Heredity and lifestyle in the determination of between-subject variation in thyroid hormone levels in euthyroid men

Short Title: Determinants of thyroid function

Greet Roef\textsuperscript{1}, Youri Taes\textsuperscript{1}, Kaatje Toye\textsuperscript{1}, Stefan Goemaere\textsuperscript{1}, Tom Fiers\textsuperscript{2}, Alain Verstraete\textsuperscript{2}, Jean-Marc Kaufman\textsuperscript{1}.

1. Department of Endocrinology and Metabolic Bone diseases, Ghent University Hospital
2. Department of Clinical chemistry, microbiology and immunology, Ghent University Hospital

Address for correspondence:

Dr. Greet Roef
Dept. Endocrinology
Ghent University Hospital
De Pintelaan 185
9000 Ghent / Belgium
Tel: ++ 32 9 332 55 95
Fax: ++ 32 9 332 3817
E-mail: Greet.Roef@ugent.be

Word Count: (253); (3739)
Nr. of figures: 4; Nr. of Tables: 4
Key words: thyroid hormone levels, heritability, common genetic variation, lifestyle-related determinants, urinary iodine
Abstract

Objective

Variation in thyroid hormone (TH) concentrations between subjects is greater than with time in a single subject, suggesting an individual set-point for thyroid function. We have previously shown that TH levels within normal range are associated with clinical indices such as bone mass, BMI, and heart rate. The aim of this study in young men was therefore to gain insight into the determinants of variation in TH levels among healthy subjects.

Methods

Healthy male siblings (n=941, 25-45 yrs) were recruited in a cross-sectional, population-based study; a history or treatment of thyroid disease and thyroid auto-immunity were exclusion criteria. A complete assessment of TH status was performed (TSH, (F)T4, (F)T3, thyroperoxidase and thyroglobulin antibodies, reverse T3 (rT3), TBG and urinary iodine levels). Genotyping was performed by TaqMan and KASP (KBiosciences) genotyping assays.

Results

(F)T4, rT3 and TBG had heritability estimates between 80 and 90%. Estimates were lower for (F)T3 (60%) and lowest for TSH (49%).

Significant associations were observed between different Single Nucleotide Polymorphisms (SNPs) in the thyroid pathway and TSH, FT4, ratio FT3/FT4 and rT3. Nevertheless, these SNPs only explain a limited part of the heredity. As to age and lifestyle-related factors, (F)T3 was negatively related to age and education level, positively to smoking and BMI (all p<0.0001) but not substantially to urinary iodine concentrations. Smoking was also negatively related to TSH and positively to FT4.

Conclusion
Both genetic and lifestyle-related factors play a role in determining between-subject variation in TH levels in euthyroid young men, although genetic factors seem most important.
Introduction

In healthy subjects, TSH and thyroid hormone concentrations can show substantial differences between individuals (inter-individual variation), whereas variability is much less in the same individual over a prolonged period of time (intra-individual variation). This suggests an individual set-point for pituitary-thyroid axis function (1). We and others have shown that this between-subject variation in thyroid hormone levels, although within the normal range, is nevertheless associated with a number of clinical parameters such as bone mass, BMI, metabolic indices and heart rate (2-5). The physiological basis of the setpoint of this axis is poorly understood (6). Twin studies demonstrate a moderately strong genetic influence (7-11). In recent years, several of the genes involved in this regulation have been identified, with common genetic variation in Phosphodiesterase 8B (PDE8B), Thyroid-Stimulating Hormone Receptor (TSHR), Deiodinase 1 (DIO1) and Capping Protein Muscle Z-line beta (CAPZB) being associated with circulating thyroid hormones (12-22). Besides, some of these single nucleotide polymorphism (SNPs) in the thyroid hormone pathway have also been associated with clinical characteristics as well such as lean mass, bone density, hypertension and insulin resistance (23-28). Nevertheless, most of the heritability remains unexplained, suggesting that many more regulatory genes remain to be identified and/or that rare genetic variants may be important (6).

Besides genetic factors, age and lifestyle-related factors can as well have an important influence on thyroid hormone levels. Aging (29, 30), smoking (31, 32), body composition (3, 33) and differences in iodine intake (34-36) were all reported to influence TSH and thyroid hormone concentrations, although not all studies agree on the magnitude of the effects.

Few studies have investigated genetic together with lifestyle-related determinants of thyroid function (8, 11). The present study therefore investigates the contribution of genetics, i.e. both heritability in general as well as the effects of specific SNPs in the thyroid hormone pathway, together with lifestyle-related factors in the determination of between-subject variation in thyroid
hormone levels in a well-characterized population of 941 healthy euthyroid brothers between 25 and 45 yrs old.
Methods

Study design and population

Participants were recruited from the population registries of the semi-rural to suburban communities around Ghent, Belgium. Whereas the initial focus of this study in healthy young men was on the determinants of sex steroid levels and peak bone mass, its scope has been extended to include the determinants and clinical correlates of thyroid hormone levels. Inclusion criteria and study design were described previously (37, 38). Men aged 25-45 years old were contacted by direct mailing, briefly describing the study purpose and were asked if they had a brother within the same age range also willing to participate. Finally, a sample of 1114 men agreed to participate. After exclusions, 1001 men were included in the study: 435 brother pairs, 25 families with 3 brothers and 2 families with 4 brothers. 92 men were included as single participants, when their brother could not participate in the study. All analyses were done taking into account the family structure. The maximal age difference within brother pairs was arbitrarily set at 12 years. All participants were in good health and completed questionnaires about previous illnesses, smoking (never/former/present smoker), food intake (calcium intake), education (years of education), physical activity and medication use. Exclusion criteria were defined as illnesses or medication use affecting body composition, hormones or bone metabolism: current or prolonged (>3 months) use of glucocorticosteroids, anti-androgens, vitamin D supplements, insulin, thyroxin, previous or current use of anti-epileptic drugs, hypogonadism, hyperthyroidism, cystic fibrosis, malabsorption or eating disorders, disorders of collagen metabolism or bone development, chronic renal failure, alcohol abuse and autoimmune rheumatoid disease. All subjects were tested for the presence of thyroid auto-antibodies and those with serum levels above the clinical cut-off (TPOAb >35 U/L or TgAb >115 U/L) were additionally excluded (60 persons or 5.3% of our population), leaving 941 subjects. The study protocol was approved by the ethical committee of the Ghent University Hospital and written informed consent was obtained from all participants. Smoking habits were registered as current or previous smoking.
Body weight was measured in light indoor clothing without shoes. Standing height was measured using a wall-mounted Harpenden stadiometer (Holtain Ltd., Crymuch, UK).

**Biochemical determinations**

Venous blood and urine samples were obtained between 08:00 and 10:00 h after overnight fasting. All serum samples were stored at –80°C until batch analysis. Thyroid tests included thyroid stimulating hormone (TSH), free thyroxin (FT4), free triiodothyronine (FT3), total T3 (TT3), total T4 (TT4), reverse T3 (rT3) and thyroid binding globulin (TBG) as well as thyroperoxidase antibodies (TPOAb) and thyroglobulin antibodies (TgAb). All measurements with the exception of TBG and rT3 were performed using immuno-electrochemiluminescence (Roche reagents) on Modular E or Cobas 411 (Roche Diagnostics GmbH, Mannheim, Germany). TBG and rT3 were measured by a radioimmunoassay (DIAsource ImmunoAssays S.A., Nivelles, Belgium). A commercial radioimmunoassay was used to determine serum levels of E2 (Clinical assay, Diasorin s.r.l., Saluggia, Italy).

Urine samples were stored at -18°C until assayed. Urinary iodine was determined using inductively coupled plasma mass spectrometry (ICP-MS) on a Perkin Elmer Elan DRC-e equipped with a standard cross-flow nebulizer and a Dynamic Reaction Cell (Perkin Elmer). Results are quantitatively reported if the concentrations exceed the limit of quantification, i.e. 4 µg/L, samples above the upper limit of quantification (500 µg/L) were diluted prior to re-analysis. The laboratory participates in the external quality control program Quebec Multi-Element External Quality Assessment Scheme (Canada).

Creatinine in urine was determined by the kinetic Jaffé-method on a Cobas 411 (Roche Diagnostics GmbH, Mannheim, Germany). The µg iodine/g creatinine ratio was calculated by dividing urinary iodine by urinary creatinine and multiplying the result by 100. Since we studied only Caucasian men of a particular age category (25-45 yrs), additional adjustment for sex, race or age was not necessary (39).

The intra- and interassay CV % were below 10% for all measurements.

**Genotyping**
The SNPs in DIO1 (rs11206244 and rs2235544), DIO2 (rs225014) and TRHR (rs7832552) were determined by TaqMan Pre-Designed SNP Genotyping assays (Applied Biosystems, Foster City, CA, USA) which were run on the StepOne System (Applied Biosystems, Foster City, CA, USA). Genotyping was successful in over 98% of the 4 SNPs, with error rates of approximately 0.5%.

All other SNPs were genotyped by KBiosciences using KASPar technology (LGC, Middlesex, UK). KASP is a competitive allele-specific polymerase chain reaction incorporating a fluorescent resonance energy transfer quencher cassette. Genotyping was successful for over 97% of the sample across the 5 SNPs, with error rates of approximately 0.5%. None of the SNPs deviated significantly from Hardy-Weinberg equilibrium.

Statistics

Descriptives are expressed as mean ± standard deviation or median [1st -3rd quartile] when criteria for normality were not fulfilled (Kolmogorov-Smirnov) and dependent variables (hormone concentrations) were log-transformed in subsequent linear models. Linear mixed-effects modeling with random intercepts and a simple residual correlation structure for random effects was used to evaluate cross-sectional relationships in our study population, taking the interdependence of measurements within families into account. Parameters of fixed effects were estimated via restricted maximum likelihood estimation and reported as standardized estimates of effect size (β) with their respective standard error of the mean. Estimates for the SNPs were calculated using an additive model. Significance levels for associations were set at p-values ≤ 0.05. P-values in figures result from ANOVA. Statistical analyses were performed using Spotfire S+ 8.1 (Insightful, Seattle, WA, USA) and MedCalc (Mariakerke, Belgium).

Taking advantage of the family structure of the dataset, the polygenic program in SOLAR 4.0 (Southwest Foundation for Biomedical Research, San Antonio, TX) was used to estimate the (shared) heritability of thyroid hormone concentrations (40).
Results

Men with positive TPO or TG antibodies were excluded from further analyses, as stated in the methods section. Thyroid hormone concentrations for men without thyroid auto-immunity are given in Table 1, together with the other descriptive parameters of the population.

Genetic determinants of thyroid function

Heritability

Heritability estimates for thyroid hormone concentrations are listed in Table 2. Estimates for FT4, TT4, rT3 and TBG are all high and in the same range between 80 and 90%. The estimates for FT3 and TT3 are considerably lower (60%). The lowest heritability estimate is observed for TSH (49%).

Single Nucleotide Polymorphisms (SNPs) in the thyroid hormone pathway

Associations between the presence of SNPs in the thyroid hormone pathway and concentrations of TSH, FT4, FT3, TT4, TT3, rT3 and the ratio FT3/FT4, are given in Table 3 and Figure 1, 2 and 3. A total of 9 SNPs were determined. (Log) TSH is highly significantly positively associated with rs4704397 in PDE8B (explaining 1.5% of variation in an unadjusted model), and negatively with the presence of rs13063628 in THRB, although the latter association is less significant (explaining 0.5% of the variation). Two SNPs in the TSHR (rs10149689 and rs12050077) are negatively associated with FT4 concentrations (both explaining 1% of the variation in an unadjusted model) (Figure 2). The other SNP in TSHR, rs1991517, does not show associations with thyroid hormone concentrations. Significant associations with FT4 concentrations are observed for 2 SNPs in DIO1; a positive association for rs11206244 and a negative association for rs2235544 (both explaining 0.5% of variation). In agreement with this negative association with FT4, this last SNP (rs2235544) also shows a negative association with total T4-levels ($\beta=-0.10 \pm 0.05$, $p=0.03$). In addition, these SNPs are positively associated with the ratio FT3/FT4, explaining 1% of variation (Figure 3). Finally, several associations with reverse T3-concentrations are observed: strong associations with the two SNPs in
DIO1, rs11206244 (positive) and rs2235544 (negative) (Figure 3), and borderline-significant associations for the two SNPs in TSHR, rs10159689 and rs12050077 (negative) and for the SNP in DIO2 (negative).

Since we replicated previously studied SNPs, we did not adjust significance levels for multiple testing. Nevertheless, it can be mentioned that adjustment for multiple comparisons according to Bonferroni would require a p-value of 0.006 (0.05 divided by 9) for significance. According to this criterion, only the associations of the SNP in PDE8B with TSH and of rT3 and the FT3/FT4 ratio with the SNPs in DIO1 are significant.

**Age and lifestyle-related determinants of thyroid function**

Associations between parameters of thyroid function and age and different lifestyle-related covariates in unadjusted models are listed in Table 4. Even though the age range in this cohort is rather narrow and only young to middle-aged men (25-45 yrs) are considered, age is negatively associated to all parameters of thyroid function and explains between 2 and 3.5% of the variation in TH parameters.

Smoking is negatively associated with TSH and positively with all other thyroid parameters. It explains 1% of the variation in TSH and (F) T4 and 3% of the variation in (F) T3. Additional adjustment for TBG or Estradiol (E2) does not change the observed associations between smoking and thyroid parameters (data not shown).

BMI is positively associated with FT3, TT3, TBG and rT3, explaining about 2% of the variation in these parameters.

The median level of iodine in urine is 0.074µg/ml (0.054-0.099), indicative of a mild iodine deficiency in this cohort. Spot urinary iodine concentrations are not related to any parameter of thyroid hormone status, except for a negative relation with rT3 concentrations (explaining 0.5% of the variation). The urinary iodine/creatinine ratio, however, is significantly negatively associated with FT4 and rT3 levels (explaining 0.5 en 2% of the variation respectively). There is also a non-significant
trend towards a negative association with TT4. Besides, (F) T4, FT3 and rT3 levels are significantly different between subjects from different quartiles of urinary iodine/creatinine ratio, with a trend for lower concentrations with a higher quartile (Figure 4). Furthermore, the ratio is positively associated with calcium intake in this population (β=0.12 ± 0.03, p<0.0001), indicative for a positive association between intake of dairy products and urinary iodine. Urinary iodine levels or serum TSH and thyroid hormones were not related to the season in which the examinations took place.

Education level is negatively associated to (F) T3, the ratio FT3/FT4 and TBG, as well as to BMI (β=-0.10 ± 0.03, p=0.002) and smoking (β=-0.30 ± 0.08, p<0.0001). It explains 3% of the variation in (F) T3 and the ratio FT3/FT4. Nevertheless, the associations between education and thyroid parameters remain significant after adjustment for smoking and BMI. Besides, a higher level of physical activity during the job is positively associated to (F) T3 (β=0.18, p<0.0001) and negatively to FT4 (β=-0.13, p<0.0001), whereas the exertion of sport in the free time is negatively related to (F) T3 (β=-0.15, p<0.0001) and (F) T4 (β=-0.09, p=0.001). Sport exertion in free time is also negatively associated with BMI, whereas a physical active job does not influence BMI (data not shown). When the different lifestyle-related determinants are considered together in a multiple regression model, the observed associations from the unadjusted models remain largely significant (data not shown).
Discussion

In this study, we have investigated genetic and lifestyle-related determinants of thyroid hormone function in a well characterized population of 941 healthy, euthyroid young to middle-aged brothers. Our observations on heritability, with the highest estimates for (F) T4, TBG and rT3, lower estimates for (F)T3 and the lowest estimate for TSH, are partially in agreement with earlier findings. Reported estimates range from 32 to 65% for TSH, from 37 to 65% for FT4 and from 23 to 67% (7, 10, 11). The observation of the lowest estimate for TSH is in agreement with observations from Samollow et al. (11). Nevertheless, other studies found relatively higher estimates for TSH than for (free) thyroid hormones (41), or estimates lying within the same range as for free thyroid hormones (7). Some authors hypothesize that variation in serum TSH concentrations is under a stronger genetic influence and lesser environmental influence than (F) T4 and (F) T3 (10). We could not confirm this hypothesis, although we did observe lower heritability estimates for (F) T3 than for (F) T4, which suggests a stronger influence of lifestyle and other environmental factors on T3 levels compared to T4. The generally somewhat higher heritability estimates in the present study might result from the fact that a sib-pair design is less able to discriminate between shared environmental and genetic effects than twin studies, resulting in an overestimation of heritability, but also from the fact that we studied a well characterized rather homogenous population.

In view of the high heritability estimates for most thyroid parameters, which indicate that a substantial part of the between-subject variation has a genetic basis, we assessed in our cohort of healthy euthyroid young men, the possible contribution of a number of different SNPs in genes involved in thyroid regulation and previously reported to be associated with thyroid hormone levels in other populations. We observe robust significant associations between 2 SNPs in DIO1 (in linking disequilibrium, $r^2=0.41$) and both the ratio FT3/FT4 and rT3 levels. There was also a weaker association of (F) T4 levels with these SNPs. The observed associations for the 2 SNPs are in opposite
direction and in agreement with previous studies, which described a higher deiodinase activity for SNP rs2235544 and a lower deiodinase activity for rs11206244, respectively (15, 22).

We observed a rather unexpected negative association between the presence of rs225014 in DIO2 and rT3 levels. Since this SNP has been reported to be associated with a lower D2 activity (28, 42), one rather might expect higher than lower rT3 levels. We do not have a physiological explanation for this observation. Furthermore, the presence of the SNP has been linked to a lower femoral neck BMD, insulin resistance and hypertension. Nevertheless, none of these studies observed an association between this SNP and circulating thyroid hormones (23, 24, 28, 42).

We observe no association between the rs7832552 polymorphism in the TRHR gene and circulating TH levels or TSH. A genome-wide study has previously identified a significant association between the presence of this SNP and higher lean body mass (25), but to the best of our knowledge, no other study has investigated associations with thyroid hormone levels so far.

The highly significant association between rs4704397, a SNP in PDE8B, and higher TSH levels has previously been observed in several large cohorts (12, 14, 21, 43, 44). We also have recently confirmed this positive association with TSH as well as a negative association with FT3 and FT4 levels in another large cohort (45). However, the latter negative relations with free thyroid hormones could not be confirmed in the present study, possibly due the smaller size of the present cohort and ensuing lower power.

At variance with previous reports of associations of SNPs in the TSH-receptor (46, 47), we do not observe an associations between 3 SNPs in TSHR with TSH levels in our study. Nevertheless, in our cohort, rs10149689 and rs12050077 in TSHR are negatively associated with FT4-levels, which points out towards an effect of these polymorphisms on TSH action, resulting in a decreased thyroid function.
The SNP in \textit{THRB}, rs13063628, has been associated with higher TSH levels in Danish twins, but this could not be confirmed in the Rotterdam study (48). Surprisingly, in our study, we even observe a negative association between the presence of this SNP and TSH levels, although the significance level of the association was rather low.

A SNP in \textit{CAPZB}, rs10917469, has been related to TSH levels as well, but this SNP was not determined in our cohort. However, in agreement with our observations for the other SNPs, this SNP also explained only about 1% of the variation in TSH levels (16).

Obviously, there are numerous candidate genes implicated in the complex determination of circulating thyroid hormone levels. The here identified contributing SNPs explain each only a very small fraction of the genetic determination of between-subject variability, hereby underlining the polygenetic character of the heritability of thyroid hormone levels. In addition, the discrepancy between the high heritability estimates and low r-squared of studied SNPs might also be caused by gene-environment interactions or even by epigenetic phenomena. Indeed, evidence suggests that epigenetics link genetics and environment in shaping endocrine function. With regard to thyroid hormone regulation, the expression of the sodium iodide symporter was shown to be regulated by cytosine methylation of its promotor. Besides, epigenetic regulation of \textit{TSHR} has been demonstrated (49).

Beside genetics, we assessed the role in the determination of thyroid hormone levels of age and lifestyle-related variables, including BMI, smoking, education, sport exertion and iodine intake. Notwithstanding the rather narrow age range in our study population, we observe inverse associations between increasing age and TSH as well as thyroid hormones. Previous studies on this topic have not been straightforward: both an increase as well as a decrease in TSH with progressing age have been observed (29, 30, 34, 50). Differences in iodine status of a population might be an explanation for this paradox as well as the thoroughness of exclusion of subjects with thyroid auto-immunity. Our finding of a clear effect of age in a relatively young, healthy population is noteworthy.
Smoking is associated with slightly higher thyroid hormone levels (both free and total) together with lower TSH levels. A larger influence of smoking on (F) T3 compared to (F) T4 levels is observed, as well as a higher R². In our population, smoking is also positively associated with TBG and rT3. These results are in line with previous studies from Asvold et al (32) and Soldin et al (31). Proposed mechanisms are activation of the sympathetic nervous system by nicotine resulting in a positive effect on thyroid hormone secretion (51); higher TBG and testosterone levels together with lower estradiol levels in smokers (52); or a direct stimulating effect of smoking on the thyroid gland (51). Since the here observed associations between smoking and thyroid parameters remained significant after adjustment for TBG and estradiol, we have no reason to believe that indirect effects through sex steroids are the mediators for the observed associations.

We discussed associations between BMI and other parameters of body composition with thyroid hormone levels in a previous paper (3). Briefly, we have observed that a less favorable body composition (with a higher BMI, higher fat and lower muscular mass) is associated with higher T3 levels and an increased FT3/FT4 ratio, possibly mediated by higher leptin levels. In keeping with these findings, we report here an inverse relation between years of education and T3 levels, which might be related to a healthier lifestyle (with a lower caloric intake and lower smoking rate) in subjects with a higher education. Besides, we observe an inverse relation between free thyroid hormones and physical activity, which might be related to our findings on body composition and thyroid hormones.

Median fasting morning urinary iodine levels in our cohort are in agreement with acknowledged mild iodine deficiency in Belgium (53, 54). Uncorrected urinary iodine levels were not associated to thyroid parameters in this cohort, in line with a previous large study (NHANES III) (35). However, the iodine/creatinine ratio was negatively associated with FT4 and rT3 in our study. Studies longitudinally evaluating subjects after iodine fortification have shown a rather surprising positive association between urinary iodine excretion and TSH levels (34, 50). In iodine-sufficient populations, findings
suggest a positive linear relation between iodine levels and TSH (36); whereas, in subjects with urinary iodine excretion below 50µg/24 h (moderate iodine deficiency), a negative correlation between urinary iodine and TSH was observed (55). Nevertheless, on a population basis, the impact of iodine on thyroid hormone levels seems limited in comparison with other known covariates (35).

Iodine intake is related to the consumption of dairy products in our population, in agreement with a previous study suggesting that milk is the main source of iodine in Belgium (54). However, we do not observe seasonal variation in urinary iodine or thyroid hormone levels, in contrast with a recent study of Moreno-Reyes et al (53).

The major strength of our study is the extensive phenotypic characterization of the population, together with information on both genetic as well as lifestyle-related determinants. A limitation is the absence of thyroid ultrasound measurements, not allowing us to give information on thyroid size or morphology. Besides, due to this absence of thyroid ultrasound measurements, it cannot be excluded that a number of subjects without thyroid antibodies still suffered from a Hashimoto thyroiditis, since up to 15% of the subjects with Hashimoto lack thyroid antibodies. Another limitation is that we only have one sample of urinary iodine from each subject. Since there is a considerable day to day variation in urinary iodine excretion, this might have biased our results.

In conclusion, in this study we investigated genetic together with lifestyle-related determinants of thyroid levels in a population of healthy, euthyroid young men. We have shown moderate to high heritability estimates, with identified contributing gene polymorphisms each explaining only a small part of between subject variability. Besides genetics, age and lifestyle-related factors, such as smoking, education level and body composition, are significant co-determinants of thyroid hormone levels; whereas iodine intake was found to only minimally influence circulating thyroid hormone. Noteworthy is that (F) T3 appears to be influenced to a larger extent by environmental factors than the other studied thyroid function indices.
Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This work was supported by a grant from the Fund for Scientific Research – Flanders (FWO-Vlaanderen grant #G.0662.08). Y Taes is holder of a post-doctoral fellowship of the Research Foundation – Flanders (FWO).

Acknowledgments

The authors are grateful to Kaatje Toye, Kathelyne Mertens, Magda Becqué, Eric Vandersypt and Eric Van de Velde for their excellent technical assistance. They also thank all the volunteers who participated as study subjects.
References

1. Andersen S, Pedersen KM, Bruun NH & Laurberg P. Narrow individual variations in serum T(4) and T(3) in normal subjects: a clue to the understanding of subclinical thyroid disease. *J Clin Endocrinol Metab* 2002 **87** 1068-1072.

2. Roef GL, Taes YE, Kaufman JM, Van Daele C, De Buyzere ML, Gilibeht TC & Rietzschel ER. Thyroid hormone levels within reference range are associated with heart rate, cardiac structure and function in middle-aged men and women. *Thyroid* 2013.


4. Murphy E, Gluer CC, Reid DM, Felsenberg D, Roux C, Eastell R & Williams GR. Thyroid function within the upper normal range is associated with reduced bone mineral density and an increased risk of nonvertebral fractures in healthy euthyroid postmenopausal women. *J Clin Endocrinol Metab* 2010 **95** 3173-3181.


Peeters RP, van Toor H, Kloostra W, de Rijke YB, Kuiper GG, Uitterlinden AG & Visser TJ. Polymorphisms in thyroid hormone pathway genes are associated with plasma TSH and iodothyronine levels in healthy subjects. *J Clin Endocrinol Metab* 2003 **88** 2880-2888.


Peeters RP, van der Deure WM & Visser TJ. Genetic variation in thyroid hormone pathway genes; polymorphisms in the TSH receptor and the iodothyronine deiodinases. *Eur J Endocrinol* 2006 **155** 655-662.


26 Peeters RP, van den Beld AW, van Toor H, Uitterlinden AG, Janssen JA, Lamberts SW & Visser TJ. A polymorphism in type I deiodinase is associated with circulating free insulin-like growth factor I levels and body composition in humans. *J Clin Endocrinol Metab* 2005 90 256-263.


31 Soldin OP, Goughenour BE, Gilbert SZ, Landy HJ & Soldin SJ. Thyroid hormone levels associated with active and passive cigarette smoking. *Thyroid* 2009 19 817-823.


Table 1: Population characteristics and descriptives of studied variables

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± SD/ Median (IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>34 (30-39)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>179.6 ± 6.5</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>79 (73-87.5)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.6 (22.7-26.9)</td>
</tr>
<tr>
<td>Smokers</td>
<td>24%</td>
</tr>
<tr>
<td>TSH (mU/l)</td>
<td>1.6 (1.2-2.1)</td>
</tr>
<tr>
<td>FT4 (ng/dl)</td>
<td>1.4 ± 0.2</td>
</tr>
<tr>
<td>FT3 (pg/dl)</td>
<td>383 ± 40</td>
</tr>
<tr>
<td>TT4 (µg/dl)</td>
<td>8.5 ± 1.5</td>
</tr>
<tr>
<td>TT3 (ng/dl)</td>
<td>129 ± 20</td>
</tr>
<tr>
<td>TBG (mg/dl)</td>
<td>1.8 ± 0.3</td>
</tr>
<tr>
<td>rT3 (ng/dl)</td>
<td>0.29 ± 0.07</td>
</tr>
<tr>
<td>Urinary iodine (µg/l)</td>
<td>74 (54-99)</td>
</tr>
<tr>
<td>Urinary creatinine (mg/dl)</td>
<td>167 (126-213)</td>
</tr>
<tr>
<td>Iodine/creatinine (µg/g)</td>
<td>0.05 (0.04-0.06)</td>
</tr>
</tbody>
</table>

General characteristics, thyroid hormone parameters and parameters of iodine intake of all study participants (n=941). Total population consisted of 1001 subjects. Subjects with positive thyroid autoimmunity were excluded (60 subjects or 5%), leaving 941 subjects. Variables are given as mean ± SD; when there was a non-Gaussian distribution, data were presented as median (first to third quartile). Conversion factor for FT₃ from pg/dl to pmol/l, for TT₃ from ng/dl to nmol/l, and for rT₃ from ng/dl to nmol/l is 0.0154; conversion factor for FT₄ from ng/dl to pmol/l and for TT₄ from µg/dl to nmol/l is 12.87; conversion factor for TBG from mg/dl to mg/l is 0.1.
Table 2

Heritability estimates (unadjusted) + standard deviation

<table>
<thead>
<tr>
<th>Thyroid parameter</th>
<th>Heritability ($h^2$)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log(TSH)</td>
<td>0.49 ± 0.09</td>
<td>$3.8 \times 10^{-9}$</td>
</tr>
<tr>
<td>FT4</td>
<td>0.89 ± 0.08</td>
<td>$3.6 \times 10^{-25}$</td>
</tr>
<tr>
<td>TT4</td>
<td>0.80 ± 0.08</td>
<td>$1.1 \times 10^{-20}$</td>
</tr>
<tr>
<td>FT3</td>
<td>0.60 ± 0.09</td>
<td>$1.5 \times 10^{-11}$</td>
</tr>
<tr>
<td>TT3</td>
<td>0.58 ± 0.09</td>
<td>$3.7 \times 10^{-11}$</td>
</tr>
<tr>
<td>TBG</td>
<td>0.82 ± 0.08</td>
<td>$7.1 \times 10^{-22}$</td>
</tr>
<tr>
<td>rT3</td>
<td>0.88 ± 0.08</td>
<td>$5.5 \times 10^{-25}$</td>
</tr>
</tbody>
</table>
Table 3: Associations between SNPs in the thyroid hormone pathway and thyroid hormone parameters.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Polymorphism</th>
<th>Minor allele</th>
<th>Log(TSH)</th>
<th>FT4</th>
<th>FT3</th>
<th>FT3/FT4</th>
<th>rT3</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>β (SEM)</td>
<td>p</td>
<td>β (SEM)</td>
<td>p</td>
<td>β (SEM)</td>
<td>p</td>
</tr>
<tr>
<td>PDE8B</td>
<td>rs4704397</td>
<td>A</td>
<td>0.18 (0.05)</td>
<td>0.0002</td>
<td>-0.08 (0.05)</td>
<td>0.1</td>
<td>-0.02 (0.05)</td>
<td>0.7</td>
</tr>
<tr>
<td>TRHR</td>
<td>rs7832552</td>
<td>T</td>
<td>-0.05 (0.05)</td>
<td>0.3</td>
<td>-0.04 (0.05)</td>
<td>0.5</td>
<td>0.01 (0.05)</td>
<td>0.9</td>
</tr>
<tr>
<td>TSHR</td>
<td>rs10149689</td>
<td>G</td>
<td>0.07 (0.05)</td>
<td>0.1</td>
<td>-0.13 (0.04)</td>
<td>0.09</td>
<td>-0.04 (0.05)</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>rs12050077</td>
<td>A</td>
<td>0.07 (0.05)</td>
<td>0.2</td>
<td>-0.12 (0.05)</td>
<td>0.01</td>
<td>-0.05 (0.05)</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>rs1991517</td>
<td>G</td>
<td>-0.10 (0.08)</td>
<td>0.2</td>
<td>-0.04 (0.08)</td>
<td>0.6</td>
<td>0.2 (0.08)</td>
<td>0.9</td>
</tr>
<tr>
<td>DIO1</td>
<td>rs11206244</td>
<td>T</td>
<td>0.04 (0.05)</td>
<td>0.4</td>
<td>0.11 (0.05)</td>
<td>0.03</td>
<td>-0.07 (0.05)</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>rs2235544</td>
<td>C</td>
<td>-0.09 (0.05)</td>
<td>0.06</td>
<td>-0.11 (0.05)</td>
<td>0.02</td>
<td>0.06 (0.05)</td>
<td>0.2</td>
</tr>
<tr>
<td>DIO2</td>
<td>rs225014</td>
<td>G</td>
<td>0.02 (0.05)</td>
<td>0.8</td>
<td>-0.08 (0.05)</td>
<td>0.09</td>
<td>-0.03 (0.05)</td>
<td>0.5</td>
</tr>
<tr>
<td>THRB</td>
<td>rs13063628</td>
<td>A</td>
<td>-0.15 (0.07)</td>
<td>0.03</td>
<td>0.01 (0.07)</td>
<td>0.9</td>
<td>-0.02 (0.07)</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Results from linear mixed effect modeling, with the different thyroid hormone parameters as dependent and the polymorphisms as independent. Beta’s are standardized and reported together with their standard deviation. Associations were unadjusted.

PDE8B: Phosphodiesterase 8 beta; TRHR: thyrotropin-releasing hormone receptor; TSHR: thyroid stimulating hormone receptor; DIO1: Deiodinase 1; DIO2: Deiodinase 2, THRB: thyroid hormone receptor beta.

R² is given for the significant associations. When there was no significant association, not applicable (NA) was reported.
Table 4

Associations between age and possible lifestyle-related determinants and the different thyroid hormone parameters

<table>
<thead>
<tr>
<th>Determinant</th>
<th>log(TSH)</th>
<th>FT4</th>
<th>TT4</th>
<th>FT3</th>
<th>TT3</th>
<th>FT3/FT4</th>
<th>TBG</th>
<th>rT3</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>-0.13 ± 0.03</td>
<td>-0.18 ± 0.03</td>
<td>-0.11 ± 0.03</td>
<td>-0.15 ± 0.03</td>
<td>-0.05 ± 0.03</td>
<td>0.06 ± 0.03</td>
<td>-0.02 ± 0.03</td>
<td>-0.24 ± 0.03</td>
<td>2-3.5%</td>
</tr>
<tr>
<td></td>
<td>p=0.0003</td>
<td>p&lt;0.0001</td>
<td>p=0.002</td>
<td>p&lt;0.0001</td>
<td>p=0.12</td>
<td>p=0.07</td>
<td>p=0.5</td>
<td>p&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td>-0.23 ± 0.08</td>
<td>0.24 ± 0.07</td>
<td>0.25 ± 0.07</td>
<td>0.36 ± 0.07</td>
<td>0.47 ± 0.08</td>
<td>0.04 ± 0.07</td>
<td>0.19 ± 0.07</td>
<td>0.17 ± 0.07</td>
<td>1-3%</td>
</tr>
<tr>
<td></td>
<td>p=0.003</td>
<td>p=0.0009</td>
<td>p=0.0006</td>
<td>p&lt;0.0001</td>
<td>p&lt;0.0001</td>
<td>p=0.6</td>
<td>p=0.01</td>
<td>p=0.02</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>-0.04 ± 0.03</td>
<td>-0.06 ± 0.03</td>
<td>0.02 ± 0.03</td>
<td>0.15 ± 0.03</td>
<td>0.15 ± 0.03</td>
<td>0.17 ± 0.03</td>
<td>0.09 ± 0.03</td>
<td>-0.09 ± 0.03</td>
<td>2%</td>
</tr>
<tr>
<td></td>
<td>p=0.3</td>
<td>p=0.6</td>
<td>p=0.6</td>
<td>p&lt;0.0001</td>
<td>p&lt;0.0001</td>
<td>p=0.005</td>
<td>p=0.009</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urinary iodine (µg/ml)</td>
<td>-0.04 ± 0.03</td>
<td>-0.03 ± 0.03</td>
<td>-0.03 ± 0.03</td>
<td>-0.01 ± 0.03</td>
<td>-0.02 ± 0.03</td>
<td>0.02 ± 0.03</td>
<td>-0.05 ± 0.03</td>
<td>-0.06 ± 0.03</td>
<td>0.5%</td>
</tr>
<tr>
<td></td>
<td>p=0.2</td>
<td>p=0.3</td>
<td>p=0.3</td>
<td>p=0.8</td>
<td>p=0.5</td>
<td>p=0.5</td>
<td>p=0.1</td>
<td>p=0.04</td>
<td></td>
</tr>
<tr>
<td>Iodine&lt;sub&gt;urine&lt;/sub&gt;/</td>
<td>-0.02 ± 0.03</td>
<td>-0.08 ± 0.03</td>
<td>-0.05 ± 0.03</td>
<td>-0.04 ± 0.03</td>
<td>-0.03 ± 0.03</td>
<td>0.05 ± 0.03</td>
<td>-0.02 ± 0.03</td>
<td>-0.10 ± 0.03</td>
<td>0.5-2%</td>
</tr>
<tr>
<td>Creatinine&lt;sub&gt;urine&lt;/sub&gt; (µg/mg)</td>
<td>p=0.5</td>
<td>p&lt;0.007</td>
<td>p=0.07</td>
<td>p=0.2</td>
<td>p=0.4</td>
<td>p=0.1</td>
<td>p=0.5</td>
<td>p&lt;0.0007</td>
<td></td>
</tr>
<tr>
<td>Education (yrs)</td>
<td>0.05 ± 0.03</td>
<td>0.04 ± 0.03</td>
<td>-0.01 ± 0.03</td>
<td>-0.17 ± 0.03</td>
<td>-0.14 ± 0.03</td>
<td>-0.16 ± 0.03</td>
<td>-0.07 ± 0.03</td>
<td>0.05 ± 0.03</td>
<td>3%</td>
</tr>
<tr>
<td></td>
<td>p=0.1</td>
<td>p=0.3</td>
<td>p=0.7</td>
<td>p&lt;0.0001</td>
<td>p&lt;0.0001</td>
<td>p&lt;0.0001</td>
<td>p=0.02</td>
<td>0.09</td>
<td></td>
</tr>
</tbody>
</table>

Results from linear mixed-effect modeling, with the different thyroid hormones used as dependents and the lifestyle-related determinants as independents. Standardized estimates are reported. Associations were unadjusted.

R² is given for the significant associations. When there was no significant association, not applicable (NA) was reported.
TSH-levels according to genotype for the rs4704397 polymorphism in PDE8B. P-value results from ANOVA.

TSH (mU/L)

Genotype rs4704397

A:A  G:A  G:G

p = 0.0009

254x190mm (96 x 96 DPI)
TSH and FT4-levels (ANOVA) according to genotype of SNPs in TSHR (rs10149689 and rs12050077). For rs10149689, genotype 0 refers to AA, 1 to AG and 2 to GG. For rs12050077, genotype 0 refers to GG, 1 to GA and 2 to AA.
FT4, rT3 and ratio FT3/FT4 according to genotype of SNPs in DIO1 (rs11206244 and rs2235544). P-values result from ANOVA. For rs11206244, genotype 0 refers to CC, 1 to CT and 2 to TT; for rs2235544, genotype 0 refers to AA, 1 to AC and 2 to CC.
FT3, FT4, TT4 and rT3 levels according to quartiles of the ratio Iodine/Creatinine. P-values result from ANOVA (Unadjusted model).

254x190mm (96 x 96 DPI)