Pooled estimate	Pooled SE	P-value	I <sup>2</sup>	Pooled estimate	Pooled SE	P-value	I <sup>2</sup>
L-HDL-C	Total cholesterol in large HDL	-0.016	0.011	1.43E-01	2.1	0.000	0.038	9.93E-01	0.0
L-HDL-CE	Cholesterol esters in large HDL	-0.013	0.011	2.30E-01	1.4	0.002	0.037	9.47E-01	0.0
L-HDL-FC	Free cholesterol in large HDL	-0.019	0.010	6.03E-02	3.5	0.003	0.037	9.31E-01	0.0
L-HDL-TG	Triglycerides in large HDL	0.022	0.018	2.12E-01	1.6	-0.018	0.023	4.25E-01	0.6
M-HDL-P	Concentration of medium HDL particles	0.019	0.016	2.40E-01	1.4	-0.037	0.022	9.13E-02	2.9
M-HDL-L	Total lipids in medium HDL	0.010	0.016	5.23E-01	0.4	-0.024	0.019	2.12E-01	1.6
M-HDL-PL	Phospholipids in medium HDL	0.007	0.018	6.96E-01	0.2	-0.028	0.021	1.79E-01	1.8
M-HDL-C	Total cholesterol in medium HDL	0.006	0.010	5.14E-01	0.4	-0.013	0.020	5.37E-01	0.4
M-HDL-CE	Cholesterol esters in medium HDL	0.004	0.010	7.09E-01	0.1	-0.008	0.019	6.82E-01	0.2
M-HDL-FC	Free cholesterol in medium HDL	0.014	0.016	3.96E-01	0.7	-0.017	0.016	3.04E-01	1.1
M-HDL-TG	Triglycerides in medium HDL	0.042	0.018	1.86E-02	5.5	-0.070	0.017	5.31E-05	16.3
S-HDL-P	Concentration of small HDL particles	0.028	0.013	2.52E-02	5.0	-0.044	0.016	5.09E-03	7.9
S-HDL-L	Total lipids in small HDL	0.024	0.013	6.72E-02	3.4	-0.037	0.016	1.97E-02	5.4
S-HDL-PL	Phospholipids in small HDL	0.010	0.015	5.09E-01	0.4	-0.045	0.014	8.92E-04	11.0
S-HDL-C	Total cholesterol in small HDL	0.016	0.013	2.28E-01	1.5	0.014	0.017	4.01E-01	0.7
S-HDL-CE	Cholesterol esters in small HDL	0.007	0.013	5.68E-01	0.3	0.025	0.019	1.98E-01	1.7
S-HDL-FC	Free cholesterol in small HDL	0.027	0.011	1.48E-02	5.9	-0.036	0.017	4.22E-02	4.1
S-HDL-TG	Triglycerides in small HDL	0.046	0.014	1.05E-03	10.7	-0.040	0.032	2.12E-01	1.6
VLDL-D	Mean diameter for VLDL particles	0.049	0.015	1.04E-03	10.8	-0.043	0.025	8.53E-02	3.0
LDL-D	Mean diameter for LDL particles	-0.022	0.014	1.12E-01	2.5	0.032	0.017	6.67E-02	3.4

Supplementary Table 3. Continued.

Metabolic particle	Description	TSH			fT4				
		Pooled estimate	Pooled SE	P-value	I <sup>2</sup>	Pooled estimate	Pooled SE	P-value	I <sup>2</sup>
HDL-D	Mean diameter for HDL particles	-0.017	0.012	1.46E-01	2.1	0.004	0.037	9.04E-01	0.0
Serum-C	Serum total cholesterol	0.022	0.011	5.41E-02	3.7	-0.013	0.010	1.85E-01	1.8
VLDL-C	Total cholesterol in VLDL	0.041	0.014	2.69E-03	9.0	-0.049	0.020	1.31E-02	6.2
Remnant-C	Remnant cholesterol (non-HDL, non-LDL-cholesterol)	0.033	0.013	1.15E-02	6.4	-0.032	0.016	4.78E-02	3.9
LDL-C	Total cholesterol in LDL	0.011	0.013	4.11E-01	0.7	-0.007	0.014	5.88E-01	0.3
HDL-C	Total cholesterol in HDL	-0.009	0.009	3.47E-01	0.9	0.000	0.033	9.91E-01	0.0
HDL2-C	Total cholesterol in HDL2	-0.015	0.009	1.05E-01	2.6	-0.005	0.036	8.86E-01	0.0
HDL3-C	Total cholesterol in HDL3	0.024	0.013	6.48E-02	3.4	-0.005	0.013	7.22E-01	0.1
EstC	Esterified cholesterol	0.025	0.011	1.85E-02	5.6	-0.018	0.010	7.05E-02	3.3
FreeC	Free cholesterol	0.009	0.011	4.20E-01	0.7	0.001	0.010	9.59E-01	0.0
Serum-TG	Serum total triglycerides	0.051	0.013	1.31E-04	14.6	-0.055	0.025	2.88E-02	4.8
VLDL-TG	Triglycerides in VLDL	0.052	0.013	5.35E-05	16.3	-0.043	0.027	1.20E-01	2.4
LDL-TG	Triglycerides in LDL	0.030	0.010	2.07E-03	9.5	-0.041	0.010	4.34E-05	16.7
HDL-TG	Triglycerides in HDL	0.053	0.019	5.04E-03	7.9	-0.081	0.010	1.85E-15	63.2
DAG	Diacylglycerol	0.052	0.010	8.76E-07	24.2	-0.067	0.014	8.75E-07	24.2
DAG/TG	Ratio of diacylglycerol to triglycerides	0.031	0.011	4.07E-03	8.3	-0.046	0.011	2.48E-05	17.8
TotPG	Total phosphoglycerides	0.031	0.013	1.68E-02	5.7	-0.042	0.014	2.38E-03	9.2
TG/PG	Ratio of triglycerides to phosphoglycerides	0.050	0.010	1.76E-07	27.3	-0.041	0.030	1.67E-01	1.9
PC	Phosphatidylcholine and other cholines	0.031	0.014	2.82E-02	4.8	-0.025	0.017	1.48E-01	2.1
SM	Sphingomyelins	0.009	0.009	3.60E-01	0.8	0.018	0.010	5.47E-02	3.7

Supplementary Table 3. Continued.

Metabolic particle	Description	TSH			fT4		
		Pooled estimate	Pooled SE	P-value	Pooled estimate	Pooled SE	P-value
TotCho	Total cholines	0.021	0.012	8.74E-02	-0.024	0.016	1.31E-01
ApoA1	Apolipoprotein A-I	0.010	0.009	2.77E-01	-0.016	0.029	5.72E-01
ApoB	Apolipoprotein B	0.035	0.012	3.62E-03	-0.040	0.018	2.69E-02
ApoB/ApoA1	Ratio of apolipoprotein B to apolipoprotein A-I	0.031	0.012	1.18E-02	-0.030	0.028	2.93E-01
TotFA	Total fatty acids	0.048	0.012	1.10E-04	-0.044	0.014	1.79E-03
FALen	Estimated description of fatty acid chain length. not actual carbon number	0.019	0.011	7.92E-02	-0.006	0.011	5.64E-01
UnsatDeg	Estimated degree of unsaturation	-0.015	0.019	4.40E-01	0.034	0.027	2.11E-01
DHA	22:6. docosahexaenoic acid	0.045	0.010	6.67E-06	0.020	0.016	2.10E-01
LA	18:2. linoleic acid	0.033	0.012	6.58E-03	-0.020	0.010	5.03E-02
CLA	Conjugated linoleic acid	0.033	0.024	1.62E-01	-0.056	0.012	2.40E-06
FAw3	Omega-3 fatty acids	0.043	0.010	1.08E-05	-0.011	0.015	4.39E-01
FAw6	Omega-6 fatty acids	0.035	0.011	1.88E-03	-0.017	0.010	8.77E-02
PUFA	Polyunsaturated fatty acids	0.035	0.012	3.35E-03	-0.018	0.010	7.92E-02
MUFA	Monounsaturated fatty acids; 16:1, 18:1	0.049	0.015	1.24E-03	-0.043	0.018	1.73E-02
SFA	Saturated fatty acids	0.042	0.010	4.25E-05	-0.064	0.010	1.83E-10
DHA/FA	Ratio of 22:6 docosahexaenoic acid to total fatty acids	0.019	0.013	1.30E-01	0.039	0.020	5.49E-02
LA/FA	Ratio of 18:2 linoleic acid to total fatty acids	-0.025	0.013	5.73E-02	-0.003	0.030	9.18E-01
CLA/FA	Ratio of conjugated linoleic acid to total fatty acids	0.017	0.025	4.99E-01	-0.038	0.011	4.90E-04
FAw3/FA	Ratio of omega-3 fatty acids to total fatty acids	-0.002	0.025	9.44E-01	0.026	0.013	5.31E-02

Supplementary Table 3. Continued.

Metabolic particle	Description	TSH				fT4			
		Pooled estimate	Pooled SE	P-value	I <sup>2</sup>	Pooled estimate	Pooled SE	P-value	I <sup>2</sup>
FAw6/FA	Ratio of omega-6 fatty acids to total fatty acids	-0.034	0.013	9.91E-03	6.7	0.012	0.032	7.10E-01	0.1
PUFA/FA	Ratio of polyunsaturated fatty acids to total fatty acids	-0.037	0.015	1.71E-02	5.7	0.022	0.031	4.69E-01	0.5
MUFA/FA	Ratio of monounsaturated fatty acids to total fatty acids	0.032	0.014	2.45E-02	5.1	-0.010	0.020	6.35E-01	0.2
SFA/FA	Ratio of saturated fatty acids to total fatty acids	-0.011	0.017	4.99E-01	0.5	-0.042	0.024	7.87E-02	3.1
Glc	Glucose	0.029	0.022	1.82E-01	1.8	-0.053	0.034	1.18E-01	2.5
Lac	Lactate	0.014	0.012	2.36E-01	1.4	0.040	0.015	8.09E-03	7.0
Pyr	Pyruvate	0.022	0.018	2.21E-01	1.5	-0.001	0.025	9.70E-01	0.0
Cit	Citrate	0.036	0.010	3.62E-04	12.7	0.011	0.010	2.83E-01	1.2
Ala	Alanine	0.034	0.012	5.01E-03	7.9	-0.011	0.017	5.12E-01	0.4
Gln	Glutamine	-0.036	0.010	2.71E-04	13.3	0.000	0.018	9.82E-01	0.0
His	Histidine	-0.003	0.010	7.68E-01	0.1	-0.052	0.016	8.51E-04	11.1
Ile	Isoleucine	0.045	0.016	4.81E-03	8.0	-0.075	0.030	1.12E-02	6.4
Leu	Leucine	0.024	0.018	1.65E-01	1.9	-0.066	0.030	2.53E-02	5.0
Val	Valine	0.015	0.017	3.70E-01	0.8	-0.063	0.032	4.72E-02	3.9
Phe	Phenylalanine	0.033	0.010	9.77E-04	10.9	-0.059	0.024	1.40E-02	6.0
Tyr	Tyrosine	0.011	0.013	3.71E-01	0.8	-0.045	0.012	1.71E-04	14.1
Ace	Acetate	0.013	0.010	2.08E-01	1.6	-0.031	0.025	2.15E-01	1.5
AcAce	Acetoacetate	0.002	0.012	8.76E-01	0.0	0.063	0.017	3.16E-04	13.0
bOHBut	3-hydroxybutyrate	-0.007	0.012	5.41E-01	0.4	0.067	0.026	1.02E-02	6.6
Crea	Creatinine	0.078	0.009	1.17E-17	73.2	0.029	0.012	1.18E-02	6.3

Supplementary Table 3. Continued.

Metabolic particle	Description	TSH			fT4		
		Pooled estimate	Pooled SE	P-value	Pooled estimate	Pooled SE	P-value
Alb	Albumin	0.060	0.010	2.40E-09	0.053	0.028	5.73E-02
Gp	Glycoprotein acetyls. mainly a1-acid glycoprotein	0.047	0.010	3.13E-06	0.036	0.029	2.09E-01
				35.6	21.7		3.6
							1.6

Abbreviations: TSH; thyroid stimulating hormone, fT4; free thyroxine, SE; standard error. Pooled estimates, standard errors and p-values were derived from a random effects model meta-analysis of study-level multivariable regression adjusted for age, sex, body mass index and smoking status.

**Supplementary Table 4.** Second stage associations between metabolomic markers associated with TSH and fT4 in Mendelian randomization analyses and Bruker platform

Metabolic particle	Bruker name	Bruker description	Mendelian randomization			Bruker platform		
			Pooled estimate	Pooled SE	P-value	Estimate	SE	P-value
<b>TSH results</b>								
XXL-VLDL-P			0.028	0.024	2.47E-01	N.A.	N.A.	N.A.
XXL-VLDL-L			0.023	0.023	3.32E-01	N.A.	N.A.	N.A.
XXL-VLDL-PL	V1PL	VLDL1 Phospholipids (mg/dL)	0.035	0.024	1.38E-01	0.077	0.031	1.35E-02
XXL-VLDL-C	V1CH	VLDL1 Cholesterol (mg/dL)	N.A.	N.A.	N.A.	0.089	0.032	5.65E-03
XXL-VLDL-FC	V1FC	VLDL1 Free Cholesterol (mg/dL)	N.A.	N.A.	N.A.	0.094	0.032	3.43E-03
XXL-VLDL-TG	V1TG	VLDL1 Triglycerides (mg/dL)	0.040	0.023	8.51E-02	0.080	0.031	9.79E-03
XL-VLDL-P			0.023	0.023	3.15E-01	N.A.	N.A.	N.A.
XL-VLDL-L			0.020	0.024	4.07E-01	N.A.	N.A.	N.A.
XL-VLDL-C	V2CH	VLDL2 Cholesterol (mg/dL)	N.A.	N.A.	N.A.	0.087	0.033	9.42E-03
XL-VLDL-FC	V2FC	VLDL2 Free Cholesterol (mg/dL)	N.A.	N.A.	N.A.	0.089	0.034	9.67E-03
XL-VLDL-TG	V2TG	VLDL2 Triglycerides (mg/dL)	0.027	0.024	2.65E-01	0.094	0.032	3.85E-03
L-VLDL-P			0.029	0.022	1.96E-01	N.A.	N.A.	N.A.
L-VLDL-L			0.026	0.023	2.61E-01	N.A.	N.A.	N.A.
L-VLDL-PL	V3PL	VLDL3 Phospholipids (mg/dL)	0.027	0.022	2.07E-01	0.079	0.034	1.85E-02
L-VLDL-C	V3CH	VLDL3 Cholesterol (mg/dL)	0.031	0.023	1.71E-01	0.080	0.034	1.85E-02
L-VLDL-CE			0.031	0.022	1.56E-01	N.A.	N.A.	N.A.
L-VLDL-FC	V3FC	VLDL3 Free Cholesterol (mg/dL)	0.030	0.023	1.89E-01	0.084	0.034	1.25E-02
L-VLDL-TG	V3TG	VLDL3 Triglycerides (mg/dL)	0.037	0.022	9.54E-02	0.080	0.033	1.76E-02
M-VLDL-P			0.031	0.023	1.72E-01	N.A.	N.A.	N.A.
M-VLDL-L			0.038	0.023	9.25E-02	N.A.	N.A.	N.A.
M-VLDL-PL	V4PL	VLDL4 Phospholipids (mg/dL)	0.041	0.022	6.46E-02	0.060	0.034	7.47E-02

Supplementary Table 4. Continued.

Metabolic particle	Bruker name	Bruker description	Mendelian randomization				Bruker platform	
			Pooled estimate	Pooled SE	P-value	Estimate	SE	P-value
M-VLDL-C	V4CH	VLDL4 Cholesterol (mg/dL)	0.042	0.021	5.28E-02	0.052	0.034	1.30E-01
M-VLDL-CE			0.038	0.022	8.26E-02	N.A.	N.A.	N.A.
M-VLDL-FC	V4FC	VLDL4 Free Cholesterol (mg/dL)	0.044	0.023	5.19E-02	0.066	0.034	5.35E-02
M-VLDL-TG	V4TG	VLDL4 Triglycerides (mg/dL)	0.042	0.022	6.00E-02	0.057	0.034	9.29E-02
S-VLDL-P			0.045	0.022	3.97E-02	N.A.	N.A.	N.A.
S-VLDL-L			0.048	0.022	2.93E-02	N.A.	N.A.	N.A.
S-VLDL-PL	V5PL	VLDL5 Phospholipids (mg/dL)	0.051	0.021	1.76E-02	0.059	0.036	1.07E-01
S-VLDL-FC	V5FC	VLDL5 Free Cholesterol (mg/dL)	0.045	0.021	3.77E-02	0.026	0.047	5.80E-01
S-VLDL-TG	V5TG	VLDL5 Triglycerides (mg/dL)	0.043	0.022	5.47E-02	0.041	0.034	2.29E-01
XS-VLDL-TG	V6TG	VLDL6 Triglycerides (mg/dL)	0.031	0.022	1.60E-01	0.020	0.036	5.74E-01
IDL-TG	IDTG	IDL Triglycerides (mg/dL)	0.008	0.022	7.08E-01	0.084	0.033	1.18E-02
S-HDL-TG	H4TG	HDL4 Triglycerides (mg/dL)	0.000	0.022	9.87E-01	0.058	0.035	9.29E-02
VLDL-D			0.030	0.023	1.99E-01	N.A.	N.A.	N.A.
Serum-TG	TPTG	Total Triglycerides (mg/dL)	0.041	0.022	6.54E-02	0.095	0.032	2.75E-03
VLDL-TG	VLTG	VLDL Triglycerides (mg/dL)	N.A.	N.A.	N.A.	0.087	0.032	5.89E-03
TotFA			0.055	0.024	2.24E-02	N.A.	N.A.	N.A.
DHA			0.023	0.026	3.74E-01	N.A.	N.A.	N.A.
FAw3			0.038	0.026	1.44E-01	N.A.	N.A.	N.A.
MUFA			0.036	0.024	1.34E-01	N.A.	N.A.	N.A.
Cit			-0.032	0.021	1.31E-01	N.A.	N.A.	N.A.
Gln			0.042	0.023	5.93E-02	N.A.	N.A.	N.A.

Supplementary Table 4. Continued.

Metabolic particle	Bruker name	Bruker description	Mendelian randomization				Bruker platform		
			Pooled estimate	Pooled SE	P-value	Estimate	SE	P-value	
Phe			-0.029	0.022	1.84E-01	N.A.	N.A.	N.A.	N.A.
Crea			0.046	0.024	5.20E-02	N.A.	N.A.	N.A.	N.A.
Alb			-0.001	0.022	9.49E-01	N.A.	N.A.	N.A.	N.A.
Gp			-0.013	0.023	5.71E-01	N.A.	N.A.	N.A.	N.A.
<b>fT4 results</b>									
XXL-VLDL-C	V1CH	VLDL1 Cholesterol (mg/dL)	N.A.	N.A.	N.A.	-0.056	0.032	7.57E-02	
XXL-VLDL-FC	V1FC	VLDL1 Free Cholesterol (mg/dL)	N.A.	N.A.	N.A.	-0.052	0.032	1.03E-01	
XL-VLDL-C	V2CH	VLDL2 Cholesterol (mg/dL)	N.A.	N.A.	N.A.	-0.062	0.033	5.77E-02	
XL-VLDL-FC	V2FC	VLDL2 Free Cholesterol (mg/dL)	N.A.	N.A.	N.A.	-0.057	0.033	8.60E-02	
IDL-TG	IDTG	IDL Triglycerides (mg/dL)	-0.012	0.032	6.97E-01	-0.081	0.032	1.26E-02	
M-HDL-TG	H3TG	HDL3 Triglycerides (mg/dL)	N.A.	N.A.	N.A.	-0.094	0.034	6.15E-03	
S-HDL-PL	H4PL	HDL4 Phospholipids (mg/dL)	N.A.	N.A.	N.A.	-0.111	0.036	1.85E-03	
LDL-TG	LDTG	LDL Triglycerides (mg/dL)	N.A.	N.A.	N.A.	-0.064	0.032	4.75E-02	
HDL-TG	HDTG	HDL Triglycerides (mg/dL)	N.A.	N.A.	N.A.	-0.094	0.034	5.30E-03	
His			-0.009	0.035	7.99E-01	N.A.	N.A.	N.A.	N.A.
Tyr			0.014	0.035	6.86E-01	N.A.	N.A.	N.A.	N.A.
AcAce			0.044	0.033	1.81E-01	N.A.	N.A.	N.A.	N.A.

Abbreviations: TSH; thyroid stimulating hormone, fT4; free thyroxine, SE; standard error; N.A.; not available.

Pooled estimates, standard errors and p-values for Mendelian randomization were derived from a fixed effects model meta-analysis of study-level inverse-variance weighted analysis.

Effect estimates, standard errors and p-values for the Bruker platform were derived from a single study multivariable regression analysis adjusted for age, sex, body mass index and smoking status.



**Supplementary Table 5.** Sensitivity analyses for metabolomic markers associated with TSH and fT4 in a restricted population without thyroid medication, lipid-lowering medication or history of diabetes

Metabolic particle	Pooled estimate	Pooled SE	P-value	I <sup>2</sup>
<b>TSH results</b>				
XXL-VLDL-P	0.041	0.019	3.29E-02	4.55
XXL-VLDL-L	0.040	0.020	4.63E-02	3.97
XXL-VLDL-PL	0.041	0.020	4.75E-02	3.93
XXL-VLDL-C	0.038	0.019	4.39E-02	4.06
XXL-VLDL-CE	0.036	0.017	3.77E-02	4.32
XXL-VLDL-FC	0.042	0.020	3.21E-02	4.59
XXL-VLDL-TG	0.041	0.020	3.71E-02	4.35
XL-VLDL-P	0.041	0.020	3.71E-02	4.34
XL-VLDL-L	0.037	0.021	7.65E-02	3.14
XL-VLDL-C	0.037	0.020	5.85E-02	3.58
XL-VLDL-CE	0.037	0.019	5.17E-02	3.79
XL-VLDL-FC	0.041	0.019	3.72E-02	4.34
XL-VLDL-TG	0.040	0.020	5.01E-02	3.84
L-VLDL-P	0.040	0.021	5.14E-02	3.80
L-VLDL-L	0.032	0.023	1.61E-01	1.97
L-VLDL-PL	0.038	0.021	7.38E-02	3.20
L-VLDL-C	0.037	0.021	7.73E-02	3.12
L-VLDL-CE	0.037	0.019	4.33E-02	4.08
L-VLDL-FC	0.039	0.022	7.09E-02	3.26
L-VLDL-TG	0.036	0.022	1.03E-01	2.65
M-VLDL-P	0.039	0.019	4.12E-02	4.17
M-VLDL-L	0.032	0.021	1.36E-01	2.22
M-VLDL-PL	0.038	0.019	4.58E-02	3.99
M-VLDL-C	0.033	0.020	1.03E-01	2.66
M-VLDL-CE	0.031	0.018	8.76E-02	2.92
M-VLDL-FC	0.037	0.021	8.23E-02	3.02
M-VLDL-TG	0.036	0.020	7.61E-02	3.15
S-VLDL-P	0.030	0.018	8.71E-02	2.93
S-VLDL-L	0.024	0.018	1.95E-01	1.68
S-VLDL-PL	0.020	0.020	3.22E-01	0.98
S-VLDL-FC	0.028	0.017	1.04E-01	2.65
S-VLDL-TG	0.031	0.019	9.84E-02	2.73
XS-VLDL-TG	0.025	0.016	1.20E-01	2.41
IDL-TG	0.006	0.017	7.34E-01	0.12
S-LDL-TG	0.010	0.023	6.45E-01	0.21

**Supplementary Table 5.** Continued.

Metabolic particle	Pooled estimate	Pooled SE	P-value	I <sup>2</sup>
S-HDL-TG	0.022	0.023	3.41E-01	0.91
VLDL-D	0.038	0.022	8.49E-02	2.97
Serum-TG	0.023	0.023	3.32E-01	0.94
VLDL-TG	0.033	0.021	1.08E-01	2.59
DAG	0.033	0.018	6.90E-02	3.31
TG/PG	0.024	0.018	1.79E-01	1.81
TotFA	0.021	0.023	3.76E-01	0.78
DHA	0.038	0.013	3.86E-03	8.35
FAw3	0.034	0.013	8.05E-03	7.02
MUFA	0.010	0.029	7.20E-01	0.13
SFA	0.022	0.024	3.60E-01	0.84
Cit	0.028	0.013	3.27E-02	4.56
Gln	-0.032	0.013	1.56E-02	5.85
Phe	0.034	0.013	1.16E-02	6.37
Crea	0.073	0.012	3.33E-10	39.47
Alb	0.064	0.013	1.19E-06	23.60
Gp	0.037	0.017	2.90E-02	4.77
<b>ft4 results</b>				
XXL-VLDL-C	-0.058	0.025	1.74E-02	5.66
XXL-VLDL-CE	-0.057	0.023	1.26E-02	6.23
XXL-VLDL-FC	-0.061	0.025	1.69E-02	5.71
XL-VLDL-C	-0.060	0.025	1.76E-02	5.64
XL-VLDL-CE	-0.061	0.025	1.26E-02	6.23
XL-VLDL-FC	-0.057	0.026	2.62E-02	4.94
IDL-TG	-0.051	0.016	1.12E-03	10.61
L-LDL-TG	-0.052	0.013	6.49E-05	15.95
S-LDL-TG	-0.062	0.013	2.11E-06	22.49
M-HDL-TG	-0.081	0.019	2.13E-05	18.07
S-HDL-PL	-0.039	0.024	1.08E-01	2.59
LDL-TG	-0.051	0.013	8.53E-05	15.44
HDL-TG	-0.094	0.013	3.50E-12	48.38
DAG	-0.075	0.023	1.10E-03	10.65
DAG/TG	-0.051	0.014	3.57E-04	12.75
CLA	-0.062	0.020	1.72E-03	9.83
SFA	-0.057	0.016	3.34E-04	12.87
CLA/FA	-0.045	0.019	1.73E-02	5.66

**Supplementary Table 5.** Continued.

<b>Metabolic particle</b>	<b>Pooled estimate</b>	<b>Pooled SE</b>	<b>P-value</b>	<b>I<sup>2</sup></b>
His	-0.052	0.016	1.02E-03	10.79
Tyr	-0.037	0.017	3.09E-02	4.66
AcAce	0.080	0.015	2.47E-07	26.63

Abbreviations: TSH; thyroid stimulating hormone, fT4; free thyroxine, SE; standard error, N.A.; not available.

Pooled estimates, standard errors and p-values were derived from a random effects model meta-analysis of study-level multivariable regression adjusted for age, sex, body mass index and smoking status excluding all participants who used thyroid therapy or lipid-lowering medication or had diabetes mellitus.

Supplementary Table 6. Sensitivity analyses for Mendelian randomization analyses of metabolomic markers and TSH and fT4

Metabolic particle	Bruker name	MR Egger			WME			MR Bruker platform		
		Pooled estimate	Pooled SE	P-value	Pooled estimate	Pooled SE	P-value	Estimate	SE	P-value
<b>TSH results</b>										
XXL-VLDL-P		0.033	0.061	5.84E-01	0.022	0.033	5.03E-01	N.A.	N.A.	N.A.
XXL-VLDL-L		0.004	0.058	9.39E-01	0.019	0.032	5.52E-01	N.A.	N.A.	N.A.
XXL-VLDL-PL	V1PL	0.027	0.059	6.41E-01	0.036	0.033	2.79E-01	-0.036	0.103	7.28E-01
XXL-VLDL-C	V1CH	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	0.037	0.103	7.20E-01
XXL-VLDL-FC	V1FC	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	-0.017	0.092	8.52E-01
XXL-VLDL-TG	V1TG	0.049	0.058	4.01E-01	0.031	0.032	3.23E-01	-0.040	0.113	7.21E-01
XL-VLDL-P		0.003	0.056	9.57E-01	0.022	0.033	5.00E-01	N.A.	N.A.	N.A.
XL-VLDL-L		0.005	0.059	9.26E-01	0.019	0.034	5.89E-01	N.A.	N.A.	N.A.
XL-VLDL-C	V2CH	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	0.047	0.112	6.75E-01
XL-VLDL-FC	V2FC	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	0.040	0.098	6.86E-01
XL-VLDL-TG	V2TG	0.007	0.057	9.08E-01	0.022	0.032	4.88E-01	0.031	0.112	7.78E-01
L-VLDL-P		0.028	0.055	6.16E-01	0.014	0.033	6.66E-01	N.A.	N.A.	N.A.
L-VLDL-L		0.010	0.058	8.62E-01	0.016	0.033	6.32E-01	N.A.	N.A.	N.A.
L-VLDL-PL	V3PL	0.027	0.054	6.17E-01	0.024	0.032	4.50E-01	0.015	0.111	8.91E-01
L-VLDL-C	V3CH	0.043	0.057	4.54E-01	0.022	0.033	5.04E-01	0.029	0.120	8.07E-01
L-VLDL-CE		0.046	0.055	3.97E-01	0.058	0.033	7.68E-02	N.A.	N.A.	N.A.
L-VLDL-FC	V3FC	0.026	0.057	6.50E-01	0.034	0.032	2.93E-01	0.037	0.103	7.19E-01
L-VLDL-TG	V3TG	0.037	0.056	5.07E-01	0.023	0.033	4.85E-01	0.050	0.113	6.57E-01
M-VLDL-P		-0.015	0.056	7.88E-01	0.029	0.033	3.80E-01	N.A.	N.A.	N.A.
M-VLDL-L		0.006	0.057	9.22E-01	0.030	0.033	3.69E-01	N.A.	N.A.	N.A.
M-VLDL-PL	V4PL	0.021	0.055	6.96E-01	0.041	0.033	2.14E-01	-0.011	0.132	9.34E-01

Supplementary Table 6. Continued.

Metabolic particle	Bruker name	MR Egger			WME			MR Bruker platform		
		Pooled estimate	Pooled SE	P-value	Pooled estimate	Pooled SE	P-value	Estimate	SE	P-value
M-VLDL-C	V4CH	0.021	0.053	6.93E-01	0.013	0.032	6.85E-01	-0.014	0.132	9.18E-01
M-VLDL-CE	V4FC	-0.001	0.054	9.91E-01	0.015	0.034	6.56E-01	N.A.	N.A.	N.A.
M-VLDL-FC	V4FC	0.022	0.056	6.99E-01	0.034	0.032	2.82E-01	-0.008	0.132	9.54E-01
M-VLDL-TG	V4TG	0.028	0.056	6.15E-01	0.044	0.032	1.72E-01	0.035	0.133	7.92E-01
S-VLDL-P	V4TG	-0.001	0.054	9.86E-01	0.032	0.033	3.36E-01	N.A.	N.A.	N.A.
S-VLDL-L	V5PL	-0.001	0.054	9.80E-01	0.028	0.033	3.96E-01	N.A.	N.A.	N.A.
S-VLDL-PL	V5PL	0.008	0.053	8.77E-01	0.023	0.032	4.61E-01	-0.062	0.139	6.54E-01
S-VLDL-FC	V5FC	0.003	0.054	9.51E-01	0.011	0.033	7.46E-01	0.120	0.150	4.22E-01
S-VLDL-TG	V5TG	0.024	0.055	6.59E-01	0.015	0.033	6.45E-01	0.066	0.136	6.28E-01
XS-VLDL-TG	V6TG	0.008	0.061	8.90E-01	0.027	0.033	4.04E-01	0.164	0.123	1.81E-01
IDL-TG	IDTG	-0.044	0.054	4.13E-01	0.015	0.031	6.35E-01	0.105	0.118	3.77E-01
S-HDL-TG	H4TG	-0.015	0.055	7.82E-01	-0.009	0.031	7.70E-01	0.027	0.144	8.51E-01
VLDL-D	VLDL-D	0.003	0.057	9.53E-01	0.018	0.033	5.89E-01	N.A.	N.A.	N.A.
Serum-TG	TPTG	0.037	0.055	5.03E-01	0.027	0.033	4.16E-01	0.045	0.109	6.82E-01
VLDL-TG	VLTG	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	0.040	0.102	6.98E-01
TotFA	TotFA	-0.009	0.059	8.85E-01	0.064	0.036	7.56E-02	N.A.	N.A.	N.A.
DHA	DHA	0.060	0.063	3.37E-01	0.036	0.036	3.21E-01	N.A.	N.A.	N.A.
FAw3	FAw3	0.034	0.064	5.96E-01	0.028	0.038	4.61E-01	N.A.	N.A.	N.A.
MUFA	MUFA	-0.025	0.059	6.69E-01	0.025	0.036	4.96E-01	N.A.	N.A.	N.A.
Cit	Cit	-0.063	0.052	2.29E-01	-0.057	0.030	6.04E-02	N.A.	N.A.	N.A.
Gln	Gln	-0.080	0.055	1.43E-01	0.001	0.031	9.62E-01	N.A.	N.A.	N.A.
Phe	Phe	-0.106	0.053	4.75E-02	-0.049	0.031	1.13E-01	N.A.	N.A.	N.A.

Supplementary Table 6. Continued.

Metabolic particle	Bruker name	MR Egger			WME			MR Bruker platform		
		Pooled estimate	Pooled SE	P-value	Pooled estimate	Pooled SE	P-value	Estimate	SE	P-value
Crea		0.008	0.058	8.93E-01	0.019	0.032	5.54E-01	N.A.	N.A.	N.A.
Alb		-0.020	0.056	7.14E-01	-0.018	0.033	5.75E-01	N.A.	N.A.	N.A.
Gp		-0.008	0.057	8.93E-01	-0.005	0.033	8.87E-01	N.A.	N.A.	N.A.
<b>fT4 results</b>										
XXL-VLDL-C	V1CH	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	0.247	0.166	1.36E-01
XXL-VLDL-CE		N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
XXL-VLDL-FC	V1FC	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	0.158	0.161	3.25E-01
XL-VLDL-C	V2CH	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	0.216	0.148	1.43E-01
XL-VLDL-FC	V2FC	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	0.213	0.144	1.40E-01
IDL-TG	IDTG	-0.008	0.076	9.13E-01	0.000	0.043	9.93E-01	0.311	0.176	7.72E-02
M-HDL-TG	H3TG	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	0.169	0.207	4.13E-01
S-HDL-PL	H4PL	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	-0.131	0.207	5.26E-01
LDL-TG	LDTG	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	0.180	0.182	3.22E-01
HDL-TG	HDTG	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	0.048	0.209	8.20E-01
His		-0.109	0.081	1.79E-01	-0.080	0.042	5.65E-02	N.A.	N.A.	N.A.
Tyr		0.057	0.085	5.03E-01	0.031	0.041	4.45E-01	N.A.	N.A.	N.A.
AcAce		-0.093	0.078	2.30E-01	0.008	0.046	8.69E-01	N.A.	N.A.	N.A.

Abbreviations: TSH; thyroid stimulating hormone, fT4; free thyroxine, SE; standard error, MR; Mendelian randomization; WME; Weighted Median Estimator, N.A.; not available.

Pooled estimates, standard errors and p-values for Mendelian randomization sensitivity analyses MR Egger and WME analyses were derived from a fixed effects model meta-analysis of study-level analyses.

Effect estimates, standard errors and p-values for Mendelian randomization on the Bruker platform were derived from a single study inverse-variance weighted analysis.

**Supplementary Table 7.** Associations between metabolomic markers associated with TSH and fT4 and biochemical thyroid dysfunction

Metabolic particle	Hypothyroidism			Hyperthyroidism			I <sup>2</sup>
	Pooled estimate	Pooled SE	P-value	Pooled estimate	Pooled SE	P-value	
<b>TSH results</b>							
XXL-VLDL-P	0.373	0.147	1.14E-02	-0.157	0.135	2.43E-01	1.36
XXL-VLDL-L	0.376	0.147	1.04E-02	-0.157	0.136	2.47E-01	1.34
XXL-VLDL-PL	0.359	0.147	1.46E-02	-0.162	0.137	2.38E-01	1.39
XXL-VLDL-C	0.403	0.155	9.20E-03	-0.139	0.133	2.95E-01	1.10
XXL-VLDL-CE	0.422	0.158	7.64E-03	-0.126	0.133	3.46E-01	0.89
XXL-VLDL-FC	0.420	0.175	1.63E-02	-0.174	0.132	1.88E-01	1.73
XXL-VLDL-TG	0.385	0.153	1.20E-02	-0.156	0.142	2.72E-01	1.21
XL-VLDL-P	0.403	0.166	1.49E-02	-0.181	0.143	2.06E-01	1.60
XL-VLDL-L	0.427	0.172	1.27E-02	-0.176	0.147	2.32E-01	1.43
XL-VLDL-C	0.441	0.171	9.89E-03	-0.153	0.132	2.47E-01	1.34
XL-VLDL-CE	0.455	0.175	9.44E-03	-0.157	0.132	2.35E-01	1.41
XL-VLDL-FC	0.443	0.177	1.24E-02	-0.147	0.132	2.66E-01	1.24
XL-VLDL-TG	0.444	0.183	1.52E-02	-0.200	0.150	1.82E-01	1.78
L-VLDL-P	0.388	0.163	1.71E-02	-0.171	0.137	2.12E-01	1.56
L-VLDL-L	0.399	0.160	1.29E-02	-0.193	0.140	1.68E-01	1.90
L-VLDL-PL	0.437	0.181	1.58E-02	-0.178	0.133	1.80E-01	1.80
L-VLDL-C	0.430	0.170	1.16E-02	-0.185	0.132	1.59E-01	1.99
L-VLDL-CE	0.445	0.173	1.00E-02	-0.160	0.132	2.26E-01	1.46
L-VLDL-FC	0.428	0.180	1.73E-02	-0.204	0.132	1.21E-01	2.41
L-VLDL-TG	0.397	0.167	1.78E-02	-0.190	0.140	1.73E-01	1.85



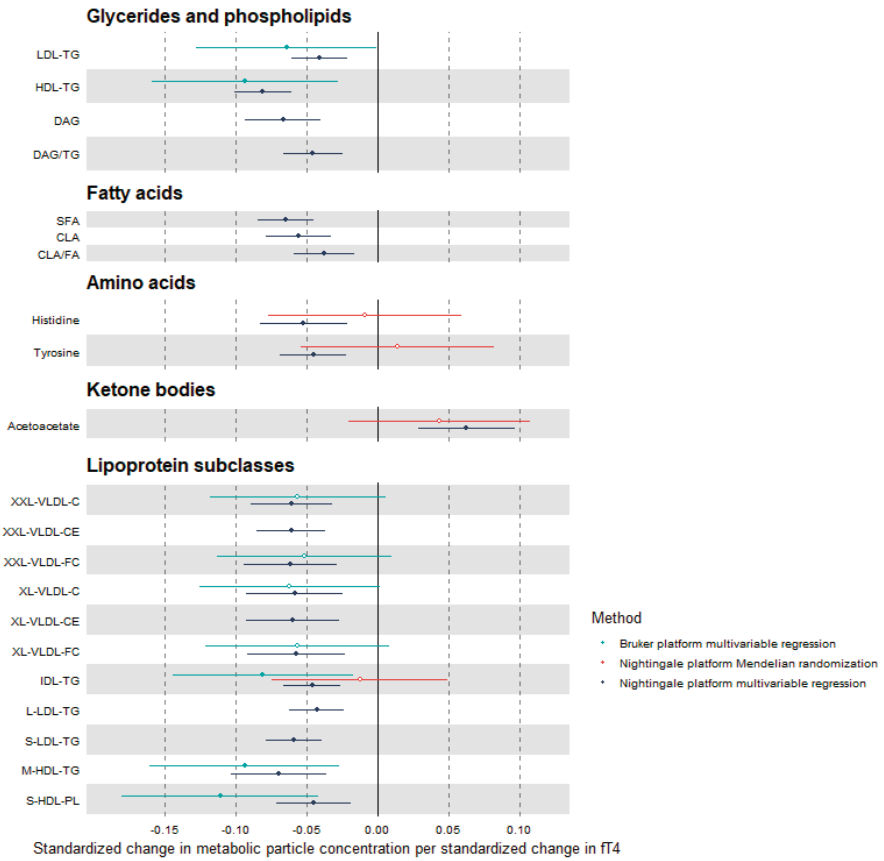




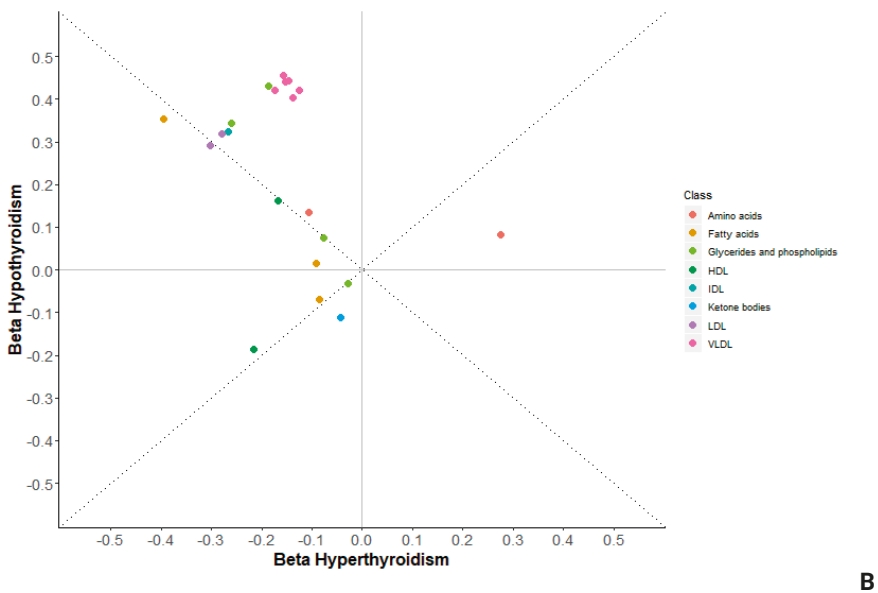
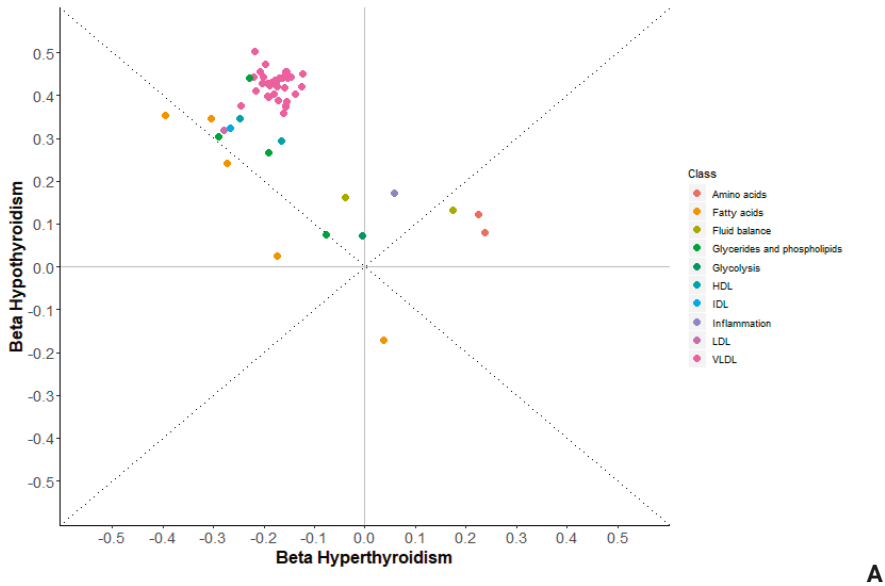
**Supplementary Table 8.** Results for sensitivity analyses for MR on thyroid status and CAD

	IVW			WME			MR Egger			MR-PRESSO		
	Odds Ratio (95% CI)	P-value	Odds Ratio (95% CI)	Odds Ratio (95% CI)	P-value	Odds Ratio (95% CI)	Intercept (95% CI)	P-value	P-value	Global P-value	Distortion P-value	
<b>TSH</b>												
CARDIoGRAM	1.09 (0.97;1.21)	0.13	1.07 (0.95;1.21)	0.27	0.96 (0.73;1.26)	0.78	0.01 (-0.01;0.03)	0.34	<0.001	<0.001	0.67	
UK Biobank	1.01 (0.96;1.06)	0.71	1.03 (0.98;1.09)	0.25	1.02 (0.91;1.15)	0.71	-0.00 (-0.01;0.01)	0.80	<0.001	<0.001	0.67	
FinnGen	1.05 (0.97;1.13)	0.20	1.12 (1.01;1.24)	0.01	0.98 (0.81;1.19)	0.83	0.01 (-0.01;0.02)	0.44	0.13	0.13	N.A.	
Overall	1.03 (0.99;1.07)	0.16	1.06 (1.00;1.12)	0.04	1.00 (0.91;1.10)	0.92	N.A.	N.A.	N.A.	N.A.	N.A.	
<b>ft4</b>												
CARDIoGRAM	0.94 (0.81;1.08)	0.39	1.09 (0.90;1.30)	0.38	1.11 (0.77;1.61)	0.58	-0.01 (-0.04;0.01)	0.35	0.11	0.11	N.A.	
UK Biobank	1.01 (0.97;1.05)	0.71	0.99 (0.92;1.06)	0.70	1.03 (0.93;1.14)	0.52	-0.00 (-0.01;0.01)	0.59	0.54	0.54	N.A.	
FinnGen	1.01 (0.90;1.13)	0.90	1.01 (0.89;1.16)	0.85	0.90 (0.68;1.17)	0.43	0.01 (-0.01;0.03)	0.36	0.09	0.09	N.A.	
Overall	1.00 (0.96;1.04)	0.89	1.00 (0.95;1.05)	0.98	1.02 (0.93;1.12)	0.65	N.A.	N.A.	N.A.	N.A.	N.A.	

Abbreviations: IVW; Inverse-variance weighted, WME; Weighted Median Estimator, MR-PRESSO; Mendelian randomization pleiotropy residual sum and outlier, TSH; thyroid stimulating hormone, ft4; free thyroxine, N.A.; not applicable.



**Supplementary Figure 1.** Second stage associations between fT4 and 21 metabolomic markers. Estimates derived from multivariable regression and Mendelian randomization analyses on Nightingale platform and multivariable regression analysis on Bruker metabolomics platform.



**Supplementary Figure 2.** Association of thyroid dysfunction with metabolomic markers identified for TSH and fT4 in first stage

A) Associations between hypo- and hyperthyroidism and 52 metabolomic markers associated with TSH

B) Associations between hypo- and hyperthyroidism and 21 metabolomic markers associated with fT4

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# CHAPTER 10

General discussion and future perspectives

## GENERAL DISCUSSION

In this thesis, we aimed to investigate the potential causality of associations between circulating concentrations of thyroid parameters and markers of age-related diseases. We conducted multi-cohort studies, Mendelian randomization and a combination of those two techniques in an attempt to approach a causal estimate in the absence of sufficiently large randomized trials.

The research described in this thesis was not performed in isolation, but within a larger framework of diverse disciplines and fields of research. Fundamental research into physiology and the pathophysiological mechanisms of diseases is essential for gaining deeper understanding of disease etiologies. Studies involving human participants are often not suitable for this type of research. Within the THYRAGE project, several novel techniques were developed with cultured cells and with model organisms such as mice and tadpoles.<sup>1, 2</sup> These advanced techniques have contributed greatly to the knowledge of thyroid hormone action at the cellular level. In the brain and skeleton, the presence of specific thyroid hormone transporters was shown to be an essential regulator of maintenance and repair.<sup>3, 4</sup> Moreover, the balanced expression of deiodinases (enzymes activating and deactivating thyroid hormones) proved crucial for growth of skeletal muscle and of malignant tumors.<sup>5, 6</sup> However, the most striking common finding in all of these different tissues has been the importance of spatiotemporal fine-tuning of thyroid hormone levels dependent on the current needs of tissues and even at single-cell level.<sup>7</sup> Thus, thyroid hormones do appear to play a causal role in tissue maintenance and repair, although the accuracy of timing and tuning might be more important than the overall thyroid status. We hypothesized that loss of tissue maintenance and repair contributes to the aging process and to the development of age-related disease, which was further elaborated in **Chapter 2**. By extension, we hypothesized that thyroid hormones might also be causally involved in aging and age-related diseases.

Translation of fundamental research to human subjects is complex. Classical observational studies can be performed to assess whether similar relationships are apparent. Since the most universal characteristic of aging is increasing mortality<sup>8</sup>, we first explored the association between thyroid parameters and mortality. As described in **Chapter 3**, we observed a lower mortality in nonagenarians with relatively more active thyroid hormone (fT3) and relatively less inactive thyroid hormone (fT4) in the circulation. As this observation was irrespective of familial longevity, we concluded that those parameters might be a universal characteristic of longevity. Interestingly, circulating TSH levels were not associated with mortality in this population. These observations were in line with another study in a younger cohort with a mean age of 65 years; here higher

fT4 was also consistently associated with higher mortality while associations with TSH were inconsistent.<sup>9</sup>

In addition, we were interested whether thyroid status was associated with age-related diseases. Within the thyroid studies collaboration several large multi-cohort studies have been performed using individual participant data; these studies have indicated associations between thyroid dysfunction and coronary heart disease, heart failure, fractures and anemia.<sup>10-13</sup> We replicated the association between thyroid dysfunction and anemia based on clinical diagnosis registration in **Chapter 6**. As the literature for the relationship between thyroid status and cognitive decline had been inconclusive<sup>14-17</sup>, we performed a multicohort study using individual participant data described in **Chapter 4**. In this study we did not find evidence for an association between a single measurement of thyroid dysfunction and cognitive function, future cognitive decline or developing dementia. These negative findings were in line with another multicohort study among individuals of 80 years or older, in which no association was observed between subclinical or overt thyroid dysfunction and cognitive function and various other functional outcomes.<sup>18</sup> Overall, the classic observational studies so far did show some associations between thyroid status and age-related diseases though causality cannot be ascertained with these designs.

In addition to classical observational studies, Mendelian randomization can be applied as an alternative approach to investigate potential causal associations. In Mendelian randomization, genetic variation is used as a natural experiment to allocate traits randomly, presumably independent of other characteristics.<sup>19</sup> This independence of traits is an assumption based on Mendel's second law, though in practice it has been difficult to prove with more complex traits. Nevertheless, the types of bias encountered are generally different from classical observational studies, which at least offers a complementary source of evidence. In **Chapter 5**, we assessed whether genetically determined variation in TSH was associated with bone mineral density as an intermediate outcome for osteoporosis. We did not observe an association, which could be interpreted as no relationship between long-term slightly lower TSH within the reference range and bone strength. A more recent study using Mendelian randomization also did not find an association between genetically determined hyperthyroidism and bone mineral density, though it is important to realize that individuals who develop hyperthyroidism are usually treated successfully to euthyroidism.<sup>20</sup> Another disease we explored with Mendelian randomization was anemia in **Chapter 6**, for which we did not find any association with genetically determined variation in circulating levels of TSH or fT4. We did however find some indications that intracellular regulation of thyroid hormone levels may play a role in developing anemia. For diabetes mellitus, several Mendelian randomization studies have

been conducted to assess potential causal effects of thyroid status, though with mixed results.<sup>21-23</sup> An added level of complexity is the interplay of both thyroid function and diabetes mellitus with obesity. A recent Mendelian randomization study provided some evidence that obesity leads to an increase in circulating TSH and fT3 but not fT4, though no associations were observed vice versa.<sup>24</sup> In **Chapter 7**, we attempted to disentangle the association of thyroid status, body mass index and diabetes mellitus using Mendelian randomization. Overall we did not observe an association of genetically determined thyroid status and diabetes mellitus, though higher TSH might be protective for diabetes mellitus only in individuals with an intrinsically lower risk of high body mass. In **Chapter 8** and **9** we investigated the association between genetically determined thyroid status and risk of coronary artery disease. Upon first exploration in **Chapter 8**, no association was found between genetically determined TSH or fT4 and coronary artery disease. However, this first study had certain important limitations; the variation explained in TSH and fT4 by the genetic instruments was only modest and the study population for the outcome was of mixed ancestry. Therefore we revisited the same hypothesis, but with stronger genetic instruments in a larger and more homogeneous population in **Chapter 9**. Despite the more rigorous design, we only found a slight increase in risk of coronary artery disease with increase in TSH. These findings are in line with other Mendelian randomization which also demonstrated a negligible increase in risk of coronary artery disease.<sup>22, 25, 26</sup> With all the negative study outcomes, one might wonder whether the technique of Mendelian randomization is actually valid for assessing the role of thyroid status. As a kind of positive control, we assessed in **Chapter 9** the association between genetically determined thyroid status and a metabolomic profile including a vast array of lipid particles. Here, we observed associations between higher TSH and higher levels of very low-density lipoprotein (VLDL) subclasses and components, triglycerides, and triglyceride content of lipoproteins which were similar across multiple methods including Mendelian randomization. Multiple previous Mendelian randomization studies all identified positive associations between TSH and low-density lipoprotein (LDL) cholesterol and total cholesterol, while no associations for high-density lipoprotein (HDL) cholesterol or circulating triglycerides were observed.<sup>22, 23, 27, 28</sup> Although these results do not fully match ours, it is important to note that our study used a Nuclear Magnetic Resonance panel which gives different insights from traditional lipid panels as used in the previous studies. Regardless, these studies do emphasize that robust and biologically plausible associations can be obtained from Mendelian randomization studies. Interestingly, two recent independent studies did reveal that higher genetically determined TSH might decrease the risk of developing dementia.<sup>29, 30</sup> Although the risk estimates were small and the data used in these studies were partly overlapping, the congruence is striking. These findings do shed a new light on our null finding in **Chapter 4**. It could be that the Mendelian

randomization assesses the role of long term exposure to slightly higher TSH in relation to developing dementia, which might be more influential and possibly more relevant than the snapshot value for thyroid status used in our study. On the other hand, the Mendelian randomization studies might be biased by horizontal pleiotropy. The genetic variants associated with thyroid status have been mapped to various functional pathways, including growth factors and transporters but a considerable share has a yet unknown function.<sup>31</sup> Especially the variants involved in transcription and growth factors might have effects on the brain independent of thyroid status, while the genetic variants of unknown function also cannot be ruled out for having thyroid hormone independent effects on the brain. Lastly, these two studies could also report mere chance findings. Of course, all these considerations are also applicable to the research described in this thesis, which is why multiple branches of research are required before rigorous conclusions can be drawn regarding causality.

Definitive answers regarding causality of thyroid status in age-related disease were expected to be provided by the TRUST and IEMO 80-plus thyroid trial; two parallel multicenter randomized placebo-controlled trials of levothyroxine treatment for subclinical hypothyroidism in older adults.<sup>32,33</sup> Both TRUST, which included individuals of 65 years or older in Switzerland, Ireland, the United Kingdom and the Netherlands, and the IEMO 80-plus thyroid trial, which included individuals of 80 years or older in Switzerland and the Netherlands, did not find evidence for benefit or harm of levothyroxine treatment compared to placebo for subclinical hypothyroidism.<sup>34,35</sup> Also among participants with higher burden of hypothyroidism symptoms, treatment with levothyroxine did not bring about any measurable changes when compared to placebo.<sup>36</sup> In line with these null-findings, the more in-depth sub-studies also did not find any difference between the participants treated with levothyroxine and those treated with placebo regarding cardiovascular outcomes, depression or bone health.<sup>37-39</sup> Hence, it seems that minor changes in the hypothalamic-pituitary-thyroid axis do not cause any major changes in hypothyroidism complaints nor in risk of common diseases among older adults.

#### *Future perspectives*

Translation from fundamental research to human subjects is challenging, but translation from research to clinical practice is yet another leap. The most straightforward would be that if treatment with levothyroxine is equivalent to placebo for subclinical hypothyroidism in older adults, treatment should not be initiated. The advice not to treat subclinical hypothyroidism except for a few special circumstances was indeed published shortly after the trial results.<sup>40</sup> In response to the trial results, many patients wondered whether they could discontinue their levothyroxine treatment which had been initiated for subclinical

hypothyroidism. Discontinuation is different from not initiating treatment, though it seems logical that an ineffective treatment could be discontinued safely. Therefore, further research is required to investigate whether and how discontinuation can be performed safely.

In addition, we might need to take a further step back and reconsider our screening methodologies. Currently, in screening of thyroid function TSH levels are measured first, followed by fT4 levels if the TSH level is outside the reference range. The vast majority of individuals with TSH levels outside the reference range have fT4 levels within the reference range, thus resulting in many diagnoses of subclinical hypothyroidism. If we decide not to treat subclinical hypothyroidism, we might not need to test for it either. Alternatively, targeted screening might need to change focus to fT4 levels with subsequent TSH level measurement if fT4 levels are outside of the reference range, which would theoretically only diagnose individuals with overt thyroid dysfunction for whom treatment indication is unequivocal. Further research is needed to assess whether this screening method would be effective and to investigate how many cases of clinically relevant thyroid dysfunction would be missed. Moreover, cost-effectiveness might also need to be addressed since the price of fT4 testing is higher than that for TSH testing in most clinical chemical laboratories in the Netherlands.

On the other hand, for the individuals who do need treatment for hypothyroidism there might also still be room for improvement. The current treatment entails supplementation of T4, with the assumption that deiodination in the peripheral tissues will compensate to produce sufficient T3. However, full compensation to physiological balance is often not achieved.<sup>41</sup> A considerable proportion of patients are dissatisfied with their treatment.<sup>42</sup> Theoretically, these could be patients who suffer from insufficient T3 production despite normalization of TSH and fT4. Multiple randomized clinical trials failed to prove benefit of adding T3 to levothyroxine treatment.<sup>42</sup> Nevertheless, patient organizations in the United Kingdom vocally protested when T3 prescriptions were withdrawn on NHS advice due to lack of evidence and high economic cost. Although the trials were negative, it is important to mention that most were underpowered and might not have included patients who were dissatisfied with levothyroxine treatment.<sup>42</sup> Economic analysis of this lack of evidence has resulted in an advice that a rigorous trial is economically worthwhile, despite all previous efforts.<sup>43</sup> Hence, a properly powered trial with patients selected for having persistent complaints while being optimally treated with levothyroxine might still benefit the field.

In the more distant future, research that is currently further removed from the clinic may also contribute to patient care. For instance, thyroid status might be used as a marker to aid predictions for individual patients. Especially in geriatric

medicine, weighing potential benefits and burden of treatment for an individual patient is becoming more common in daily practice. However, estimating an individual patient's prognosis remains a difficult task. Various tools have been developed that are aiming to help these predictions, such as the APOP screener for older adults in the emergency department.<sup>44</sup> There are several other environments and situations where individualized vulnerability estimates are desirable, most importantly around major treatment decisions. Thyroid status might add to individual patients' characterization in this context. However, its added value to other estimators of frailty is yet to be established.

Lastly, in the long term, we might not just want to tailor medical treatment to the individual but also to individual cell types or even cells in certain states. As the fundamental research in model organisms has shown, cells have differential needs when going through different states. With the continuous progress in the delivery of medication in the body through techniques such as specialized peptides and mRNA vaccines, new treatment opportunities arise. Although speculating, in the distant future thyroid hormone antagonists aimed at neural progenitor cells might aid remyelination after flare ups of multiple sclerosis or thyroid hormone agonists aimed specifically at cancer cells might inhibit tumor growth.

In conclusion, targeting thyroid status to alter the aging process is most likely an illusion, nevertheless multiple clinically relevant questions around thyroid function in older individuals remain to be answered.

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# CHAPTER 11

Appendices

Nederlandse samenvatting

List of publications

PhD Portfolio

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Dankwoord

## NEDERLANDSE SAMENVATTING

### *Achtergrond*

Wereldwijd neemt de levensverwachting toe. Hierdoor bereiken steeds meer mensen een hoge leeftijd, en doordat er tegelijkertijd relatief minder kinderen worden geboren ontstaat vergrijzing. Hoewel het gezegde luidt “ouderdom komt met gebreken”, zijn er opvallend grote verschillen tussen mensen in de gebreken waar zij mee te maken krijgen en de leeftijd waarop deze ontstaan. In **Hoofdstuk 2** brengen we het idee naar voren dat de capaciteit van het lichaam voor onderhoud en schadeherstel wellicht een rol speelt bij het ontwikkelen van ouderdomsgerelateerde ziekten. In dit hoofdstuk stellen we ook manieren voor waarop dat onderhoud en die schade zouden kunnen worden gemeten voor de botten, het kraakbeen, de spieren en de hersenen.

### *De werking van de schildklier*

De daaropvolgende hoofdstukken van dit proefschrift zijn gericht op het ontrafelen van de rol van schildklierhormonen bij het ontstaan van ouderdomsgerelateerde ziekten. De schildklier is een klein orgaan in de hals dat schildklierhormonen produceert. Schildklierhormonen stimuleren de stofwisseling en de rijping van nieuwe cellen (de bouwstenen van het lichaam) door het hele lichaam. De schildklier bepaalt niet zelf hoe veel schildklierhormonen nodig zijn, hiervoor worden signaalstoffen gestuurd vanuit de hersenen. In de hersenen stemmen de hypothalamus en de hypofyse de aansturing van de schildklier af op de hoeveelheid schildklierhormonen in het bloed en op andere signalen, zoals onder andere stress en de voedingstoestand.<sup>1</sup> De signaalstof van de hypofyse naar de schildklier kunnen we meten in het bloed, en heet schildklierstimulerend hormoon (thyroid-stimulating hormone; TSH). De schildklier reageert hier normaal gesproken op door inactief schildklierhormoon (thyroxine; T4) en een klein beetje actief schildklierhormoon (triiodothyronine; T3) uit te scheiden. De meeste organen in het lichaam bevatten speciale enzymen (deiodasen), hiermee wordt inactief T4 naar actief T3 omgezet wanneer ze dat nodig hebben. Samen houden de schildklier en de hypofyse de hoeveelheid schildklierhormonen in het bloed in evenwicht. Hoe goed dit samenspel werkt, kan worden bepaald door het meten van de waarden van het TSH en de T4 in het bloed. Als deze uit balans raken hebben we het over een schildklierziekte: hypothyreoïdie als de schildklier te traag werkt waarbij het TSH te hoog is en de T4 te laag, hyperthyreoïdie als de schildklier te hard werkt waarbij het TSH te laag is en de T4 te hoog. Met name bij oudere mensen komt subklinische hypothyreoïdie vaak voor waarbij het TSH te hoog is maar de T4 normaal, het lijkt dan alsof de schildklier meer stimulatie nodig heeft of minder kan produceren dan de hersenen nastreven. Subklinische hyperthyreoïdie waarbij het TSH te laag is maar de T4 normaal komt minder vaak voor. Er is veel verschil tussen mensen hoe de schildklierhormonen

zijn afgesteld, sommige mensen hebben van nature een wat trager afgestelde schildklier dan anderen. Hierbij speelt onder andere genetica een rol. Daarnaast hebben omgevingsfactoren ook een grote invloed, waardoor ook binnen een persoon de schildklierhormoonwaardes de ene keer wat hoger kunnen zijn dan de andere keer.<sup>2</sup>

#### *Schildklierhormonen en ouderdomsgerelateerde ziekten*

In **Hoofdstuk 3** en **4** hebben we bij groepen oudere mensen onderzocht of de schildklierhormoonwaarden in het bloed een verband hadden met hun kans op overlijden en met hun geheugen. In **Hoofdstuk 3** zagen we dat 90-plussers die meer actief schildklierhormoon (T3) in hun bloed hadden gemiddeld langer leefden dan 90-plussers met lagere T3 gehalten in hun bloed. Dit zagen we zowel bij 90-plussers uit families waar veel familieleden een hoge leeftijd bereiken als bij 90-plussers uit normale families. Daarmee lijkt een hoger T3 gehalte een voorspeller van relatief langer leven op zeer hoge leeftijd. In **Hoofdstuk 4** hebben we in verschillende groepen oudere mensen van over de hele wereld onderzocht of afwijkende schildklierhormoonwaarden in het bloed samengaan met meer geheugenproblemen. In grote groepen mensen met mild afwijkende schildklierwaarden, passend bij subklinische hypothyreoïdie en subklinische hyperthyreoïdie, hebben we geen verband gevonden met een groter risico op geheugenproblemen of op het ontwikkelen van dementie in vergelijking met mensen zonder afwijkende schildklierwaarden. Voor de mensen met schildklierziekten, waarbij beide metingen van de schildklierfunctie (TSH en T4) afweken, konden we geen uitspraken doen, doordat deze groepen te klein waren.

In **Hoofdstuk 5, 6** en **7** hebben we het verband onderzocht tussen de genetische afstelling van schildklierhormoonwaardes en botontkalking (osteoporose, lage botdichtheid), bloedarmoede (anemie, laag gehalte van rode bloedcellen) en suikerziekte (diabetes mellitus). In **Hoofdstuk 5** vonden we in een grote groep Europeanen dat de genetisch bepaalde afstelling van de schildklierhormoonwaardes geen relatie had met de dichtheid van de botten; daarmee lijkt de variatie die van nature optreedt in de schildklierhormoonwaardes geen risico te vormen voor verlies van kracht van de botten. In **Hoofdstuk 6** onderzochten we de rol van schildklierhormonen bij het ontstaan van anemie. In Britse proefpersonen van middelbare leeftijd vonden we dat mensen met een schildklierziekte vaker anemie hadden dan mensen zonder schildklierziekte. We zagen echter geen verband tussen genetische afstelling van de schildklierhormonen en het risico op anemie. Het lijkt erop dat de verstoring in schildklierhormoonwaardes die ontstaat bij schildklierziekten het risico op anemie vergroot, maar het kan ook zijn dat mensen met schildklierziekten kwetsbaarder zijn dan mensen zonder schildklierziekten voor andere oorzaken van anemie. In **Hoofdstuk 7** hebben we de ingewikkelde wisselwerking onderzocht tussen

schildklierhormonen, overgewicht en diabetes mellitus. In het algemeen was er geen verband tussen de genetische afstelling van schildklierhormonen en risico op diabetes mellitus. Mogelijk hebben mensen die op basis van hun genetica een iets hoger afgesteld TSH hebben een lager risico op diabetes mellitus, maar dit vonden we alleen in mensen met een genetisch laag risico op overgewicht en niet in mensen met een hoger risico op overgewicht. Wat dit betekent moet nog verder worden onderzocht.

In **Hoofdstuk 8** en **9** hebben we het verband tussen genetisch bepaalde schildklierhormoonwaardes en het risico op hart- en vaatziekten onderzocht. In **Hoofdstuk 8** vonden we in een grote groep mensen uit Europa, de VS en Azië geen verband tussen genetische afstelling van schildklierhormonen en het risico op ziekten van de kransslagaders om het hart (coronaire hartziekte). In **Hoofdstuk 9** hebben we dit opnieuw en uitgebreider onderzocht, dat kon mede doordat er een groter aantal genen van invloed op de schildklierwaarden waren ontdekt. In dit hoofdstuk zagen we dat in een grote groep Europeanen een langzamer afgesteld schildkliersysteem samengaat met een specifiek vetstofwisselingspatroon. Dit specifieke patroon van verschillende vetten in het bloed wordt vaker gezien bij mensen die coronaire hartziekten ontwikkelen. Hierbij passend vonden we dat mensen met een genetisch relatief langzamer afgesteld schildkliersysteem een iets hoger risico hadden op coronaire hartziekte, maar het risico was niet veel groter dan voor mensen met een relatief sneller afgesteld schildkliersysteem. Op groepsniveau is dit verschil in risico verwaarloosbaar.

### *Toekomstperspectieven*

Wat betekent dit alles voor de toekomst? Op basis van de onderzoeken die in dit proefschrift zijn beschreven lijken schildklierhormonen geen grote rol te spelen bij ouderdomsgerelateerde ziekten van het brein, de botten, het bloed, diabetes mellitus en coronaire hartziekten. Bovendien zijn er in de tussentijd twee grote studies uitgevoerd om te testen of oudere mensen met subklinische hypothyreoidie baat hadden bij inname van extra schildklierhormoon in pilvorm (levothyroxine) ten opzichte van een placebo.<sup>3,4</sup> Uit beide studies bleek dat het niet uitmaakte of deelnemers levothyroxine of placebo kregen; waaruit men kan concluderen dat bij deze patiëntengroep levothyroxine niet helpt.<sup>5,6</sup> De richtlijnen zijn nu ook veranderd: als bij een ouder persoon subklinische hypothyreoidie wordt geconstateerd, wordt nu alleen nog in uitzonderlijke gevallen gestart met behandeling.<sup>7</sup> Er zijn heel veel oudere mensen die levothyroxine gebruiken, waarvan een deel zal zijn gestart op basis van subklinische hypothyreoidie in het verleden. De volgende stap is om te onderzoeken of deze mensen veilig kunnen stoppen met hun behandeling. Daarnaast is het goed om opnieuw te overwegen hoe we screening voor schildklierziekten moeten uitvoeren; met de huidige

screeningsmethode wordt veel subklinische hypothyreoïdie aangetoond welke we vervolgens niet behandelen. Extra onderzoek zou moeten worden uitgevoerd om de veiligheid en kosteneffectiviteit te beoordelen van screening op basis van schildklierhormoon T4 in plaats van het aansturende TSH. Aan de andere kant valt er voor de mensen die behandeling van hypothyreoïdie nodig hebben mogelijk ook nog verbetering te behalen. Veel patiënten met hypothyreoïdie zijn ontevreden met hun behandeling en hebben een lagere kwaliteit van leven dan mensen zonder schildklierziekte.<sup>8</sup> In levothyroxine zit alleen het inactieve schildklierhormoon T4, wat in het lichaam moet worden omgezet naar actief schildklierhormoon T3. Mogelijk hebben de mensen die niet goed reageren op levothyroxine baat bij een combinatie van T4 en T3 medicatie. Gedegen onderzoek hiernaar kan mogelijk een grote groep patiënten meer kwaliteit van leven bieden. Op de langere termijn kan onderzoek wat nu nog verder van klinische praktijk af staat, mogelijk ook bijdragen aan betere patiëntenzorg. Wellicht kunnen schildklierwaarden in de toekomst gebruikt worden ter ondersteuning van gepersonaliseerde behandelplannen. Met steeds meer kwetsbare oudere patiënten moet steeds vaker een afweging worden gemaakt tussen de voor- en nadelen van behandeling. Die voor- en nadelen vooraf voorspellen is erg moeilijk; daarvoor worden steeds meer screeningsinstrumenten ontwikkeld die zijn toegespitst op verschillende situaties. Of schildklierwaarden hierin een toegevoegde waarde kunnen hebben, zal moeten blijken uit vervolgonderzoek. In de verre toekomst gaan we naast gepersonaliseerde behandelplannen misschien ook toe naar behandelingen gericht op bepaalde soorten cellen of zelfs specifieke cellen in bepaalde fasen. Met nieuwe technieken als mRNA vaccinaties en het maken van weefsel-specifieke peptiden komen ook nieuwe behandelmogelijkheden. Vrij speculerend zouden in de toekomst schildklierhormoonremmers gericht op specifieke stamcellen in de hersenen en het ruggenmerg mogelijk kunnen bijdragen aan de behandeling van Multiple Sclerose of schildklierhormoonversterkers gericht op kankercellen kunnen bijdragen aan remming van tumorgroei.

Concluderend lijkt het onwaarschijnlijk dat het aanpassen van schildklierhormonen in het bloed het verouderingsproces en ontstaan van ouderdomsgerelateerde ziekten zal remmen. Desalniettemin zijn er nog veel vragen te onderzoeken rondom schildklierhormonen en ouderen.

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**PHD PORTFOLIO**

<b>Courses</b>	<b>Years</b>	<b>Hours</b>
PhD Introductory Meeting	2017	5
Epidemiology "An Introduction" (Rothman)	2017	84
Basic Methods and Reasoning in Biostatistics	2017	42
Using R for data analysis	2017	32
Introduction course for using local computer cluster (Shark)	2017	1.5
Practical Linux	2017	11
Basiscursus Regelgeving en Organisatie voor Klinisch onderzoekers (BROK)	2017	42
Meta-analysis	2018	22
Repeated measurements	2019	40
Workshop Artificial Intelligence	2020	4
Causal Inference (Hernan)	2020	84
<b>Attended congresses and meetings</b>	<b>Years</b>	<b>Hours</b>
Biobanking and Biomolecular Resources Research Infrastructure (BBMRI) meeting (Utrecht, the Netherlands)	2017	7
THYRAGE annual meeting (Paris, France)	2017	12
Dutch Society for Research on Ageing (DuSRA) annual meeting (Leiden, the Netherlands)	2017	8
Mendelian randomization in the age of large-scale accessible data (Bristol, United Kingdom)	2017	24
Jonge Nederlandse Vereniging voor Endocrinologie congres 2017 (Leiden, the Netherlands)	2017	12
Biobanking and Biomolecular Resources Research Infrastructure (BBMRI) meeting (Utrecht, the Netherlands)	2018	6
THYRAGE annual meeting (London, United Kingdom)	2018	12
Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) meeting (Rotterdam, the Netherlands)	2018	16
Annual Symposium of the Dutch Thyroid Research Foundation (Amsterdam, the Netherlands)	2018	6
Dutch Society for Research on Ageing (DuSRA) annual meeting (Leiden, the Netherlands)	2018	8
Jonge Nederlandse Vereniging voor Endocrinologie congres 2018 (Nijmegen, the Netherlands)	2018	12
British Geriatric Society Autumn Meeting (London, United Kingdom)	2018	12
THYRAGE steering committee meeting (Leiden, the Netherlands)	2018	5
THYRAGE annual meeting (Capri, Italy)	2019	12
LUMC Association for PhD candidates Career event (Leiden, the Netherlands)	2019	6
TRUST and IEMO trial researchers meeting (Leiden, the Netherlands)	2019	6
LUMC Internal Medicine Science day (Leiden, the Netherlands)	2019	7

## Chapter 11

4th International Mendelian Randomization Conference (Bristol, United Kingdom)	2019	18
42nd Annual Meeting of the European Thyroid Association (Budapest, Hungary)	2019	28
Jonge Nederlandse Vereniging voor Endocrinologie congres 2019 (Nijmegen, the Netherlands)	2019	8
Health Research Infrastructure meeting (Utrecht, the Netherlands)	2020	8.5
TRUST and IEMO trial researchers meeting (Bern, Switzerland)	2020	6
TRUST and IEMO trial researchers meeting (Online)	2020	3.5
THYRAGE annual meeting (Online)	2020	6
<b>Teaching activities</b>	<b>Years</b>	<b>Hours</b>
Monthly returning working groups on principles of geriatrics for Master students in preparation for their first medical internship (Introductiecoşchap)	2017-2021	50
Annually returning series of working groups for Bachelor students in medicine regarding gerontology and geriatrics (Vraagstukken Latere Levensfasen)	2017-2021	85
Annually returning supervision of an assignment on mendelian randomization for students of the Master Vitality and Aging	2017-2020	18
Supervision of Research & Evidence Group assignment for Master students	2019	2.5
<b>Student supervision</b>	<b>Years</b>	<b>Hours</b>
Rebecca Tepper (Bachelor thesis)	2017	9
Meike van der Geest and Larissa Kuijpers (Hogeschool thesis)	2017	5
Karlijn Jansen (Hogeschool thesis)	2018	4
Selma Wilting (Bachelor thesis)	2019	9
Roos den Hertog (Bachelor thesis)	2020	9
Annelies Kamphuis (Master thesis)	2020	80
<b>Awards</b>	<b>Years</b>	<b>Hours</b>
Young Investigator Fellowship of the European Atherosclerosis Society	2022	-

## CURRICULUM VITAE

Nicolet Alien van Vliet werd geboren op 28 april 1993 te Alphen aan den Rijn. In 2010 behaalde ze haar tweetalig atheneumdiploma aan het Scala college te Alphen aan den Rijn, waarna ze startte met de studie Geneeskunde aan de Universiteit Leiden. Tijdens haar bachelor heeft zij extracurriculair bijgedragen aan dataverzameling voor verschillende projecten omtrent hypofysetumoren bij de afdeling Interne geneeskunde sectie Endocrinologie binnen de onderzoeksgroep van prof. dr. N.R. Biermasz in het LUMC. Tijdens haar master heeft ze haar wetenschapsstage gedaan bij de afdeling Interne geneeskunde sectie Ouderengeneeskunde in het LUMC onder begeleiding van dr. ir. D. van Heemst en dr. E. van der Spoel. Na haar afstuderen in november 2016 keerde zij terug bij de sectie Ouderengeneeskunde om haar onderzoek voort te zetten, in het kader van een promotietraject onder leiding van prof. dr. G.J. Blauw en dr. ir. D. van Heemst. Het promotorschap werd later overgedragen aan prof. dr. S.P. Mooijaart. Gedurende haar promotie was zij nauw betrokken bij het management van het onderzoeksconsortium THYRAGE wat was gefinancierd vanuit het Europese Unie Horizon 2020 programma. Tevens heeft zij voor dit consortium een sociale media-account op Twitter opgebouwd met meer dan 400 volgers. Van juni 2019 tot september 2020 heeft zij zich naast haar promotie ingezet als bestuurslid voor het Promovendi Netwerk Nederland. Daarna heeft zij van september 2020 tot april 2021 naast haar promotie telefoondiensten gedraaid bij het Regionaal Coördinatiecentrum Patiënten Spreiding voor overplaatsingen van patiënten met COVID-19 tussen ziekenhuizen in Nederland. Sinds mei 2021 werkt zij als arts niet in opleiding (ANIOS) bij het Haaglanden Medisch Centrum: tot en met augustus 2022 bij de afdeling Interne geneeskunde en vanaf september 2022 bij de afdeling Intensive Care.

## DANKWOORD

Allereerst wil ik de deelnemers bedanken van de vele verschillende studies die zijn gebruikt in dit proefschrift. Zonder hen was dit onderzoek niet mogelijk geweest.

Mijn promotor prof. Mooijaart, mijn voormalig promotor prof. Blauw en mijn copromotor dr. ir. van Heemst wil ik bedanken voor hun bijdragen de afgelopen jaren. Simon, dankjewel dat jij de taak van het promotorschap over wilde nemen. Gerard Jan, bedankt voor de kritische vragen en het bewaken van de samenhang van dit proefschrift. Diana, dankjewel voor deze fantastische kans die je me hebt gegeven om in jouw team te promoveren. Ik heb veel van je mogen leren over de technische, taalkundige maar ook strategische aspecten van de wetenschap.

Dit proefschrift was niet tot stand gekomen zonder het THYRAGE consortium. Ik wil alle consortium collega's bedanken voor het delen van hun kennis en inzichten en voor de inspirerende jaarlijkse meetings. In het bijzonder bedank ik Charlotte dat ik bij het projectmanagement betrokken mocht zijn.

Ik wil mijn collega's van de sectie Ouderengeneeskunde bedanken met wie ik deze wetenschappelijke reis samen heb mogen beleven. Evie, dankjewel voor het wegwijs maken tijdens mijn wetenschapsstage en de fijne samenwerking daarna. Raymond, dankjewel dat ik altijd bij je kon aankloppen (en in coronatijd kon bellen) voor advies of gewoon een gezellig kopje thee. Stella, bedankt voor je tomeloze geduld en wijze raad bij ons gezamenlijk project in de Thyroid Studies Collaboration. Ania, het was zeer leerzaam om met je te werken voor het onderwijsblok Vraagstukken Latere Levensfasen en het Introductiecoschap. Ik bedank mijn kamergenoten Maxime, Laura, Marjan, Lisa, Jiao, Marelina en Leon voor het laagdrempelige overleg en de vele gezellige momenten op C7-125. Marian en Christine, bedankt voor jullie hulp bij het plannen van de vele onmogelijke afspraken en de verdere ondersteuning.

Ik bedank mijn collega's van de TRUST en IEMO trials dat ik bij deze trials en de bijbehorende biobank betrokken mocht zijn, het was een bijzonder leerzame ervaring. In het bijzonder wil ik collega's Jacobijn, Wendy, Rosalinde, Robert en Laurien bedanken voor de prettige samenwerking de afgelopen jaren.

Rianne, Leonie, Marlinda, Justin, Irina, Faiez, Inge en Natasja; ik ben dankbaar dat we ook na de studie met elkaar in contact zijn gebleven. Ik hoop dat we nog vele mijlpalen met elkaar mogen delen.

Ik wil mijn bestuursgenoten bedanken voor de mooie ervaring bij het Promovendi Netwerk Nederland. Lucille, je was een hartstochtelijke voorzitter en een bijzonder

fijn persoon om samen mee de barricades op te gaan. Kimberley, Bram, Carola, Tjitske, Roel, Rob, Reinder, Peta en Tess; ik vond het een plezier om met jullie samen te werken en te sparren over de toekomst van promovendi in Nederland.

Papa en mama, jullie waren er de afgelopen jaren altijd met een luisterend oor en leven mee met elke stap. Dankjulliewel voor al jullie steun en aanmoediging.

Wouter, jouw relativeringsvermogen en humor hebben menige traan doen vervagen. Het is altijd een plezier om bij jou thuis te komen.



