Are serum thyrotropin levels within the reference range associated with endothelial function?

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Aims

High serum thyrotropin (TSH) levels within the reference range might be associated with an increased cardiovascular risk. In the present study, we investigated the association between serum TSH levels and flow-mediated dilation (FMD) as a measure of endothelial dysfunction.

Methods and results

The study population comprised 1364 subjects (670 women) aged 25–85 years with serum TSH levels between 0.25 and 2.12 mIU/L recruited from 5-year follow-up of the Study of Health in Pomerania. No interventions were performed. Measurements of FMD and nitrate-mediated dilation (NMD) were performed in the supine position using standardized ultrasound techniques. FMD and NMD values below the median of each distribution were considered decreased. Analyses adjusted for age, sex, smoking, and systolic and diastolic blood pressure revealed a non-significant inverse trend between serum TSH levels and FMD ($P = 0.130$). Subjects with serum TSH levels above the highest quartile had lower median FMD values relative to subjects with serum TSH levels below the lowest quartile (4.86 vs. 5.43%, $P < 0.05$). A linear inverse trend between serum TSH levels and decreased FMD barely missed statistical significance ($P = 0.138$). Subjects with high serum TSH levels had higher odds of decreased FMD relative to subjects with low serum TSH levels (odds ratio 1.42; 95% confidence interval 1.02; 1.96; $P < 0.05$). These associations were more pronounced in men than in women. There were no such associations for NMD.

Conclusion

Serum TSH levels within the upper reference range are associated with impaired endothelial function. Our findings contribute to the discussion on whether the upper TSH reference limit should be redefined.

Keywords

Thyroid function • Endothelial function • Epidemiology

Introduction

Thyroid function has major effects on cardiovascular function. A widened pulse pressure, left ventricular hypertrophy, and cardiac arrhythmias are major clinical signs of overt hyperthyroidism.1 Patients with overt hypothyroidism commonly have a reduced heart rate and an increased risk of atherosclerosis due to impaired lipid metabolism.2 Similar associations were found for subclinical thyroid disorders, but these findings have not always been replicated.1

Significant debate is centred around the correct definition of the upper and lower reference intervals of serum thyrotropin (TSH) levels.3–5 Methodically, reference values are markers of a distribution of test results within a given population. As the distribution of serum TSH levels strongly depends on the underlying iodine supply,4 TSH reference values may substantially vary across populations.7 Against this background, it becomes obvious that TSH reference values might not necessarily be related to clinical diseases or outcomes. Thus, accumulating evidence suggests that serum TSH levels within the upper reference range are related
to an increased risk of incident hypothyroidism, hypertension, dyslipidemia, and weight gain, although not all of these associations were confirmed by other studies. Endothelial dysfunction can be assessed as decreased flow-mediated dilation (FMD) using ultrasound and represents a very early stage of atherosclerosis. Subjects with decreased FMD share common atherosclerotic risk factors with myocardial infarction or cerebrovascular atherosclerosis patients. Endothelial dysfunction predicts future cardiovascular morbidity and mortality. Some smaller studies have investigated possible associations between thyroid function and FMD. Most of these studies demonstrated high FMD values in subjects with subclinical and overt hyperthyroidism and low FMD values in subjects with subclinical and overt hypothyroidism. One study in 35 subjects also demonstrated low FMD values in subjects with serum TSH levels within the upper reference range relative to euthyroid subjects, whereas another study failed to demonstrate an association between thyroid function and endothelial function in euthyroid subjects. Putative associations between thyroid function and FMD over the whole TSH reference range of a general population have not been investigated.

In the present study, we investigated the association between serum TSH levels and endothelial dysfunction. We hypothesized that subjects with serum TSH levels in the upper reference range have lower FMD values and consequently higher odds of decreased FMD when compared with subjects with serum TSH levels within the lower reference range.

Methods
Study population
The Study of Health in Pomerania (SHIP) is a population-based investigation in West Pomerania, a region in the northeastern part of Germany. The study region is a previously iodine-deficient area with a high prevalence of iodine deficiency-related disorders such as goiter, thyroid nodules, and decreased serum TSH levels. Study details are given elsewhere. In brief, a sample from the population aged 20–79 years was drawn. The SHIP population finally comprised 4310 participants (2193 women), which corresponds to a final response of 68.8%.

Baseline examinations (SHIP-0) were conducted between October 1997 and March 2001. Between March 2002 and September 2006, the 5-year follow-up examinations (SHIP-1) were performed and contained 3300 participants (83.6% of still eligible subjects, 1711 women). The study was reviewed by a board of independent scientist and approved by the Ethics Committee of the University of Greifswald. All participants provided written informed consent.

We offered all SHIP-1 participants the opportunity to take part in a substudy with measurements of endothelial function, body plethysmography, and cardiopulmonary exercise testing. Of the 3300 SHIP-1 participants, 1693 subjects (51.3%; 861 women) agreed to take part in the side project. We excluded 172 subjects (103 women) with non-usable ultrasound images, 9 subjects (eight women) who refused blood draws, 148 subjects (80 women) with serum TSH levels outside the reference range, and 4 subjects (3 women) with anti-thyroid medication. This resulted in a final study population of 1360 subjects (667 women) aged 25–85 years, who were available for the present analysis.

Measurements
Sociodemographic and medical characteristics were assessed by computer-assisted personal interviews. According to tobacco consumption, participants were categorized into current (one or more cigarettes per day), occasional (less than one cigarette per day), former, and non-smokers. Pack years for current and former smokers were calculated by multiplying the duration of smoking in years with the amount of packs smoked daily (20 cigarettes were defined as one pack). Data on medication were collected using the anatomical therapeutic chemical code. We considered anti-thyroid preparations as exclusion criterion and included anti-hypotensives, peripheral vasodilators, beta-blockers, calcium channel blockers, drugs acting on the renin–angiotensin system, statins, and non-steroidal anti-inflammatory drugs as potential confounders.

Height and weight were measured for the calculation of body mass index (BMI = weight (kg)/height2 (m2)). After a 3 min rest period, the systolic and diastolic blood pressures were measured three times in the right arm of seated subjects using a digital blood pressure monitor (HEM-705CP, Omron Corporation, Tokyo, Japan), with each reading followed by an additional 3 min rest period. One of two differently sized cuffs was applied according to the circumference of the participant’s arm. The mean of the second and third measurements was calculated and used for the present analyses.

Non-fasting blood samples were taken between 7 a.m. and 4 p.m. Serum TSH levels were analysed by immunnochemiluminescent procedures [Immulite 2000, Third generation, Diagnostic Products Corporation (DPC), Los Angeles, IL, USA]. We used the serum TSH reference range of 0.25–2.12 mIU/L that has been recently established for this region. Serum anti-thyroperoxidase (anti-TPO) was measured by an enzyme immunoassay (VARELSIA, Elia Medizintechnik GmbH, Freiburg, Germany). The detection limit of this assay was 1 IU/mL. LDL cholesterol was determined via lipoprotein electrophoresis in agarose gels with subsequent enzymatic cholesterol staining applying the Spife 3000 System from Helena Laboratories (Progen Biotechnik GmbH, Heidelberg, Germany).

Endothelial dysfunction was assessed by FMD. Examinations were performed in a supine position by two observers. The subject’s right arm was comfortably immobilized. The brachial artery diameter was measured recorded 3–7 cm above the antecubital fossa using a 10 MHz linear array transducer ultrasound system (Cypress, Siemens AG, Erlangen, Germany). After the resting scan, a pneumatic cuff placed around the forearm 10 cm distal to the ultrasound location was inflated above a pressure of 220 mmHg for 5 min. Diameter measurements were repeated 60 s after cuff deflation. FMD was calculated by the ratio between brachial diameters before and after inflation of the pneumatic cuff and is expressed as % diameter. Nitrate-mediated dilation (NMD) was taken 3 min after sublingual administration of nitroglycerin (400 μg) in 1096 subjects (465 women). Examinations were performed and read by two observers. All ultrasound measurements in SHIP underlie strict quality management. Intra-reader, inter-reader, intra-observer, inter-reader, and inter-observer variabilities are evaluated in certification procedures. Before data collection, 25 images are measured twice by each participating reader, and 12 volunteers are examined twice by each participating observer. Figure 1 gives an example of an inter-observer certification, which was performed before data collection. During data collection, observer certification procedures are repeated semi-annually for six volunteers. At least 24 h is required between two readings and examinations, respectively. The number of images and volunteers, respectively, was arbitrarily defined before the beginning of the study and has been proven satisfactory from experience to demonstrate relevant reader and observer agreement.
Serum TSH and endothelial function

1.10–2.12 mIU/L). Inter-group comparisons were made using two-

or absolute numbers as indicated. The study population was divided

differences. All measurements of intra-reader, intra-observer, inter-

Statistical analysis

The power analysis was based on the assumptions that a total number

LDL cholesterol and glucose levels, and use of medication did not

Also, there was a non-significant linear inverse trend \( P = 0.130 \) in age-

(smoking, BMI, and blood pressure) and laboratory variables were

in various orders. Thereafter, variables on medication listed

In age- and sex-adjusted analyses, there was a non-significant linear

Also, there was a non-significant linear inverse trend \( P = 0.138 \) in age-

in various orders. BMI, serum LDL cholesterol and glucose levels, and use of medication did not

levels and decreased FMD. In the final model, this

inversely in age- and sex-adjusted analyses for the association between

Figure 1 Example for an inter-observer examination with respect to flow-mediated dilation (FMD) plotted according to Bland and Altman.\(^{45}\) Two observers (OBS1 and OBS2) examined independently from each other the FMD in 12 volunteers. The figure indicates the mean FMD of each volunteer examined by the two observers (x-axis) and the absolute FMD difference measured by the two observers relative to the mean FMD of each volunteer (y-axis). The plot allows assessment for systematic error and its direction by the mean difference (green line) as well as the random error by the 1.96 standard deviation (purple lines). The 95% confidence intervals for each estimate are given as dotted lines.

Results

Subjects with high serum TSH levels within the reference range were older, more commonly non-smokers, and less commonly current smokers and had a higher diastolic blood pressure when compared with those with low serum TSH levels within the reference range. Subjects in the different serum TSH groups were similar with respect to sex, BMI, systolic blood pressure, positive anti-TPO antibodies, and serum LDL cholesterol and glucose levels. With respect to medication, subjects with higher serum TSH levels used more commonly beta-blockers than those with low serum TSH levels. Individuals with serum TSH levels between the second and third quartiles received more often statins relative to those with low serum TSH levels (Table 1).

In 319 subjects (23.5%), the quality of FMD images was rated as excellent, good, or adequate. FMD and NMD values below the median of each distribution were considered decreased.
The trend narrowly missed statistical significance ($P = 0.074$). Subjects with high serum TSH levels within the reference range had higher odds for decreased FMD relative to those with low serum TSH levels within the reference range (Table 2). None of the results outlined in Table 3 was substantially affected when positive serum anti-TPO antibodies were added to the final models as further independent variables.

We performed sex-stratified analyses in the full models to explore possible gender differences. In men, the inverse trends between serum TSH levels within the reference range and FMD ($P = 0.037$) or decreased FMD ($P = 0.041$) attained statistical significance. Relative to men with serum TSH levels below the lowest quartile, men with high serum TSH levels above the highest quartile had significantly lower FMD values [adjusted mean 3.81

### Table 1  Selected characteristics in subjects with different serum thyrotropin levels

<table>
<thead>
<tr>
<th>Serum thyrotropin levels (mIU/L)</th>
<th>n</th>
<th>0.25–0.54</th>
<th>0.55–0.78</th>
<th>0.79–1.09</th>
<th>1.10–2.12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male)</td>
<td></td>
<td>339</td>
<td>342</td>
<td>343</td>
<td>336</td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-smoker</td>
<td>181</td>
<td>(53.4%)</td>
<td>172 (50.3%)</td>
<td>172 (50.1%)</td>
<td>168 (50.0%)</td>
</tr>
<tr>
<td>Ex-smoker</td>
<td>108</td>
<td>(32.0%)</td>
<td>107 (31.3%)</td>
<td>116 (33.8%)</td>
<td>107 (31.8%)</td>
</tr>
<tr>
<td>Occasional smoker</td>
<td>8</td>
<td>(2.4%)</td>
<td>7 (2.0%)</td>
<td>10 (2.9%)</td>
<td>17 (5.1%)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>86</td>
<td>(25.4%)</td>
<td>74 (21.6%)</td>
<td>78 (22.7%)</td>
<td>60 (17.9%)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td></td>
<td>27.7 (4.1)</td>
<td>27.7 (4.8)</td>
<td>27.8 (4.8)</td>
<td>28.0 (4.8)</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td></td>
<td>131.3 (19.3)</td>
<td>130.1 (17.3)</td>
<td>129.4 (17.7)</td>
<td>132.0 (17.7)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td></td>
<td>81.1 (10.0)</td>
<td>81.7 (10.2)</td>
<td>81.6 (10.1)</td>
<td>82.9 (10.2)*</td>
</tr>
<tr>
<td>Positive anti-TPO antibodies²</td>
<td></td>
<td>8 (2.4%)</td>
<td>12 (3.6%)</td>
<td>15 (4.4%)</td>
<td>18 (5.4%)</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td></td>
<td>3.50 (0.92)</td>
<td>3.50 (1.03)</td>
<td>3.53 (1.08)</td>
<td>3.59 (1.05)</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td></td>
<td>5.41 (1.37)</td>
<td>5.33 (1.18)</td>
<td>5.26 (1.28)</td>
<td>5.27 (0.98)</td>
</tr>
<tr>
<td>Use of anti-hypotensives</td>
<td></td>
<td>1 (0.3%)</td>
<td>3 (0.9%)</td>
<td>3 (0.9%)</td>
<td>2 (0.6%)</td>
</tr>
<tr>
<td>Use of peripheral vasodilators</td>
<td></td>
<td>6 (1.8%)</td>
<td>4 (1.2%)</td>
<td>2 (0.6%)</td>
<td>3 (0.9%)</td>
</tr>
<tr>
<td>Use of beta-blockers</td>
<td></td>
<td>88 (26.0%)</td>
<td>62 (18.1%)</td>
<td>69 (20.1%)</td>
<td>55 (16.4%)*</td>
</tr>
<tr>
<td>Use of calcium channel blockers</td>
<td></td>
<td>28 (8.3%)</td>
<td>32 (9.4%)</td>
<td>22 (6.4%)</td>
<td>19 (5.7%)</td>
</tr>
<tr>
<td>Use of drugs acting on the renin–angiotensin system</td>
<td>80 (23.6%)</td>
<td>73 (21.3%)</td>
<td>62 (18.1%)</td>
<td>61 (18.2%)</td>
<td></td>
</tr>
<tr>
<td>Use of statins</td>
<td></td>
<td>49 (14.5%)</td>
<td>32 (9.4%)</td>
<td>33 (9.6%)</td>
<td>3.5 (10.4%)</td>
</tr>
<tr>
<td>Use of non-steroidal anti-inflammatory drugs</td>
<td>34 (10.0%)</td>
<td>29 (8.5%)</td>
<td>29 (8.5%)</td>
<td>30 (8.9%)</td>
<td></td>
</tr>
</tbody>
</table>

TSH, thyrotropin; LDL, low density lipoprotein; BMI, body mass index. Data are given as numbers (percentage) or mean (standard deviation).

¹P < 0.05; x²-test (nominal data) or Student’s t-test (interval data). Comparisons were performed separately against the group with low serum.

²Data do not sum up to 100% due to missing values.

### Table 2  Flow- and nitrate-mediated dilation in subjects with different serum thyrotropin levels

<table>
<thead>
<tr>
<th>Serum TSH levels (mIU/L)</th>
<th>n</th>
<th>0.25–0.54</th>
<th>0.55–0.78</th>
<th>0.79–1.09</th>
<th>1.10–2.12</th>
</tr>
</thead>
<tbody>
<tr>
<td>FMD (%)</td>
<td></td>
<td>339</td>
<td>342</td>
<td>343</td>
<td>336</td>
</tr>
<tr>
<td>4.92 (3.97)</td>
<td></td>
<td>5.18 (3.63)</td>
<td>5.10 (3.51)</td>
<td>4.94 (4.33)</td>
<td></td>
</tr>
<tr>
<td>Decreased FMD</td>
<td></td>
<td>171 (50.4%)</td>
<td>169 (49.4%)</td>
<td>166 (48.4%)</td>
<td>174 (51.8%)</td>
</tr>
<tr>
<td>NMD (%)</td>
<td></td>
<td>13.34 (6.98)</td>
<td>13.72 (7.03)</td>
<td>14.93 (7.22)*</td>
<td>14.50 (6.67)*</td>
</tr>
<tr>
<td>Decreased NMD</td>
<td></td>
<td>118 (45.0%)</td>
<td>129 (48.5%)</td>
<td>141 (53.0%)</td>
<td>143 (53.6%)</td>
</tr>
</tbody>
</table>

FMD, flow-mediated dilation; NMD, nitrate-mediated dilation. Data are given as numbers (percentage) or mean (standard deviation).

¹P < 0.05; x²-test (nominal data) or Student’s t-test (interval data). Comparisons were performed separately against the group with low serum.

²P < 4.43.

³NMD < 13.21%.
Serum TSH levels and endothelial function

P

serum TSH levels and both FMD (subjects with excellent FMD image quality. Linear trends between

Table 4

Likewise, there was no association between serum TSH levels

or sex-stratified analyses did not yield any significant differences. Likewise, there was no association between serum TSH levels and decreased NMD (Table 4).

With respect to NMD, there was no trend over the exposure groups, neither in the age- and sex-adjusted models ($P = 0.780$) nor in the final model ($P = 0.888$). Also, inter-group comparisons or sex-stratified analyses did not yield any significant differences. Likewise, there was no association between serum TSH levels and decreased NMD (Table 4).

In sensitivity analyses, we restricted the full FMD models to 319 subjects with excellent FMD image quality. Linear trends between serum TSH levels and both FMD ($P = 0.085$) and decreased FMD ($P = 0.068$) barely missed statistical significance in the final model. Subjects with serum TSH levels above the highest quartile of the reference range had lower FMD values and higher odds for decreased FMD relative to subjects with serum TSH levels below the lowest quartile of the reference range, whereby the mean OR estimate was higher relative to the whole study population. Similar to analyses in the whole population, we did not detect any association of serum TSH levels within the reference range with NMD or decreased NMD in the 269 subjects with excellent NMD image quality (Table 5).

Further sensitivity analyses were performed by varying the scales of independent variables and by replacing the smoking status variable by pack years. In addition, the time point of blood sampling was included in the analysis. All these analyses did not affect the major results materially. Likewise, the inclusion of observers or readers in the multivariable models did neither reveal an association between both variables and FMD or NMD nor a substantial effect on the major results.

Finally, we asked the question whether the association between serum TSH levels and FMD was due to the specific definition of TSH reference range. To answer this question, we re-included all

Table 3

<table>
<thead>
<tr>
<th>Serum TSH levels (mIU/L)</th>
<th>FMD (%) adjusted for age and sex</th>
<th>FMD (%) adjusted for the final model including age, sex, smoking status, and systolic and diastolic blood pressure</th>
<th>Decreased FMD, adjusted for age and sex</th>
<th>Decreased FMD, adjusted for the final model including age, sex, smoking status, and systolic and diastolic blood pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.25–0.54</td>
<td>0.55–0.78</td>
<td>0.79–1.09</td>
<td>1.10–2.12</td>
</tr>
<tr>
<td></td>
<td>5.24 (4.85; 5.62)</td>
<td>5.25 (4.87; 5.63)</td>
<td>4.92 (4.54; 5.31)</td>
<td>4.79 (4.41; 5.18)</td>
</tr>
<tr>
<td></td>
<td>5.43 (4.97; 5.89)</td>
<td>5.36 (4.90; 5.82)</td>
<td>5.04 (4.58; 5.49)</td>
<td>4.86 (4.41; 5.31)*</td>
</tr>
<tr>
<td></td>
<td>1.0 (reference)</td>
<td>1.08 (0.79; 1.49)</td>
<td>1.17 (0.85; 1.62)</td>
<td>1.34 (0.97; 1.86)</td>
</tr>
<tr>
<td></td>
<td>1.0 (reference)</td>
<td>1.12 (0.81; 1.55)</td>
<td>1.21 (0.88; 1.67)</td>
<td>1.42 (1.02; 1.96)*</td>
</tr>
</tbody>
</table>

Data are adjusted means or odds ratios (95% confidence interval).
FMD values <4.43% were considered decreased.
TSH, thyrotropin; FMD, flow-mediated dilation.

* $P < 0.05$; analysis of variance and logistic regression. Analysis of variance did not attain statistical significance if adjusted for multiple testing. Comparisons were performed against the group with low serum TSH levels.

Table 4

<table>
<thead>
<tr>
<th>Serum TSH levels (mIU/L)</th>
<th>NMD (%) adjusted for age and sex</th>
<th>NMD (%) adjusted for the final model including age, sex, smoking status, and systolic and diastolic blood pressure</th>
<th>Decreased NMD, adjusted for age and sex</th>
<th>Decreased NMD, adjusted for the final model including age, sex, smoking status, and systolic and diastolic blood pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.25–0.54</td>
<td>0.55–0.78</td>
<td>0.79–1.09</td>
<td>1.10–2.12</td>
</tr>
<tr>
<td></td>
<td>14.43 (13.65; 15.20)</td>
<td>14.01 (13.25; 14.77)</td>
<td>14.71 (13.95; 15.47)</td>
<td>14.35 (13.60; 15.11)</td>
</tr>
<tr>
<td></td>
<td>14.63 (13.74; 15.52)</td>
<td>14.10 (13.20; 15.00)</td>
<td>14.75 (13.86; 15.64)</td>
<td>14.35 (13.48; 15.22)</td>
</tr>
<tr>
<td></td>
<td>1.0 (reference)</td>
<td>0.95 (0.66; 1.37)</td>
<td>1.02 (0.71; 1.47)</td>
<td>1.07 (0.74; 1.55)</td>
</tr>
<tr>
<td></td>
<td>1.0 (reference)</td>
<td>0.93 (0.64; 1.35)</td>
<td>0.99 (0.68; 1.43)</td>
<td>1.04 (0.71; 1.50)</td>
</tr>
</tbody>
</table>

Data are adjusted means or odds ratios (95% confidence interval).
Analysis of variance and logistic regression. Comparisons were performed against the group with low serum TSH levels.
NMD values <13.21% were considered decreased.
TSH, thyrotropin; NMD, nitrate-mediated dilation.
subjects with serum TSH levels outside the reference range. The spline given in Figure 2 did not clearly support this notion. There was no association between TSH and NMD over the whole TSH range (Figure 3).

**Discussion**

In the present study, we investigated the association between serum TSH levels within the reference range and endothelial dysfunction. Our hypothesis was partly confirmed. Although there was only a non-significant inverse trend between serum TSH levels and FMD, subjects with serum TSH levels above the upper quartile of the reference range had lower FMD values and higher odds for decreased FMD relative to subjects with serum TSH levels below the lowest quartile of the reference range. These results became clearer when analyses were restricted to subjects with excellent FMD image quality, although in these analyses, the number of included subjects was considerably reduced.

Our results are in good agreement with other studies, which demonstrate that serum TSH levels in the upper reference range are associated with incident hypothyroidism, hypertension, dyslipidaemia, and weight gain. Decreased FMD mirrors endothelial dysfunction, represents a very early stage of...

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**Table 5** Association between serum thyrotropin levels in the reference range, flow-, and nitrate-mediated dilation in subjects with excellent quality rating of images

<table>
<thead>
<tr>
<th>Serum TSH levels (mIU/L)</th>
<th>FMD (%)</th>
<th>Decreased FMD</th>
<th>NMD (%)</th>
<th>Decreased NMD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25–0.54</td>
<td>5.84 (4.97; 6.72)</td>
<td>1.0 (reference)</td>
<td>14.95 (13.26; 16.63)</td>
<td>1.0 (reference)</td>
</tr>
<tr>
<td>0.55–0.78</td>
<td>5.46 (4.30; 6.43)</td>
<td>1.42 (0.73; 2.74)</td>
<td>14.61 (12.73; 16.49)</td>
<td>0.92 (0.44; 1.94)</td>
</tr>
<tr>
<td>0.79–1.09</td>
<td>5.36 (4.33; 6.39)</td>
<td>1.63 (0.82; 3.22)</td>
<td>15.94 (13.97; 17.91)</td>
<td>1.05 (0.49; 2.23)</td>
</tr>
<tr>
<td>1.10–2.12</td>
<td>4.79 (3.91; 5.67)*</td>
<td>2.08 (1.09; 3.95)*</td>
<td>14.89 (13.24; 16.54)</td>
<td>0.80 (0.40; 1.62)</td>
</tr>
</tbody>
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Data are adjusted means or odds ratios (95% confidence interval). FMD values <4.43% and NMD values <13.21% were considered decreased. TSH, thyrotropin; FMD, flow-mediated dilation; NMD, nitrate-mediated dilation.

*P < 0.05; analysis of variance and logistic regression. Analysis of variance did not attain statistical significance if adjusted for multiple testing. Comparisons were performed against the group with low serum TSH levels.
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atherogenesis and plaque formation, and is highly associated with progression and prognosis of generalized atherosclerosis. Moreover, endothelial dysfunction predicts cardiovascular outcome. Together, these findings indicate that serum TSH levels within the upper reference range are markers for clinically relevant disorders and outcomes.

As discussed previously, the serum TSH reference range that has been recently established for our region is relatively shifted towards the left. Reasons for this shift include the previous status of iodine deficiency, the strict criteria used to select a reference population, which included thyroid ultrasound, and the specific laboratory method used for analysing TSH values. In the present study, the association between serum TSH levels and decreased FMD was observed, although we used our relatively low upper TSH reference value. Taking this knowledge into account, our results support the notion that currently generally too high, as they include a thyroid status that is already associated with significant cardiovascular alterations.

The present findings indirectly support previous studies, which demonstrate high FMD values in patients with hyperthyroidism and low FMD values in those with hypothyroidism. These findings, however, have not always been confirmed. Divergent results might be explained by the low number of patients recruited for former studies, ranging from 42 to 221 patients. Together, 660 subjects were investigated by previous studies on the association between thyroid and endothelial function. Thus, with 1364 subjects recruited, our study represents by far the largest study in this field to date.

The inverse association between serum TSH levels within the reference range and FMD was more pronounced in men than in women, thereby suggesting that endothelial function in males is more sensitive for thyroid hormone action than in females. This is in good agreement with other studies, which demonstrated that the negative effects of known cardiovascular risk factors on endothelial function are more pronounced in men than in women. It is a matter of current research to answer the question whether these disparities can be mainly explained by gender-related differences in accumulated atherosclerotic risk factors, divergent hormonal levels, or simply by distinct diameters of the brachial artery.

In contrast to FMD, NMD is a marker of endothelium-independent vasodilation. Although there was an association between serum TSH levels within the reference range and FMD in our study, we did not find such association for NMD. This strengthens the hypothesis that thyroid hormones affect endothelial function via the NO system. Thus, experimental evidence strengthens the hypothesis that thyroid hormones affect endothelial function.

FMD and NMD measurements were potentially prone to observer bias. In the daily practice of study quality management, the wish to measure potential observer differences with high precision has to be weighed up against practicability. We usually examine 12 volunteers to assess intra- and inter-observer variabilities for a priori certifications of ultrasound observers. This procedure does not take 2 days and is by experience sufficient to detect major observer differences. Thus, although the confidence intervals in the exemplary certification examination displayed in Figure 1 do not overlap, the estimates of observer differences are relatively imprecise, reflecting the limitation of the procedure. However, we do not suspect a relevant systematic observer bias in the present study, because in multivariable analyses, the observer variable neither was related to FMD and NMD nor did substantially change the results with respect to the association of interest. We certainly cannot unequivocally exclude misclassification in the definition of FMD and NMD variables due to random errors. An incorrect labelling of FMD status, however, should bias our results towards the null, thus strengthening our positive findings. The fact that the association between serum TSH levels within the reference range and FMD became clearer in subjects with excellent FMD reading quality argues for a certain underestimation of this association in the entire study population. Small NMD differences across the serum TSH groups and the lack of a dose–response relation make it unlikely that we missed a significant association between serum TSH levels within the reference range and NMD.

We conclude that serum TSH levels within the upper reference range are associated with impaired endothelial function. Our findings significantly contribute to the current discussion on whether the upper TSH reference limit should be redefined.

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