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Genetic and environmental determinants of cardiometabolic health

Bos, M.M.

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Author: Bos, M.M.

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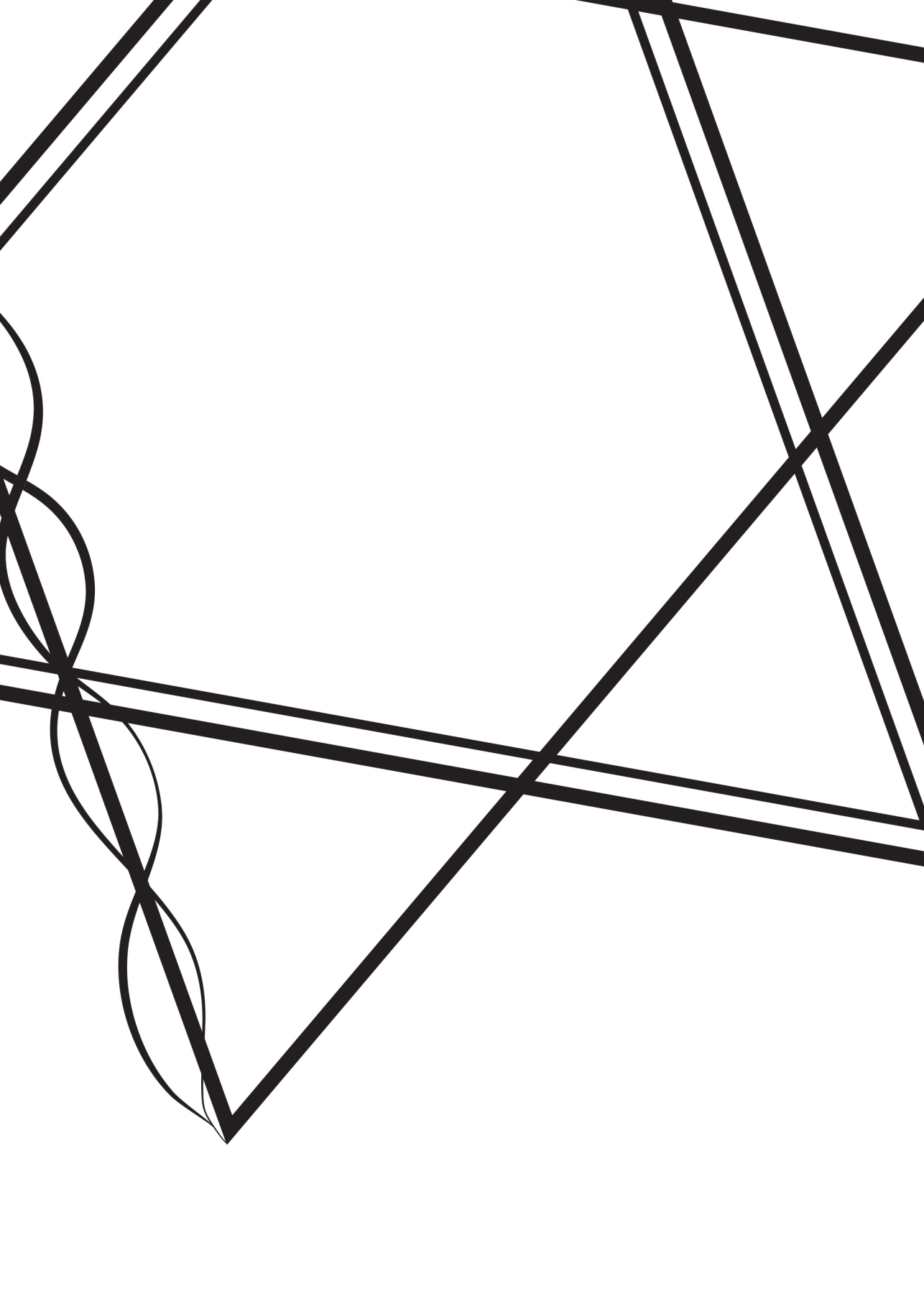
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PART IV

Thyroid status and diabetes







CHAPTER 5.1

Thyroid Signaling, Insulin Resistance,
and 2 Diabetes Mellitus:
A Mendelian Randomization Study

Maxime M Bos, Roelof AJ Smit, Stella Trompet, Diana van Heemst, Raymond Noordam

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ABSTRACT

Increasing evidence suggests an association between thyroid stimulating hormone (TSH), free thyroxine (fT4) and deiodinases with insulin resistance and type 2 diabetes mellitus (T2D). We examined whether TSH and fT4 levels and deiodinases are causally associated with insulin resistance and T2D using Mendelian randomization (MR). We selected twenty genetic variants for TSH level and four for fT4 level (identified in a GWAS meta-analysis of European-ancestry cohorts) as instrumental variables for TSH and fT4 level, respectively. We used summary data from genome-wide association studies (GWAS) on the outcomes type 2 diabetes mellitus (T2D) (DIAGRAM; 12,171 cases, 56,862 controls) and glycemic traits in nondiabetics (MAGIC; N=46,186 for fasting glucose and insulin and N=46,368 for HbA1c). To examine whether the associations between TSH/fT4 levels and the glycemic traits are causal, we combined the effects of the genetic instruments. Furthermore, we examined the associations between 16 variants in *DIO1*, *DIO2*, and *DIO3* and T2D and glycemic traits. We found no evidence of an association between the combined genetic instrumental variables for TSH and fT4 and the study outcomes. For example, we did not observe a genetically determined association between high TSH level and T2D (Odds ratio: 0.91 per standard deviation TSH increase; 95% confidence interval: 0.78;1.07). Selected genetic variants in *DIO1* (e.g., rs7527713) were associated with measures of insulin resistance. We found no evidence of a causal association between circulatory levels of TSH and fT4 with insulin resistance and T2D, but found suggestive evidence that *DIO1* affects glucose metabolism.

INTRODUCTION

Due to the increased proportion of obesity^{1,2}, and increased life expectancy³⁻⁵, type 2 diabetes mellitus (T2D) is becoming a major public health challenge. Key mechanisms involved in T2D are insulin resistance in muscle, adipose tissue and liver, and impaired insulin secretion due to deterioration of pancreatic β -cell function³. As recently described by Peppas *et al.*, there is increasing evidence for the existence of an association between endocrine disorders, like disturbed thyroid function or (subclinical) hypothyroidism, and altered glucose-insulin homeostasis⁶. For example, several studies have reported that subclinical hypothyroidism is related to insulin resistance and T2D^{7,8}.

The concentration of thyroid hormones in the circulation is regulated by the hypothalamic pituitary thyroid (HPT) axis. Thyrotropin releasing hormone (TRH) secreted by the hypothalamus regulates synthesis and release of TSH from the pituitary gland, which stimulates the production and secretion of the thyroid hormones by the thyroid gland. Via a classical feedback loop, thyroid hormones inhibit the production of hypothalamic TRH and pituitary TSH. In target tissues, type 1 and 2 deiodinases convert the prohormone thyroxine (T4) into the active hormone triiodothyronine (T3), while type 3 deiodinase converts T4 into inactive reverse triiodothyronine (rT3)^{9,10}.

Previously, it has been shown that patients with subclinical hypothyroidism have lower insulin sensitivity^{7,11}. In line, in euthyroid individuals, higher fT4 levels and/or lower TSH levels have been associated with a higher insulin sensitivity^{11,12}, and with a lower risk of developing T2D¹³. Of the deiodinases, genetic variation in deiodinase 2 has been associated with risk of T2D¹⁴, but results between studies as these are inconsistent¹⁵⁻¹⁷. These studies suggest thyroid hormone metabolism might exert an effect on insulin sensitivity and T2D, but the biological mechanisms for these observations are largely unclear. Causality of observational associations (e.g., low TSH in euthyroid individuals and higher insulin sensitivity) cannot be ascertained because of unmeasured confounding and/or reverse causality. In case of reverse causality, insulin resistance might suppress thyroid function, and consequently lead to relatively higher TSH and lower fT4 levels.

One method used to ascertain causality of observational associations, free of confounding and reverse causality, is Mendelian randomization (MR)^{18,19}. This method uses genetic variants as instrumental variables for the exposure of interest²⁰. The levels of TSH and fT4 are, in part, genetically determined²¹, and several loci have been identified in a large genome-wide association study (GWAS)²². To date, however, no studies have investigated the relation between genetic variants associated with TSH and fT4 concentration and measures of insulin resistance and T2D. On the other hand,

genetic variation in *DIO2* has been studied before in relation to T2D, but this needs to be explored in more depth to confirm earlier results. With respect to the other deiodinases, deiodinase 1 is highly expressed in liver tissue, an organ which is pivotal in glucose-insulin homeostasis²³ and IGF-1 production²⁴. Deiodinase 3 is an inactivation deiodinase associated with rT3 concentration, and thus inhibits thyroid hormone action. However, to the best of our knowledge, no studies have been performed on deiodinase 1 and 3 in relation to insulin resistance and T2D. In addition to individual participant data, Mendelian randomization studies can also be conducted using the summary statistics data of previously conducted GWAS meta-analyses²⁵. Within the present study, we aimed to investigate whether there is evidence for a causal association between circulatory TSH and fT4 levels and glycemic traits and T2D using Mendelian randomization. Furthermore, to provide more insights in the role of thyroid function in target tissues in the pathogenesis of T2D, we additionally tested genetic variants in deiodinase 1, 2, and 3 in relation to measures of insulin resistance and T2D.

MATERIALS AND METHODS

Selection of Single Nucleotide Polymorphisms associated with TSH or fT4

For the present Mendelian randomization study, we selected the lead single nucleotide polymorphisms (SNPs) for all genetic loci that have been shown to independently associate with the levels of TSH or fT4 ($p\text{-value} < 5 \times 10^{-8}$) as genetic instrumental variables for TSH and fT4 levels, respectively. These loci were extracted from a meta-analysis of genome-wide association studies (GWAS) performed by Porcu *et al.* in individuals of European ancestry²², which is the largest GWAS on TSH and fT4 level to date. This meta-analysis identified twenty independent genetic variants associated with higher serum TSH level in 26,420 individuals and four independent genetic variants associated with higher serum fT4 level in 17,520 individuals. In this meta-analysis, TSH and fT4 were standardized to approximate a normal distribution. The identified genetic variants explained a total of 5.64% and 2.30% of total variation in TSH and fT4 serum concentration, respectively. Within this GWAS analysis, all individuals with a TSH < 0.4 and > 4.0 mIU/L were excluded.

Selection of Single Nucleotide Polymorphisms associated with deiodinases

Adapted from Panicker *et al.*, we selected nine SNPs mapped in *DIO1*, three SNPs in *DIO2* and four SNPs in *DIO3*, which cover 100%, 85% and 71%, respectively, of the common HapMap-based variation in these genes (minor allele frequency $> 10\%$) with r^2 greater than 0.8²⁶.

Data sources and outcome definition

In the present study, we used T2D and measures of insulin resistance as outcomes. For this, we used publically available summary statistics datasets of two large GWAS conducted by the DIABetes, Genetics Replication and Meta-analysis (DIAGRAM) consortium and the Meta-Analyses of Glucose and Insulin-related traits Consortium (MAGIC). These datasets contain the summary level meta-analysis data of these GWAS, comprising the per-allele beta estimates of the SNPs on the outcomes, accompanying standard errors and the effect alleles. The data of the DIAGRAM consortium comprised the stage I meta-analysis of 12 different cohort studies of European ancestry²⁷, including 12,171 cases of T2D and 56,862 controls. T2D was defined based on a fasting glucose > 6.9 mmol/L, treatment with glucose-lowering agents and/or diagnosis by a general practitioner or medical specialist. The used data of MAGIC comprised a meta-analysis of 21 different cohort studies of European ancestry investigating genetic variants associated with glycemic traits²⁸. In total, 46,186 individuals without diabetes were included in the meta-analysis. From the MAGIC consortium we used all glycemic traits, i.e. fasting glucose and insulin, the homeostatic model assessment for insulin resistance

(HOMA-IR) and pancreatic β -cell function (HOMA-B) as study outcomes. Within the GWAS meta-analysis, fasting insulin, HOMA-IR and HOMA-B data were log transformed to approximate a normal distribution. Additionally, we used data from a second GWAS meta-analysis from MAGIC on HbA1c (N = 46,368)²⁹. Furthermore, we used data from MAGIC on fasting glucose and insulin that have been adjusted for body mass index (BMI), as thyroid hormone is known to be associated with BMI³⁰. Potential mediation of effects by BMI (e.g., effects of thyroid function on glucose metabolism through BMI) was accounted for in this analysis, and the direct effect could be studied.

Statistical analyses

To limit bias from including weak genetic instrumental variables, i.e. those that explain little of the variance in the exposure of interest, we calculated the F-statistic as a measure of strength for each genetic instrument. In line with the existing literature, we considered an F-statistic of 10 or more as being of sufficient strength³¹. We then explored whether any of the individual genetic instruments or genetic variants in the deiodinase genes were associated with T2D or glycemic traits. For these analyses, we corrected for multiple testing with Bonferroni based on the number of variants included in each analysis (TSH: $\alpha = 0.05/20 = 0.0025$; fT4: $\alpha = 0.05/4 = 0.0125$; DIOs: $\alpha = 0.05/16 = 0.0031$).

By performing summary level Mendelian randomization analyses, we aimed to separately combine the twenty genetic instrumental variables for TSH level and the four genetic instrumental variables for fT4 level to obtain a genetically determined (causal) association between the TSH and fT4 levels on the study outcomes (notably T2D, fasting glucose and insulin, HOMA-IR and HOMA-B, and HbA1c). The resulting estimate can be interpreted as the change in outcome per unit increase in genetically raised exposure of interest (e.g., change in log(odds) for T2D per standard deviation increase in genetically raised TSH levels). Analogous to pooling estimates from different studies in conventional meta-analysis using inverse-variance weighting (IVW), we weighted this combined estimate by the inverse of the variance of the per-allele effect on the outcome (T2D or glycemic trait) for each genetic instrument. However, the effect estimate retrieved from this analysis might be biased as some of the genetic instrumental variables could be invalid because of pleiotropy. Biological pleiotropy is a phenomenon where a genetic variant also influences other traits than the exposure of interest of the study that influence the outcome of interest, which may therefore bias the results of a Mendelian randomization study (e.g., a genetic variant known to influence TSH associates with T2D, but not solely through modifying TSH level). If these pleiotropic effects across the genetic variants do not balance out, this might skew the mean genetically determined estimate of the exposure on the outcome. Such bias is formally called 'directional pleiotropy'. To take into account potential bias in this study

caused by directional pleiotropy, we conducted two sensitivity analyses, knowing MR-Egger regression³² and weighted median estimator analyses³³. With MR-Egger analyses, we were able to formally test for the presence of directional pleiotropy³². These analyses were repeated using summary-level statistics data of the GWAS analyses on fasting glucose and insulin adjusted for body mass index. The unadjusted source codes for these methods for R, as provided online by the authors^{32, 33} were used for the calculations of the combined effect of the individual genetic instrumental variables. For the combined effect of the genetic instruments on the study outcomes, a two-sided p-value below 0.05 was considered statistically significant.

RESULTS

Effect of individual genetic instruments for TSH or fT4 levels on various study outcomes

All individual genetic instruments for both TSH and fT4 levels had an F-statistic above 10 (TSH: median 42.5; range 32.1 – 245.4; fT4: median 52.1; range 34.1 – 132.3), and were therefore considered to be of sufficient strength to be used in the present study. The associations between the individual genetic instruments for TSH levels and T2D and the glycemic traits are presented in **Figure 1**.

Of the individual genetic instruments, we observed an association between rs9472138 in *VEGFA* and T2D (**Online Supplementary Table 1**), and between rs3813582 in *MAF/LOC440389* and HOMA-IR after correction for multiple testing. Within the analysis adjusted for BMI, only rs9472138 in *VEGFA* was associated with fasting glucose after correction for multiple testing (**Figure 2**). None of the individual genetic instruments for fT4 were associated with any of the study outcomes (**Online Supplementary Figure 1**).

Combined effect of genetic instrumental variables for TSH and fT4 levels on the study outcomes

When using inverse-variance weighted analyses to combine the effects on the outcomes of the individual genetic instruments, we found no evidence that there was an association between TSH level and the risk of T2D (**Table 1 and Figure 3**; odds ratio = 0.91 per 1 standard deviation [SD] higher TSH, 95% confidence interval [CI] = 0.78; 1.07). Furthermore, again using inverse-variance weighted analyses, we found no evidence that there was an association between TSH level and the other studied glycemic traits, such as fasting glucose and insulin. The mean effect estimates remained similar with MR-Egger regression and median weighted estimator analyses. Nevertheless, we found some suggestive evidence using median weighted estimator analysis that there might be an association between a higher TSH level and lower risk of T2D (β = -0.139 [95% CI: -0.301; 0.023]). We found no evidence, using MR-Egger regression analysis, that the intercept deviated from zero, which indicates that we found no evidence that the effect estimates were biased due to directional pleiotropy. In addition, similar results were observed when we used the GWAS datasets on glycemic traits adjusted for BMI (results not shown). Similarly, we did not find evidence for an association between a higher fT4 level and any of the study outcomes (**Table 1 and Online Supplementary Figure 2**).

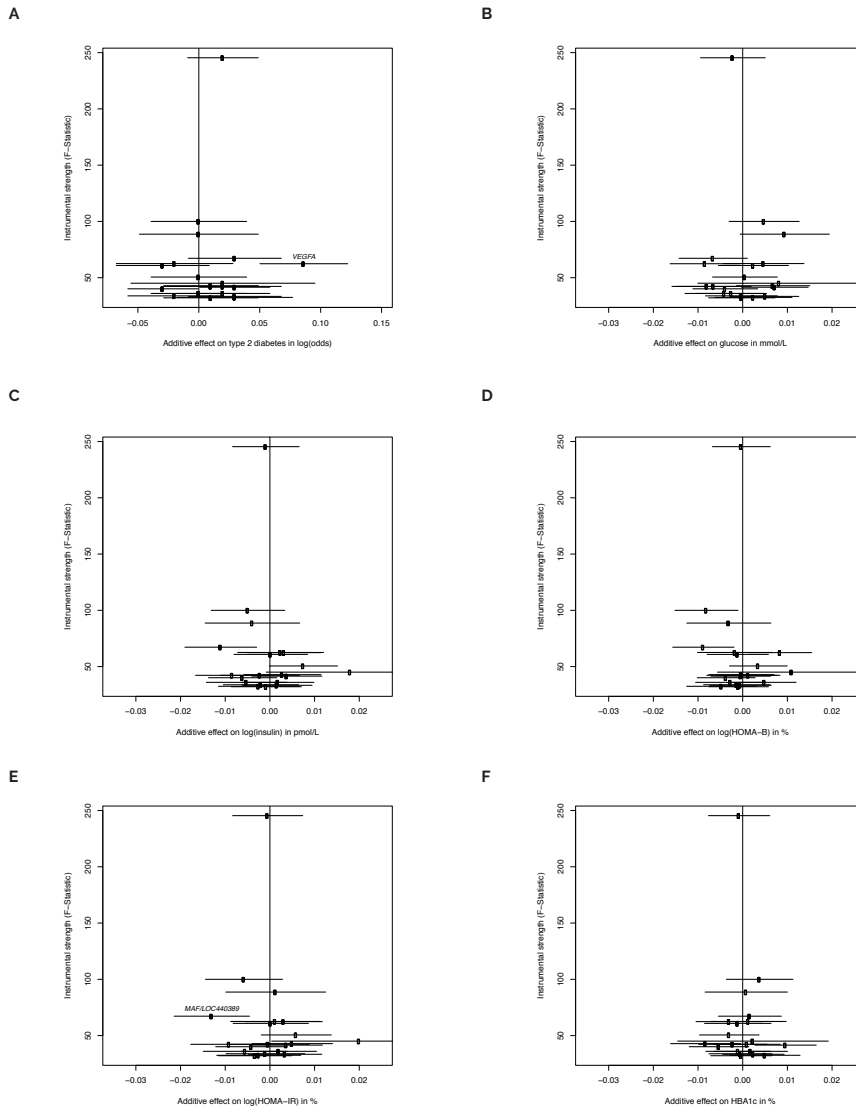


Figure 1. The effect for individual genetic instrumental variables for TSH levels and study outcomes. Results are displayed as the additive beta estimates with 95% confidence intervals. The X-axis presents the additive (per allele) effect for each of the individual genetic instrumental variables and the study outcome. Full written name of gene indicates a significant association after Bonferroni correction ($p = 0.0025$). The association between twenty genetic markers for TSH levels and (A) type 2 diabetes mellitus in log(odds), (B) fasting glucose in mmol/L per unit, (C) fasting log(insulin) in pmol/L, (D) log(homeostatic model assessment for beta cell function (HOMA-B)) in %, (E) log(homeostatic model assessment for insulin resistance (HOMA-IR)) in %, and (F) glycated hemoglobin (HbA1c) in %.

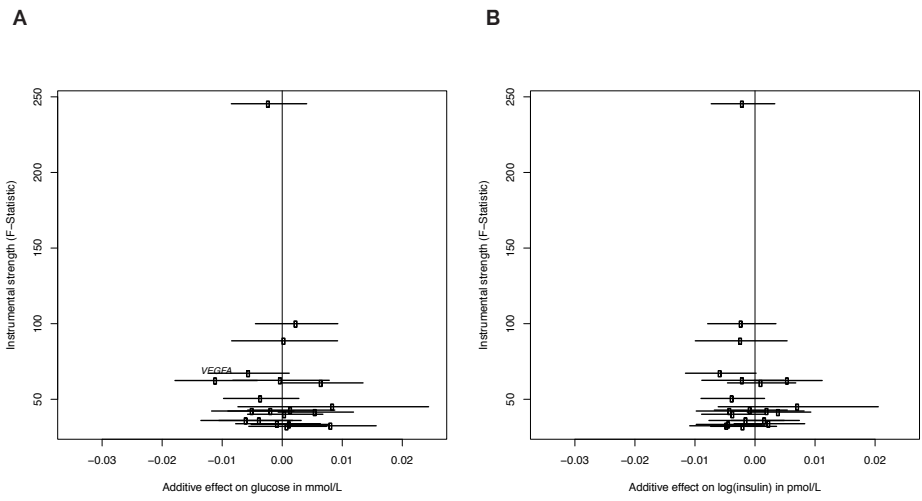


Figure 2. The effect for individual genetic instrumental variables for TSH levels and BMI adjusted fasting glucose and insulin. Results are displayed as the additive beta estimates with 95% confidence intervals. The X-axis presents the additive (per allele) effect for each of the individual genetic instrumental variable and the study outcome. Full written name of gene indicates a significant association after Bonferroni correction ($p = 0.0025$). **(A)** BMI-adjusted fasting glucose in mmol/L and **(B)** BMI-adjusted fasting log(insulin) in pmol/L.

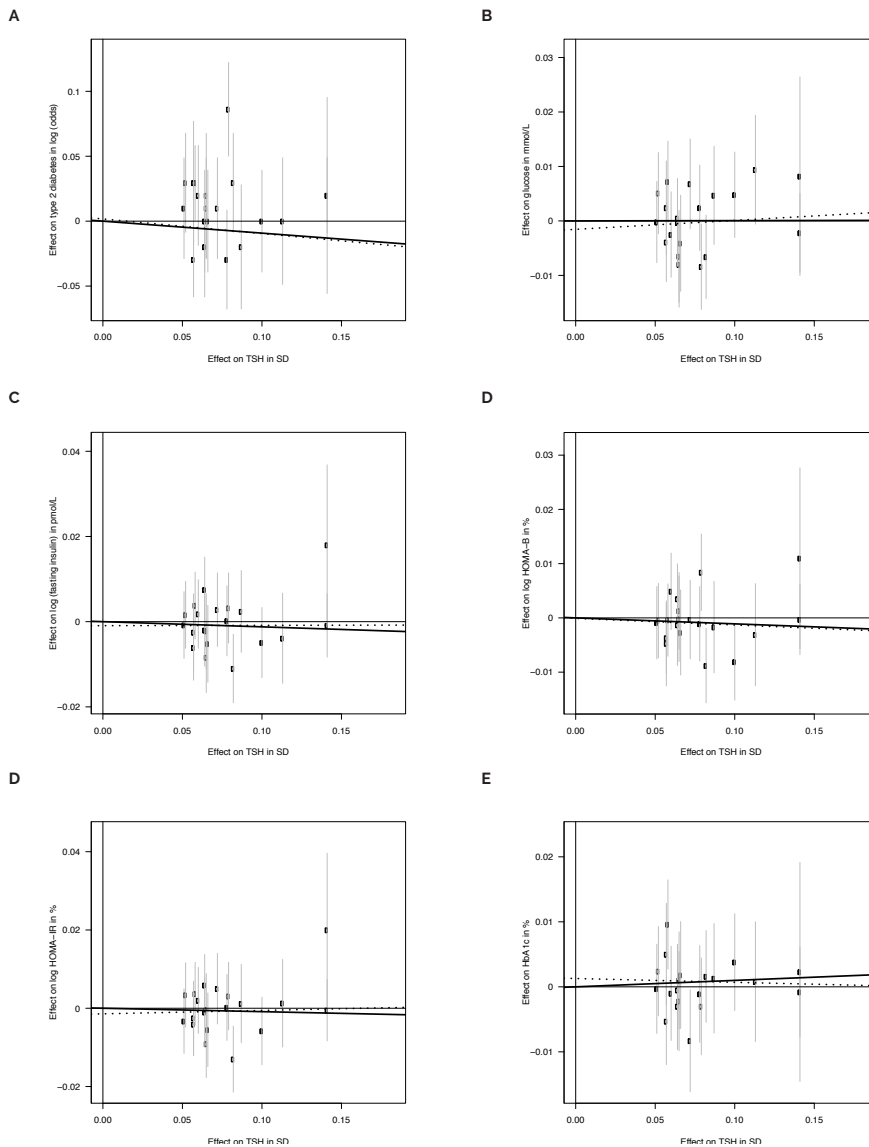


Figure 3. The combined effect of the individual genetic instrumental variables for TSH levels on the study outcomes. Results of the individual genetic instruments for TSH levels displayed as the causal effect on study outcome with 95% confidence interval per standard deviation higher serum level of TSH. The solid line represents the regression line of the inverse-variance weighted approach to combine the individual genetic instruments. The dashed line represents the regression line of the MR-Egger regression analysis to combine the individual genetic instruments. Data presented for (A) type 2 diabetes mellitus in log(odds), (B) fasting glucose in mmol/L, (C) fasting log(insulin) in pmol/L, (D) log(homeostatic model assessment for beta cell function (HOMA-B)) in %, (E) log(homeostatic model assessment for insulin resistance (HOMA-IR)) in %, and (F) glycated hemoglobin (HbA1c) in %.

Effect of genetic variation in deiodinases and the study outcomes

After correction for multiple testing (**Table 2**), the genetic variants rs11206237, rs2268181 and rs7527713 mapped in *DIO1* were significantly associated with fasting insulin ($\beta_{\text{additive}} = -0.018$ pmol/L [95%CI = -0.029; -0.007]; $\beta_{\text{additive}} = 0.019$ pmol/L [95%CI = 0.008; 0.030]; $\beta_{\text{additive}} = -0.018$ pmol/L [95%CI = -0.028; -0.008], respectively), and with HOMA-IR ($\beta_{\text{additive}} = -0.018$ pmol/L [95%CI = -0.029; -0.007]; $\beta_{\text{additive}} = 0.020$ pmol/L [95%CI = 0.009; 0.031]; $\beta_{\text{additive}} = -0.019$ [95%CI = -0.029; -0.009], respectively). In addition, rs7527713 in *DIO1* was associated with HOMA-B ($\beta_{\text{additive}} = -0.014$ [95%CI = -0.023; -0.005]). None of the three genetic variants in *DIO2* and none of the four SNPs in *DIO3* were significantly associated with T2D or any of the glycemic traits.

Table 1. Mendelian randomization estimates

	T2D mellitus in log(odds)	Glucose in mmol/L	Log(in- sulin) in pmol/L	Log(HO- MA-IR) in %	Log(HO- MA-B) in %	HbA1c in %
TSH						
IVW	-0.09 (-0.25;0.07)	0.00 (-0.03;0.03)	-0.01 (-0.04;0.02)	-0.01 (-0.04;0.02)	-0.01 (-0.04;0.01)	0.01 (-0.01;0.03)
MR Egger Intercept	0.00 (-0.04;0.04)	-0.00 (-0.01;0.01)	-0.00 (-0.01;0.00)	-0.00 (-0.01;0.01)	9.3×10^{-5} (-0.01;0.01)	0.00 (-0.00;0.01)
Estimate	-0.11 (-0.47;0.24)	0.02 (-0.06;0.10)	0.00 (-0.10;0.10)	0.01 (-0.08;0.10)	-0.01 (-0.08;0.06)	-0.01 (-0.08;0.07)
WME	-0.14 (-0.30;0.02)	-0.01 (-0.04;0.03)	-0.01 (-0.05;0.03)	-0.00 (-0.04;0.04)	-0.01 (-0.04;0.025)	0.00 (-0.03;0.03)
T4						
IVW	0.10 (-0.07;0.27)	0.00 (-0.04;0.05)	0.02 (-0.02;0.05)	0.01 (-0.03;0.05)	0.013 (-0.02;0.04)	-0.01 (-0.04;0.03)
MR Egger Intercept	0.02 (-0.04;0.07)	0.02 (-0.02;0.05)	-0.01 (-0.04;0.02)	-0.01 (-0.03;0.02)	-0.01 (-0.04;0.01)	-0.00 (-0.02;0.02)
Estimate	-0.04 (-1.33;1.25)	-0.15 (-0.47;0.18)	0.10 (-0.24;0.45)	0.06 (-0.27;0.39)	0.12 (-0.16;0.41)	0.01 (-0.26;0.27)
WME	0.09 (-0.10;0.27)	-0.02 (-0.06;0.02)	0.02 (-0.02;0.07)	0.02 (-0.03;0.07)	0.01 (-0.03;0.04)	-0.01 (-0.04;0.03)

Abbreviations: HOMA-IR, homeostatic model assessment for insulin resistance; HOMA-B, homeostatic model assessment for pancreatic beta cell function; IVW, inverse-variance weighted; WME, weighted median estimator. Data presented as beta coefficients with 95% confidence interval per standard deviation higher serum level of thyroid stimulating hormone (TSH) or free prohormone thyroxine (fT4).

Table 2. *The association between genetic variants for deiodinases and the study outcomes*

	T2D mellitus in log(odds)	Glucose in mmol/L	Log (insulin) in pmol/L	Log (HOMA-IR) in %	Log (HOMA-B) in %	HbA1c in %
<i>DIO1</i>						
11206237	0.01 (-0.04;0.06)	-0.00 (-0.01;0.01)	-0.02 (-0.03;-0.01)	-0.02 (-0.03;-0.01)	-0.01 (-0.02;-0.00)	0.00 (-0.01;0.01)
11206244	0.00 (-0.04;0.04)	-0.00 (-0.01;0.01)	0.00 (-0.01;0.01)	0.00 (-0.01;0.01)	0.00 (-0.00;0.01)	0.00 (-0.01;0.01)
2235544	0.01 (-0.02;0.05)	-0.00 (-0.01;0.00)	0.00 (-0.00;0.01)	0.00 (-0.01;0.01)	0.00 (-0.00;0.01)	-0.00 (-0.01;0.01)
2268181	0.01 (-0.04;0.06)	0.01 (-0.01;0.02)	0.02 (0.01;0.03)	0.02 (0.01;0.03)	0.01 (0.00;0.02)	-0.00 (-0.01;0.01)
2294511	0.01 (-0.03;0.05)	0.00 (-0.01;0.01)	-0.01 (-0.02;0.00)	-0.01 (-0.02;0.01)	-0.01 (-0.01;0.00)	0.00 (-0.00;0.01)
2294512	0.03 (-0.01;0.07)	-0.00 (-0.01;0.01)	-0.00 (-0.01;0.01)	-0.00 (-0.01;0.01)	-0.00 (-0.01;0.01)	-0.00 (-0.01;0.01)
4926616	0.02 (-0.03;0.06)	-0.00 (-0.01;0.01)	0.01 (-0.00;0.02)	0.01 (-0.01;0.02)	0.01 (-0.00;0.02)	-0.01 (-0.01;0.00)
731828	0.01 (-0.03;0.04)	-0.00 (-0.01;0.00)	-0.01 (-0.01;0.00)	-0.01 (-0.01;0.00)	-0.00 (-0.01;0.01)	-0.00 (-0.01;0.01)
7527713	0.02 (-0.02;0.07)	-0.00 (-0.01;0.01)	-0.02 (-0.03;-0.01)	-0.02 (-0.03;-0.01)	-0.01 (-0.02;-0.01)	0.01 (-0.00;0.01)
<i>DIO2</i>						
225011	0.00 (-0.03;0.04)	-0.00 (-0.01;0.01)	-0.00 (-0.01;0.01)	-0.00 (-0.01;0.01)	0.00 (-0.01;0.01)	-0.00 (-0.01;0.01)
225014	0.01 (-0.03;0.05)	-0.00 (-0.01;0.01)	0.00 (-0.01;0.01)	0.00 (-0.01;0.01)	0.00 (-0.01;0.01)	-0.00 (-0.01;0.00)
225015	0.01 (-0.03;0.05)	0.00 (-0.01;0.01)	0.00 (-0.01;0.01)	0.00 (-0.01;0.01)	-0.00 (-0.01;0.01)	0.00 (-0.00;0.01)
<i>DIO3</i>						
17716499	0.02 (-0.03;0.07)	0.01 (-0.00;0.01)	0.00 (-0.01;0.01)	0.00 (-0.01;0.01)	-0.00 (-0.01;0.01)	0.00 (-0.01;0.01)
7150269	0.01 (-0.03;0.05)	0.01 (0.00;0.02)	0.00 (-0.01;0.01)	0.00 (-0.01;0.01)	-0.00 (-0.01;0.01)	0.00 (-0.01;0.01)
8011440	0.01 (-0.03;0.06)	-0.01 (-0.02;0.00)	-0.00 (-0.01;0.01)	-0.00 (-0.01;0.01)	0.00 (-0.01;0.01)	-0.00 (-0.01;0.01)
945006	0.05 (-0.04;0.14)	0.00 (-0.01;0.02)	-0.01 (-0.03;0.01)	-0.01 (-0.02;0.01)	-0.00 (-0.02;0.01)	-0.01 (-0.02;0.01)

Abbreviations: HOMA-IR, homeostatic model assessment for insulin resistance; HOMA-B, homeostatic model assessment for pancreatic beta cell function. Data presented as additive beta coefficients with 95% confidence interval.

DISCUSSION

In the present study we examined whether the previously described observational associations between (subclinical) hypothyroidism, characterized by high TSH levels and fT4 levels within the normal range, with measures of insulin resistance and T2D^{7, 8} are causal using Mendelian randomization analyses. Within this study, we did not find evidence that the association between TSH and fT4 (as determined in a population with TSH>0.4 mIU/L and TSH<4.0 mIU/L) with T2D and glycemic traits (fasting glucose and insulin, HOMA-IR, HOMA-B, and HbA1c) is causal. However, although effect sizes were relatively small, we found suggestive evidence of an association between selected genetic variants in *DIO1* with glycemic traits, but not with T2D.

The lack of a genetically determined (causal) association between TSH/fT4 level and measures of glucose metabolism suggests that the previously observed association between subclinical hypothyroidism and insulin resistance might be explained by reverse causality or residual confounding. In case of reverse causality, progression of insulin resistance and T2D influences TSH and thyroid hormone levels³⁴. In addition, unmeasured or unknown factors not taken into account as confounding variables in the observational studies might have affected the observed association between subclinical hypothyroidism and insulin resistance.

In this study two genetic variants associated with TSH level showed a significant association with the study outcomes after correction for multiple testing. The genetic variant rs3813582 was significantly associated with HOMA-IR. This polymorphism is a noncoding variant in an exonic region of the *LOC440389/MAF* gene, associated with increased thyroid volume³⁵, a condition more frequently found in patients with insulin resistance³⁶. However, the allele that has been associated with higher TSH concentration²² was associated with lower insulin resistance in the data from the MAGIC consortium. Gene expression of *LOC440389/MAF* was previously found higher in thyroid tissue compared to skeletal muscle, which might suggest a more thyroid-specific function³⁵. Further studies are required to gain more information about this SNP and its potential effect on insulin resistance beyond the effects through TSH. Moreover, we observed that rs9472138 in *VEGFA* was associated with T2D and fasting glucose after adjustment for BMI. *VEGFA* encodes a growth factor important in angiogenesis, which is critical for thyroid function due to the high vascularization of this organ for the continuous supply of iodine to synthesize thyroid hormones³⁷. Genetic variation in *VEGFA* has not been significantly related to T2D in GWAS studies. Nevertheless, it might still be a novel genetic predictor for T2D. Previous work already

suggested that *VEGFA* gene variants were associated with diabetic nephropathy³⁸. Based on our results, we hypothesize that this association is independent of TSH level, and that *VEGFA* is involved in multiple biological pathways.

In the literature, there is evidence of an association between deiodinases and the onset of insulin resistance³⁴. The most studied polymorphism in the deiodinases in relation to insulin resistance and T2D is rs225014 in *DIO2*, which has a high allele frequency in various ethnic groups¹⁶. However, previous studies have shown contradictory results^{15, 17}. In this study we assessed the role of deiodinases on insulin resistance and T2D using genetic variants in the deiodinase genes that cover the gene as much as possible²⁶. We observed genetic variation in *DIO1* to be significantly associated with fasting insulin, HOMA-IR and HOMA-B. In the data from DIAGRAM and MAGIC, we did not find a significant association between the previously described polymorphism in *DIO2* and increased risk for T2D and any of the glycemic traits in individuals of European ancestry. The discrepancy in the results can be explained by the smaller sample size of the previous studies and by the differences in ethnic background of the different cohorts examining the relationship. However, we showed that there might be a minor effect of deiodinase 1 on insulin resistance. To the best of our knowledge, no data are yet available about this mechanism, which will therefore require further studies. Speculatively, polymorphisms in *DIO1* are highly expressed in liver tissue²³ and have been associated with IGF-1 and IGF-1 related endpoints, such as body height and skeletal muscle mass²⁴. Skeletal muscle is an important organ for insulin-stimulated glucose uptake and a lower relative muscle mass was found to associate with insulin resistance³⁹. However, this hypothesis should be explored in more detail in future studies.

Strengths of the present study include the large sample size of the used study populations, and the use of summary statistics data which increases efficiency. A limitation of our study was that the twenty independent loci associated with the level of TSH only explained 5.64% of the total variation in TSH concentration, and for fT4 this was only 2.30%²². Nevertheless, the F-statistics of all individual genetic instrumental variables was above 10, which should greatly limit the likelihood of weak instrumental variable bias³¹. Additional insights could be provided by the identification of additional loci that are associated with TSH and fT4 concentration. However, the effect sizes of these newly identified loci will probably be lower or they will have a smaller allele frequency. In both cases the strength of the instrumental variables will be lower, and the contribution of these genetic instrumental variables in the combined effect will be lower as well. Another limitation is that the genetic contribution to TSH and fT4 concentration was found to be different between men and women²², and (subclinical) hypothyroidism is more frequent in women than in men⁴⁰. As the datasets of the study outcomes were

not available stratified by sex, we were not able to address the research questions separately for men and women. Furthermore, as the genetic instrumental variables as well as the summary statistics originated from population of European ancestry, our results may not be generalized to populations of non-European ancestry. A last limitation might be that the GWAS on TSH and fT4 was only performed in euthyroid individuals. However, as the combined score of the twenty identified genetic variants associated with TSH was also associated with a higher risk of having an extreme high TSH level²², results might be also applicable for patients beyond the normal TSH range.

Taken together, the results in the present study indicate no causal association between genetically determined circulatory TSH and fT4 levels and higher risk of T2D. This conclusion was supported by the lack of significant association between genetically determined circulatory TSH and fT4 with glycemic traits. Moreover, we did observe an association between genetic variants in *DIO1* and insulin resistance, which might suggest that thyroid metabolism affects glucose metabolism in target tissues.

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REFERENCES

1. Ogden CL, Carroll MD, Flegal KM. Prevalence of obesity in the United States. *Jama*. 2014;312(2):189-90.
2. Ogden CL, Carroll MD, Kit BK, Flegal KM. Prevalence of childhood and adult obesity in the United States, 2011-2012. *Jama*. 2014;311(8):806-14.
3. Chen L, Magliano DJ, Zimmet PZ. The worldwide epidemiology of type 2 diabetes mellitus--present and future perspectives. *Nature reviews Endocrinology*. 2012;8(4):228-36.
4. van Dieren S, Beulens JW, van der Schouw YT, Grobbee DE, Neal B. The global burden of diabetes and its complications: an emerging pandemic. *European journal of cardiovascular prevention and rehabilitation : official journal of the European Society of Cardiology, Working Groups on Epidemiology & Prevention and Cardiac Rehabilitation and Exercise Physiology*. 2010;17 Suppl 1:S3-8.
5. Kirkman MS, Briscoe VJ, Clark N, Florez H, Haas LB, Halter JB, et al. Diabetes in older adults. *Diabetes care*. 2012;35(12):2650-64.
6. Peppas M, Koliaki C, Nikolopoulos P, Raptis SA. Skeletal muscle insulin resistance in endocrine disease. *J Biomed Biotechnol*. 2010;2010:527850.
7. Maratou E, Hadjidakis DJ, Kollias A, Tsegka K, Peppas M, Alevizaki M, et al. Studies of insulin resistance in patients with clinical and subclinical hypothyroidism. *European journal of endocrinology / European Federation of Endocrine Societies*. 2009;160(5):785-90.
8. Al Sayed A, Al Ali N, Bo Abbas Y, Alfadhli E. Subclinical hypothyroidism is associated with early insulin resistance in Kuwaiti women. *Endocr J*. 2006;53(5):653-7.
9. Bianco AC, Salvatore D, Gereben B, Berry MJ, Larsen PR. Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. *Endocrine reviews*. 2002;23(1):38-89.
10. Bianco AC, Larsen PR. Cellular and structural biology of the deiodinases. *Thyroid : official journal of the American Thyroid Association*. 2005;15(8):777-86.
11. Garduno-Garcia Jde J, Alvirde-Garcia U, Lopez-Carrasco G, Padilla Mendoza ME, Mehta R, Arellano-Campos O, et al. TSH and free thyroxine concentrations are associated with differing metabolic markers in euthyroid subjects. *European journal of endocrinology / European Federation of Endocrine Societies*. 2010;163(2):273-8.
12. Amouzegar A, Kazemian E, Gharibzadeh S, Mehran L, Tohidi M, Azizi F. Association between thyroid hormones, thyroid antibodies and insulin resistance in euthyroid individuals: A population-based cohort. *Diabetes & metabolism*. 2015;41(6):480-8.
13. Chaker L, Ligthart S, Korevaar TI, Hofman A, Franco OH, Peeters RP, et al. Thyroid function and risk of type 2 diabetes: a population-based prospective cohort study. *BMC medicine*. 2016;14(1):150.
14. Marsili A, Zavacki AM, Harney JW, Larsen PR. Physiological role and regulation of iodothyronine deiodinases: a 2011 update. *Journal of endocrinological investigation*. 2011;34(5):395-407.
15. Maia AL, Hwang SJ, Levy D, Larson MG, Larsen PR, Fox CS. Lack of association between the type 2 deiodinase A/G polymorphism and hypertensive traits: the Framingham Heart Study. *Hypertension*. 2008;51(4):e22-3.
16. Dora JM, Machado WE, Rheinheimer J, Crispim D, Maia AL. Association of the type 2 deiodinase Thr92Ala polymorphism with type 2 diabetes: case-control study and meta-analysis. *European journal of endocrinology / European Federation of Endocrine Societies*. 2010;163(3):427-34.

17. Mentuccia D, Proietti-Pannunzi L, Tanner K, Bacci V, Pollin TI, Poehlman ET, et al. Association between a novel variant of the human type 2 deiodinase gene Thr92Ala and insulin resistance: evidence of interaction with the Trp64Arg variant of the beta-3-adrenergic receptor. *Diabetes*. 2002;51(3):880-3.
18. Postmus I, Deelen J, Sedaghat S, Trompet S, de Craen AJ, Heijmans BT, et al. LDL cholesterol still a problem in old age? A Mendelian randomization study. *International journal of epidemiology*. 2015;44(2):604-12.
19. Voight BF, Peloso GM, Orho-Melander M, Frikke-Schmidt R, Barbalic M, Jensen MK, et al. Plasma HDL cholesterol and risk of myocardial infarction: a mendelian randomisation study. *Lancet*. 2012;380(9841):572-80.
20. Lawlor DA, Harbord RM, Sterne JA, Timpson N, Davey Smith G. Mendelian randomization: using genes as instruments for making causal inferences in epidemiology. *Statistics in medicine*. 2008;27(8):1133-63.
21. Medici M, Visser WE, Visser TJ, Peeters RP. Genetic determination of the hypothalamic-pituitary-thyroid axis: where do we stand? *Endocrine reviews*. 2015;36(2):214-44.
22. Porcu E, Medici M, Pistis G, Volpato CB, Wilson SG, Cappola AR, et al. A meta-analysis of thyroid-related traits reveals novel loci and gender-specific differences in the regulation of thyroid function. *PLoS genetics*. 2013;9(2):e1003266.
23. St Germain DL, Galton VA. The deiodinase family of selenoproteins. *Thyroid : official journal of the American Thyroid Association*. 1997;7(4):655-68.
24. Verloop H, Dekkers OM, Peeters RP, Schoones JW, Smit JW. Genetics in endocrinology: genetic variation in deiodinases: a systematic review of potential clinical effects in humans. *European journal of endocrinology*. 2014;171(3):R123-35.
25. Burgess S, Scott RA, Timpson NJ, Davey Smith G, Thompson SG, Consortium E-I. Using published data in Mendelian randomization: a blueprint for efficient identification of causal risk factors. *European journal of epidemiology*. 2015.
26. Panicker V, Cluett C, Shields B, Murray A, Parnell KS, Perry JR, et al. A common variation in deiodinase 1 gene DIO1 is associated with the relative levels of free thyroxine and triiodothyronine. *The Journal of clinical endocrinology and metabolism*. 2008;93(8):3075-81.
27. Morris AP, Voight BF, Teslovich TM, Ferreira T, Segre AV, Steinthorsdottir V, et al. Large-scale association analysis provides insights into the genetic architecture and pathophysiology of type 2 diabetes. *Nature genetics*. 2012;44(9):981-90.
28. Dupuis J, Langenberg C, Prokopenko I, Saxena R, Soranzo N, Jackson AU, et al. New genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk. *Nature genetics*. 2010;42(2):105-16.
29. Soranzo N, Sanna S, Wheeler E, Gieger C, Radke D, Dupuis J, et al. Common variants at 10 genomic loci influence hemoglobin A(1)C levels via glycemic and nonglycemic pathways. *Diabetes*. 2010;59(12):3229-39.
30. Manning AK, Hivert MF, Scott RA, Grimsby JL, Bouatia-Naji N, Chen H, et al. A genome-wide approach accounting for body mass index identifies genetic variants influencing fasting glycemic traits and insulin resistance. *Nature genetics*. 2012;44(6):659-69.
31. Pierce BL, Ahsan H, Vanderweele TJ. Power and instrument strength requirements for Mendelian randomization studies using multiple genetic variants. *International journal of epidemiology*. 2011;40(3):740-52.

32. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. *International journal of epidemiology*. 2015;44(2):512-25.
33. Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent Estimation in Mendelian Randomization with Some Invalid Instruments Using a Weighted Median Estimator. *Genetic epidemiology*. 2016;40(4):304-14.
34. Sandhu MS, Debenham SL, Barroso I, Loos RJ. Mendelian randomisation studies of type 2 diabetes: future prospects. *Diabetologia*. 2008;51(2):211-3.
35. Teumer A, Rawal R, Homuth G, Ernst F, Heier M, Evert M, et al. Genome-wide association study identifies four genetic loci associated with thyroid volume and goiter risk. *American journal of human genetics*. 2011;88(5):664-73.
36. Duran AO, Anil C, Gursoy A, Nar A, Inanc M, Bozkurt O, et al. Thyroid volume in patients with glucose metabolism disorders. *Arquivos brasileiros de endocrinologia e metabologia*. 2014;58(8):824-7.
37. Yamada E, Yamazaki K, Takano K, Obara T, Sato K. Iodide inhibits vascular endothelial growth factor-A expression in cultured human thyroid follicles: a microarray search for effects of thyrotropin and iodide on angiogenesis factors. *Thyroid : official journal of the American Thyroid Association*. 2006;16(6):545-54.
38. Mooyaart AL, Valk EJ, van Es LA, Bruijn JA, de Heer E, Freedman BI, et al. Genetic associations in diabetic nephropathy: a meta-analysis. *Diabetologia*. 2011;54(3):544-53.
39. Bijlsma AY, Meskers CG, van Heemst D, Westendorp RG, de Craen AJ, Maier AB. Diagnostic criteria for sarcopenia relate differently to insulin resistance. *Age (Dordr)*. 2013;35(6):2367-75.
40. Fatourechi V. Subclinical hypothyroidism: an update for primary care physicians. *Mayo Clinic proceedings*. 2009;84(1):65-71.

