Variation in normal range thyroid function affects serum cholesterol levels, blood pressure and type 2 diabetes risk: A Mendelian randomization study.

Aleksander Kuś, MD, PhD [a, b, c, *], Eirini Marouli, PhD [d, e, *], Fabiola Del Greco M., PhD [f], Layal Chaker, MD, PhD [a, b], Tomasz Bednarczuk, MD, PhD [c], Robin P. Peeters, MD, PhD [a, b], Alexander Teumer, PhD [g, h], Marco Medici, MD, PhD [a, b, i, *], Panos Deloukas, PhD [d, e, j, *]

a. Academic Center for Thyroid Diseases, Department of Internal Medicine, Erasmus Medical Center, Dr. Molewaterplein 40, 3015 CE Rotterdam, The Netherlands
b. Department of Epidemiology, Erasmus Medical Center, Dr. Molewaterplein 50, 3015 GE Rotterdam, The Netherlands
c. Department of Internal Medicine and Endocrinology, Medical University of Warsaw, Banacha 1a, 02-097 Warsaw, Poland
d. William Harvey Research Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, EC1M 6BQ London, UK
e. Centre for Genomic Health, Life Sciences, Queen Mary University of London, EC1M 6BQ London, UK
f. Institute for Biomedicine, Eurac Research, Affiliated Institute of the University of Lubeck, Via Galvani 31, 39100 Bolzano, Italy
g. Institute for Community Medicine, University Medicine Greifswald, W.-Rathenau-Str. 48, 17475 Greifswald, Germany
h. DZHK (German Center for Cardiovascular Research), partner site Greifswald, Fleischmannstr. 8, 17475 Greifswald, Germany

i. Department of Internal Medicine, Radboud University Medical Center, Geert Grooteplein Zuid 10, 6525 GA Nijmegen, The Netherlands

j. Princess Al-Jawhara Al-Brahim Centre of Excellence in Research of Hereditary Disorders (PACER-HD), King Abdulaziz University, Jeddah, Saudi Arabia

*** contributed equally**

Contact information:

Aleksander Kuś: akus@wum.edu.pl

Eirini Marouli: e.marouli@qmul.ac.uk

Fabiola Del Greco M.: fabiola.delgreco@eurac.edu

Layal Chaker: l.chaker@erasmusmc.nl

Tomasz Bednarczuk: tbednarczuk@wum.edu.pl

Robin P. Peeters: r.peeters@erasmusmc.nl

Alexander Teumer: ateumer@uni-greifswald.de

Marco Medici: marco.medici@radboudumc.nl

Panos Deloukas: p.deloukas@qmul.ac.uk

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Abstract

Background: Observational studies have demonstrated that variation in normal range thyroid function is associated with major cardiovascular risk factors, including dyslipidaemia, hypertension, type 2 diabetes (T2D), and obesity. As observational studies are prone to residual confounding, reverse causality and selection bias, we used a Mendelian randomization (MR) approach to investigate whether these associations are causal or not.

Methods: Two-sample MR analysis using data from the largest available genome-wide association studies on normal range TSH and FT4 levels, serum lipid levels, blood pressure measurements, T2D and obesity traits (body mass index (BMI) and waist-hip ratio (WHR)).

Results: A one standard deviation (SD) increase in genetically predicted TSH levels was associated with a 0.037 SD increase in total cholesterol levels (P=3.0x10^{-4}). After excluding pleiotropic instruments, we also observed significant associations between TSH levels and low-density lipoprotein levels (β=0.026 SD, P=1.9x10^{-3}), pulse pressure (β=-0.477 mmHg, P=7.5x10^{-10}) and T2D risk (OR=0.95, P=2.5x10^{-3}). While we found no evidence of causal associations between TSH or FT4 levels and obesity traits, we found that a one SD increase in genetically predicted BMI was associated with a 0.075 SD decrease in FT4 levels (P=3.6x10^{-4}).

Conclusions: Variation in normal range thyroid function affects serum cholesterol levels, blood pressure and T2D risk.

Introduction

Cardiovascular disorders (CVD) are a leading cause of mortality worldwide (1). Whereas traditional cardiovascular risk factors, such as dyslipidaemia, hypertension, type 2 diabetes (T2D) and obesity, are well-recognized, observational studies have shown that also overt and subclinical thyroid dysfunction are associated with a higher risk of CVD (2-6). More recently, even variation in normal range thyroid function has been associated with an increased risk of CVD, including atherosclerotic disease and stroke (7-10), as well as with serum lipid levels (11), blood pressure (12), T2D risk (13) and obesity (14). These findings could have important clinical implications for prevention efforts targeting cardiovascular risk (15). However, observational studies are prone to various sort of bias, including residual confounding, reverse causality and selection bias, which can affect their results and disrupt their interpretation (16). Therefore before translating these findings into clinical practice, it is essential to first clarify whether causal associations underlie these epidemiological observations (17).

An established and widely used approach to investigate whether causal relationships underlie the observed associations is to perform a Mendelian randomization (MR) study. This method involves finding genetic variants which are associated with an exposure (*e.g.* thyroid function), and then testing the association between these variants and the outcome of interest (*e.g.* CVD). The fundamental principle of MR is that if genetic variants alter the exposure that is causal for the outcome, then these genetic variants should also be associated with this outcome to the extent corresponding to their effects on the exposure (18, 19). In that way, MR uses genetic variants as proxies to evaluate the causal effect of an exposure on the outcome of interest (20).

It draws from the fact that genetic variants segregate randomly from parents to offspring, which can be compared to randomization used in clinical trials and allows to overcome potential confounding (19). As genetic variants can affect the trait of interest but not the other way around, an association between the genetically predicted exposure and the tested outcome can provide evidence for causality (20). However, this approach requires several assumptions. Most importantly, the genetic variants have to be truly associated with the exposure, and their effects on the outcome of interest has to be mediated solely by the exposure under study (20). Although a single genetic variant can be used as an instrument in MR analyses, combining the effects of multiple genetic variants that can explain a larger proportion of variance in the exposure can significantly increase the analysis power (21). As some of the variants used as instruments might potentially violate MR assumptions, several statistical methods has been proposed to adjust for these violations (22, 23).

In this study, we performed a two-sample MR to investigate the effects of variation in normal range thyroid function on established cardiovascular risk factors, including cholesterol and triglyceride levels, blood pressure, T2D risk and obesity traits (body mass index (BMI) and waist-hip ratio (WHR)). For this, summary level data from the most recent and largest genome-wide association studies (GWAS) on thyroid function and cardiovascular risk factors were used (24-28). Bidirectional MR analyses were performed to gain insight into the complex associations and potentially causal effects in both directions between thyroid function and obesity (29).

Materials and Methods

Two-sample Mendelian randomization

We performed two-sample MR analyses using the data from the most recent genome-wide association study (GWAS) on thyroid function (24), and summary-level statistics from the largest available GWAS meta-analyses on cardiovascular risk factors (detailed in the sections below; (25-28)). No ethical approval was required as all data were extracted from publically available summary statistics.

Exposures and instruments

The exposures of interest were normal range TSH and FT4 levels. Based on the results of the currently largest GWAS on thyroid function (24), we identified 61 and 31 independent ($r^2 \leq 0.01$ within windows of ±1 Mb for variants in the same locus) single nucleotide polymorphisms (SNPs) associated at a genome-wide significant level ($P < 5 \times 10^{-8}$) with TSH and FT4 levels within the reference range, respectively. Only individuals with TSH levels within their cohort-specific reference ranges were included in the GWAS on TSH and FT4 levels and subjects using thyroid medications or after thyroid surgery were excluded from these GWAS, while no information on thyroid-specific antibodies was available in that study (24). We used the identified genetic variants as potential instruments to investigate the causal relationship between normal range thyroid function and the outcomes of interest. Two variants associated with TSH levels were *a priori* excluded from all the analyses as they were highly pleiotropic (*ABO*-rs8176645) or had the same effect allele associated ($P < 0.05$) with both higher TSH levels and higher FT4 levels

within the normal range (*BCAS3*-rs1157994). Detailed data on variants used as instruments are presented in **Supplementary Tables 1 & 2**.

Outcomes of interest and datasets used

Outcomes of interest included serum lipid levels (total cholesterol, low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c) and triglyceride (TG) levels), blood pressure measurements (systolic blood pressure (SBP), diastolic blood pressure (DBP), and pulse pressure (defined as a difference between SBP and DBP)), T2D risk, and obesity parameters (BMI and WHR).

Summary data for serum lipid levels were derived from a GWAS meta-analysis in nearly 300,000 participants from the Million Veteran Program (25), available at dbGaP under the accession number phs001672. Summary data for blood pressure measurements were derived from a GWAS meta-analysis in over 750,000 participants of European ancestry, provided by the UK Biobank and ICBP Consortium (26), made available by the study authors upon request. Summary data for T2D were derived from a GWAS meta-analysis performed by the DIAGRAM Consortium, which investigated the association of 27 million genetic variants in up to 74,124 cases and 824,006 controls of European ancestry (27), available at the consortium website (https://diagram-consortium.org/downloads.html). Summary data for BMI and WHR were derived from a GWAS meta-analysis in over 800,000 participants, combining data from the UK Biobank and GIANT Consortium (28), available at the online repository (https://github.com/lindgrengroup/fatdistnGWAS).

Data on the effect/other allele, beta coefficients and standard errors (SE) for the variants associated with TSH and FT4 levels were extracted from each study for MR analyses and presented in **Supplementary Tables 1 & 2**.

Statistical analyses

Primary analyses

The primary analyses included two-sample MR analyses performed using the inverse-variance weighted (IVW) method (22). This approach requires several assumptions of which the most important are that: (i) the genetic variants used as instruments have to be truly associated with the exposure (*i.e.* TSH or FT4 levels), and (ii) the effect of the instruments on the outcome of interest (*i.e.* one of the studied cardiovascular risk factors) has to be mediated solely by the exposure under study (20). This means that weak and pleiotropic instruments should be avoided as they can strongly bias the causal estimates (30, 31). To this end, we assessed the strength of all instruments based on the F statistics (calculated as $F=\beta^2_{exposure}/SE^2_{exposure}$), which indicated no weak instruments (F statistics ranged 29.81-535.70 and 30.25-455.33 for the TSH and FT4 instruments, respectively), and we addressed the problem of potential pleiotropy in the sensitivity analyses. To control for false positive findings due to multiple testing, a conservative Bonferroni correction adjusted for the number of primary exposures and outcomes analyzed in the study was applied, and *P*-values less than 0.05/20=0.0025 were considered statistically significant. A *P*-value less than 0.05 was considered as evidence for nominal significance. All analyses evaluate the causal effects of a one standard deviation (SD)

increase in genetically predicted TSH or FT4 levels, approximately corresponding to a 1.0 mU/L and 2.2 pmol/L increase in TSH and FT4, respectively (32).

Secondary analyses

Sensitivity analyses

Sensitivity analyses were performed in order to account for potential pleiotropy in the associations between thyroid function and the outcomes of interest. First, we compared the results obtained using the IVW method with the results from MR Egger (33) and weighted median (WM) (34) methods, as the slope of the MR Egger regression may provide valid MR estimates in the presence of horizontal pleiotropy when the pleiotropic effects of the genetic variants are independent from the genetic associations with the exposure (33), while WM can provide valid MR estimates under the presence of horizontal pleiotropy when up to half of the included instruments are invalid (34). Egger intercept was also used as one of the indicators of directional pleiotropy (33). Furthermore, we used I^2 statistics and Cochran's Q test to quantify heterogeneity across the instruments, with P_{het}<0.05 indicating the presence of significant heterogeneity suggesting pleiotropy (35). We identified potentially pleiotropic variants based on their individual Q statistics and repeated the IVW MR analyses after excluding outliers extending the 99.9th (L1), 99th (L2) and 95th (L3) percentiles of a chi-squared distribution with 1 degree of freedom (23, 36). Finally, as the genetic variants associated with FT4 levels form a highly heterogeneous group with potentially diverse effects on T4 and T3 bioavailability, we also compared the results of MR analyses using as instruments two separate subsets of FT4

196 associated variants, specifically including: (i) variants within the deiodinases loci (i.e. *DIO1* and
197 *DIO2*), and (ii) other (non-deiodinase) genetic variants associated with FT4 levels in the GWAS
198 by Teumer *et al.* (24).
199
200 **Bidirectional MR on normal range thyroid function and obesity traits**
201 Bidirectional MR studies on thyroid function and obesity traits (BMI and WHR) were performed
202 to gain insight into the complex and potentially bidirectional associations between thyroid
203 function and obesity (29). A list of variants associated with BMI and WHR at a genome-wide
204 significant level ($P<5.0x10^{-8}$) and corresponding summary statistics were derived from the study
205 by Yengo *et al.* (37) and Pulit *et al.* (28), respectively. To eliminate pleiotropic effects of variants
206 primarily associated with thyroid function, we repeated MR analyses after excluding all variants
207 associated ($P<0.05$) with normal range TSH and FT4 levels, respectively.
208
209 **Power calculations**
210 To estimate the power of our study, we used a non-centrality parameter-based approach (21),
211 implemented in a publicly available mRnd web tool (http://cnsgenomics.com/shiny/mRnd/).
212 For binary outcomes (T2D), we calculated minimal odds ratio (OR) of the outcome variable per
213 standard deviation (SD) of the exposure variable (TSH and FT4 levels) that was detectable
214 (power=0.8, $\alpha=0.05$) in our study. For continuous outcomes (blood pressure measurements,
215 serum lipid levels and obesity traits), we calculated the smallest detectable regression
216 coefficient (β) for the true underlying causal association between the exposure and outcome
217 variables. Proportions of total variance in TSH and FT4 levels explained by the genetic variants

used as instruments (9.4% and 4.8%, respectively) were established based on the data from Teumer *et al.* (24). The results of power calculations are provided in **Supplementary Table 3**.

Results

The results of MR analyses investigating the association between genetically predicted normal range TSH and FT4 levels and each of the tested cardiovascular risk factors respectively are presented in **Supplementary Tables 4-8** and summarized in **Figure 1** and below.

Lipid levels

A one SD increase in genetically predicted TSH levels was associated with a 0.037 SD increase in total cholesterol levels (P=3.0x10^{-4}; **Supplementary Table 4**). Sensitivity analyses using the WM and MR Egger methods provided effect estimates of the same direction and magnitude (β=0.039 SD, P=1.3x10^{-3} and β=0.031 SD, P=0.20, respectively; **Supplementary Table 5**), while exclusion of potentially pleiotropic instruments also led to similar results (β=0.043 SD, P=1.3x10^{-6}; **Supplementary Table 4**).

Analyses of specific lipid fractions showed that the association between TSH and total cholesterol levels could be driven by the effect on LDL-c levels (β=0.022 SD, P=0.029; **Supplementary Table 4**), which was confirmed in sensitivity analyses excluding potentially pleiotropic instruments (β=0.026 SD, P=1.9x10^{-3}; **Supplementary Table 4**), and using the WM method (β=0.037 SD, P=1.1x10^{-3}; **Supplementary Table 5**).

Although we found no associations between TSH levels and HDL-c or TG levels in the primary analyses (β=0.018 SD, *P*=0.11 and β=0.015 SD, *P*=0.23, respectively; **Supplementary Table 4**), sensitivity analyses excluding potentially pleiotropic instruments showed nominally significant associations between TSH and HDL-c levels (β=0.016 SD, *P*=0.042; **Supplementary Table 4**), which was also in line with the results of sensitivity analyses using the WM method (β=0.028 SD, *P*=0.014; **Supplementary Table 5**).

No associations were found between FT4 and total cholesterol levels or any of the specific lipid fractions (**Supplementary Table 4**).

Blood pressure

TSH levels were not associated with SBP (β=-0.178 mmHg, *P*=0.24) or DBP (β=0.160 mmHg, *P*=0.080) in our primary analyses (**Supplementary Table 4**). However, after exclusion of potentially pleiotropic instruments we observed a nominally significant association between TSH levels and SBP (β=-0.255 mmHg, $P=8.6\times10^{-3}$; **Supplementary Table 4**), which was also in line with the results of sensitivity analyses using the WM method (β=-0.315 mmHg, *P*=0.013; **Supplementary Table 5**). Moreover, we observed a nominally significant association between TSH levels and pulse pressure (β=-0.322 mmHg, $P=5.1\times10^{-3}$; **Supplementary Table 4**), which was further confirmed after exclusion of potentially pleiotropic instruments (β=-0.477 mmHg, $P=7.5\times10^{-10}$; **Supplementary Table 4**). Sensitivity analyses using the WM and MR Egger methods also indicated associations between TSH levels and pulse pressure (β=-0.454 mmHg, $P=3.5\times10^{-6}$ and β=-0.518 mmHg, *P*=0.078, respectively; **Supplementary Table 5**).

No associations were found between FT4 levels and any of the blood pressure traits (**Supplementary Table 4**).

Type 2 diabetes

TSH and FT4 levels were not associated with T2D risk in our primary analyses (**Supplementary Table 4)**. However, after exclusion of potentially pleiotropic instruments we observed a significant association between TSH levels and a lower T2D risk (OR=0.95, 95%CI=0.91-0.98, P=2.5x10^{-3}, **Supplementary Table 4**), which was also supported by sensitivity analyses using the WM method (OR=0.95, 95%CI=0.91-1.00, P=0.045; **Supplementary Table 5**). Sensitivity analyses using the MR Egger method provided effect estimates of the same direction (OR=0.87, 95%CI=0.70-1.09, P=0.22; **Supplementary Table 5**).

Obesity parameters

TSH and FT4 levels were not associated with BMI or WHR, except for a nominally significant association between FT4 levels and WHR in sensitivity analyses excluding potentially pleiotropic instruments (β=-0.022 SD, P=0.026, **Supplementary Table 4**).

MR analyses with specific subsets of FT4 instruments

Although we found no significant associations in MR analyses using specific subsets of FT4 instruments (i.e. variants within the deiodinases loci and other (non-deiodinase) genetic variants associated with FT4 levels), we observed opposite effect directions in the analyses using both subsets of instruments for 9 out of 10 analyzed outcomes (**Supplementary Table 7**).

Causal effects of obesity traits on TSH and FT4 levels

To further investigate the relationship between TSH and FT4 levels and obesity traits, we performed bidirectional MR analyses assessing the effects of genetically predicted BMI and WHR on TSH and FT4 levels (**Supplementary Table 8**). While we observed no causal effects of BMI and WHR on TSH levels (β=0.022 SD, *P*=0.24, and β=0.015 SD, *P*=0.68, respectively), we found that a one SD increase in genetically predicted BMI was associated with a 0.075 SD decrease in FT4 levels ($P=3.6\times10^{-4}$). Sensitivity analyses excluding all BMI variants associated (*P*<0.05) with FT4 levels yielded similar results (β=-0.042 SD, *P*=0.020). There was also a nominal association between genetically predicted WHR and FT4 levels in the same direction (β=-0.072 SD, *P*=0.032), which disappeared in sensitivity analyses excluding instruments associated (*P*<0.05) with FT4 levels (**Supplementary Table 8**).

Discussion

This study presented the currently largest, to the best of our knowledge, MR analysis assessing causal relationships between variation in normal range thyroid function and cardiovascular risk factors. We found statistically significant associations ($P<2.5\times10^{-3}$) between TSH levels and serum cholesterol levels, blood pressure and T2D risk. In contrast, FT4 was not associated with any of the tested outcomes. While variation in normal range thyroid function did not affect BMI or WHR, secondary analyses suggested that BMI affects FT4 levels.

Variation in normal range thyroid function is causally associated with total cholesterol and LDL-c levels

Both overt and subclinical hypothyroidism have been associated with dyslipidaemia (38, 39). Moreover, normal range TSH levels have been positively associated with total cholesterol, LDL-c and TG levels, as well as negatively associated with HDL-c levels in various observational studies (11, 40, 41). Our results confirm that the associations between variation in normal range thyroid function and total cholesterol are causal, and that this can be predominantly attributed to a change in LDL-c serum levels. Although the estimated effects are relatively small (0.037 SD and 0.022 SD increase in total cholesterol and LDL-c levels, respectively, for a one SD increase in TSH levels), they might be clinically relevant as they reflect a lifelong exposure. Our results are in line with the results of *in vitro* studies showing that thyroid hormones regulate LDL-c catabolism by their effects on lipid metabolizing enzymes and LDL-c receptor expression in the liver (42, 43). Several intervention studies also demonstrated that L-thyroxine treatment reduces total cholesterol and LDL-c levels in patients with subclinical hypothyroidism (44-46), as well as in euthyroid subjects (47), while no significant effects on HDL-c or TG levels were observed in these studies (44-47). In our MR study we neither observed an effect on TG levels, while we only detected a nominally significant association between TSH and HDL-c levels. Future larger MR studies with more genetic instruments will clarify whether this is due to small effect sizes which we could not detect in our study, or whether there is no effect of variation in normal range thyroid function on HDL-c and TG levels at all.

Variation in normal range thyroid function is causally associated with blood pressure

There is evidence that thyroid disease is an important cause of secondary hypertension (48, 49). Overt hyperthyroidism is accompanied by increased cardiac output and reduced vascular resistance resulting in increased SBP, decreased DBP and increased PP (50). However, the effect of subclinical hyperthyroidism on blood pressure was not confirmed (51-53). The few studies which have investigated the effects of overt hypothyroidism on blood pressure found an association with increased DBP (54, 55), possibly due to increased vascular resistance and arterial stiffness (56). In contrast, much more data are available on the effects of subclinical hypothyroidism on blood pressure. A meta-analysis of observational studies comparing patients with subclinical hypothyroidism to euthyroid controls (N=50,147) found a minor increase in their SBP, but not DPB (57). Importantly, a large (N>30,000) population-based study even found a positive association between normal range TSH levels and SBP as well as DBP (12), which was further confirmed by a recent meta-analysis of 14 observational studies (N=96,175) (58).

In the current study, we found that within the normal range, higher TSH levels were associated with a lower pulse pressure, which was mainly driven by an inverse association with SBP. Future studies should clarify why the nominal association between normal range TSH levels and SBP observed in our study was opposite to the reported in observational studies (58). Importantly, such studies should also take potential non-linear relations into account, as the effects of hypo- and hyperthyroidism on blood pressure come together within the normal range. This is important, as observational studies have shown that increased pulse pressure is an independent predictor of cardiovascular events in patients with hypertension (59, 60), as well as a predictor for peripheral arterial disease (61).

MR analysis suggest a causal association between normal range thyroid function and T2D risk

Several observational studies have shown that thyroid disease and T2D frequently coexist in patients (62-64). A meta-analysis of observational studies in Chinese has also reported an increased risk of diabetic complications in patients with coexisting T2D and subclinical hypothyroidism (65). Both hypo- and hyperthyroidism have been associated with T2D risk and insulin resistance (66, 67), and multiple mechanisms have been suggested to play a role in this association, including intestinal glucose absorption, hepatic gluconeogenesis, and glucose utilization in peripheral tissues (68). However, the associations between variation in normal range thyroid function and T2D are less clear. Recently, a large population-based prospective study in 8,452 participants reported an increased risk of incident T2D in individuals with low-normal thyroid function (13). However, a following meta-analysis in nearly 30,000 participants did not confirm these findings (69). In 2017, Bos *et al*. performed a MR study investigating the effects of genetically predicted TSH and FT4 levels on T2D risk and glycaemic traits, and did not find causal associations (70). Compared to Bos *et al*. (70), we significantly increased statistical power by using genetic instruments which doubled the proportion of explained variance in TSH and FT4 levels, as well as by using more precise effect estimates for T2D, as based on the most recent GWAS meta-analysis including nearly 900,000 participants (74,124 cases and 824,006 controls) (27). Moreover, we performed sensitivity analyses excluding potentially pleiotropic instruments which can be a source of bias in MR analysis. Interestingly, we identified various genetic variants with pleiotropic effects on thyroid function and T2D in our sensitivity analyses using the Cochran's Q statistics. Indeed, these included variants within the *INSR* gene (encoding

the insulin receptor), *IGF2BP2* (which regulates the translation of IGF2 mRNA and has been associated with T2D susceptibility (71)), *GLIS3* (a susceptibility gene for T2D that modulates pancreatic beta cell development and apoptosis (72, 73)), *VEGFA* (essential for a proper formation of pancreatic islet structure (74)), and two variants within the *FGF7* gene (promotes proliferation of embryonic pancreatic epithelial cells (75)). The fact that our statistical analyses identified these variants as pleiotropic also makes sense from a biological perspective, as they are located in loci encoding proteins with a known role in glucose regulation. We excluded these pleiotropic variants, which were in majority associated with both higher TSH levels and higher T2D risk, to unravel the real causal association between normal range thyroid function and T2D. This showed that within the normal range higher TSH levels were associated with lower T2D risk. The carriage of genes with pleiotropic effects could therefore be an important explanation for the observed discrepancy between the results of observational studies and MR analyses.

Bidirectional MR analysis suggests a causal effect of BMI on FT4 levels

In our study, we found no evidence for a causal effect of variation in normal range thyroid function on obesity traits, represented by BMI and WHR. While we cannot exclude causal effects smaller than detectable in our study, this can also suggest that minor variation in thyroid function tests is rather a consequence than a cause of weight change. Indeed, our bidirectional MR analysis indicated that genetically predicted BMI was inversely associated with FT4 levels. Multiple observational studies showed that there is a positive association between BMI and TSH levels, even within the normal range (76). Several observational studies reported also a

positive association between BMI and FT3 levels (77-80), as well as a negative association between BMI and FT4 levels in euthyroid subjects (77, 78). A MR study performed by Taylor *et al.* found that higher BMI leads to higher FT3 levels in children, while no effect of genetically predicted BMI on FT4 levels was observed in that study (81). It was suggested that the increase in serum levels of FT3 may be a compensatory mechanism for the increase in central fat accumulation (80). Although the expression of type 1 deiodinase (DIO1) and type 2 deiodinase (DIO2) in the white adipose tissue (WAT), in comparison to DIO1 expression in the liver or DIO2 expression in the brown adipose tissue (BAT), is minimal (82, 83), it has been shown that DIO1 activity in WAT is increased in obese subjects (84). Therefore higher FT3 and lower FT4 levels in overweight and obese subjects might at least partially result from an increased peripheral conversion of FT4 to FT3 in WAT. Moreover, studies on animal models suggest that, besides its effects on central regulation of the hypothalamus-pituitary-thyroid axis (85), leptin produced in WAT may be also involved in tissue-specific regulation of deiodinase activity in other tissues (86-89). Interestingly, Araujo *et al.* showed that leptin administration restores starvation-induced decrease in DIO1 activity in the liver and the kidney (89), the main sources of circulating FT3. Although these results require further confirmation, this hypothesis would be in line with the observation that weight reduction is associated with a decrease in FT3 and an increase in FT4 serum levels in humans (90).

Strengths and limitations of the study

Strengths of the current study include the use of data from the largest available GWAS on thyroid function and the tested cardiovascular risk factors (24-28). Moreover, sensitivity

413 analyses were performed to reduce bias due to potentially pleiotropic instruments, as well as to
414 provide better insights into the analyzed associations.
415 While we observed several significant associations between genetically predicted TSH levels
416 and the tested outcomes, we found no such associations for genetically predicted FT4 levels. A
417 possible explanation for this discrepancy could be that TSH is a much more sensitive biomarker
418 for detecting small alterations in thyroid function compared to FT4 (91), as relatively modest
419 changes in FT4 concentrations result in marked excursions in TSH levels due to an inverse log-
420 linear association between both parameters (92, 93). A limitation of our study was that we had
421 less power to detect associations with FT4, as the available instruments reported in literature
422 have a lower explained variance compared to TSH levels (4.8% vs. 9.4%, respectively).
423 Therefore, our results should not be interpreted as reflecting direct (*i.e*. not mediated by
424 thyroid hormones) effects of TSH on the tested outcomes. Importantly, the available FT4
425 variants form a highly heterogeneous group, including polymorphisms within genes encoding
426 transcription factors implicated in the pituitary and thyroid development (*FOXE1*, *LHX3*), TH
427 transporters (*SLCO1B1*, *SLC17A4*), TH metabolizing enzymes (*DIO1*, *DIO2*, *AADAT*) and multiple
428 loci without a known function in the hypothalamus-pituitary-thyroid axis (94). Therefore, while
429 they all increase serum FT4 levels, they could well have differential effects on tissue T4 and T3
430 bioavailability. For example, variants in the *DIO1* gene, encoding the type 1 deiodinase (DIO1),
431 which is responsible for peripheral conversion of T4 to T3, result in higher T4 levels and lower
432 T3 levels. This leads to a net euthyroid state of the pituitary, as reflected by the absence of an
433 association with TSH levels. Consequently, while these variants can be used as instruments
434 reflecting variation in normal range FT4 levels, they should not be interpreted as being

435 instruments for increased thyroid function (95). This is supported by our sensitivity analyses in
436 which the effects of deiodinase gene variants and other variants were analysed separately.
437 These analyses showed opposite effect directions in the majority of the tested outcomes, likely
438 reflecting the differential effects of these genetic instruments on T4 and T3 bioavailability.
439 When both subsets are analyzed together, their effects could level out, resulting in a net zero
440 effect. Indeed, none of the MR studies performed so far found any evidence for associations
441 between genetically predicted FT4 levels and tested outcomes, including the recent study on
442 thyroid function and atrial fibrillation risk, which reported significant effects for TSH levels,
443 FT3:FT4 ratio and hyperthyroidism (96). This underlines the importance of having a good
444 biological understanding of the genetic instruments used in MR studies. Finally, while we
445 provide evidence for associations between variation in normal range thyroid function and
446 cholesterol levels, blood pressure and T2D risk, MR studies performed so far found no evidence
447 for a causal association between normal range thyroid function and CVD (97, 98), except for the
448 recently reported association with stroke, that was mediated *via* the risk of atrial fibrillation
449 (99). One of possible explanations is that established cardiovascular risk factors, such as
450 dyslipideamia and hypertension, are nowadays widely recognized and treated in the context of
451 primary prevention. This might limit the potential cause-and-effect relationship between
452 thyroid function and CVD in the general population that was used as a basis for the MR-
453 underlying GWAS, and consequently make it more difficult to detect the effects of variation in
454 normal range thyroid function on CVD in a MR study. Alternatively, unfavourable effects of low
455 normal thyroid function on the lipid profile observed in this study might be levelled out by the
456 beneficial effects of low normal thyroid function on pulse pressure and T2D risk, limiting in that

way the overall effect of variation in normal range thyroid function on cardiovascular risk. Future studies should further investigate this complex relationship.

Conclusions

In conclusion, our study demonstrates that variation in normal range thyroid function is causally associated with serum cholesterol levels, blood pressure and T2D risk. On the other hand, we found no evidence of causal association between variation in normal range thyroid function and the tested obesity traits. Instead, our study suggests that increased BMI might be causally associated with lower FT4 levels in euthyroid individuals. These findings provide a better insight into the complex relationships between thyroid function and CVD risk.

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Disclosure Statement

No competing financial interests exist.

Corresponding author:

Aleksander Kuś, MD, PhD

Department of Internal Medicine and Endocrinology, Medical University of Warsaw

ul. Banacha 1a, 02-097 Warsaw, Poland

tel.: +48 22 599 29 75

fax: +48 22 599 19 75

e-mail: akus@wum.edu.pl

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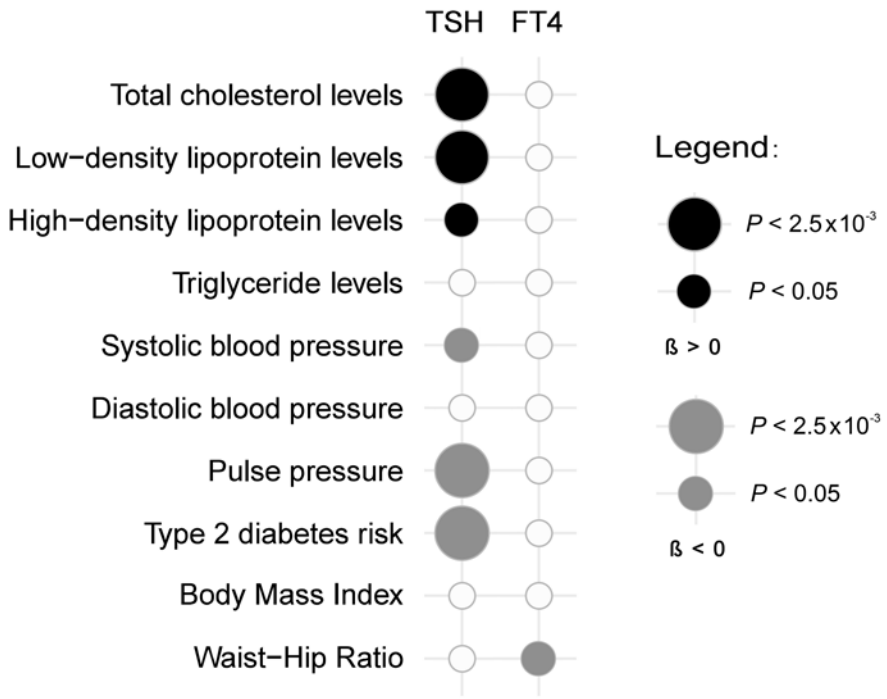

Figure 1. Heatmap of associations between normal range TSH and FT4 levels and major cardiovascular risk factors. For each pair of traits, the size of the circle corresponds to the *P*-value for the regression coefficient (β) from the Mendelian Randomization analysis using the inverse variance weighted method. Positive (direct) association is shown in black, whereas negative (inverse) association is shown in gray.