Thyroid function and the metabolic syndrome in older persons: a population-based study

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Abstract

Background: Studies suggest an association between a high TSH and (individual components of) the metabolic syndrome. Only a few studies have been performed in the general older population.

Objective: This study investigates the association between serum TSH and the metabolic syndrome in a representative sample of older persons in The Netherlands.

Design and patients: Data of the Longitudinal Aging Study Amsterdam were used, which is an ongoing cohort study in a representative sample of Dutch older persons. A total of 1187 subjects (590 men and 597 women) between the ages of 65 and 88 years participated in the study.

Measurements: Metabolic syndrome (US National Cholesterol Education Program definition) and its individual components were assessed, as well as serum TSH levels.

Results: Among the participants, the prevalence of the metabolic syndrome was 34.2%. The mean serum TSH was 1.9 mU/l. Subjects in the upper quartile with a serum TSH level above 2.28 mU/l (odds ratio (OR) 1.68; 95% confidence interval (CI) 1.19–2.37) had a significantly increased prevalence of metabolic syndrome compared with subjects in the lowest quartile with a serum TSH below 1.04 mU/l. After adjustment for confounders, age, sex, alcohol use, total physical activity, and smoking, the OR was 1.62 (95% CI 1.15–2.32).

Conclusions: Subjects with a serum TSH in the upper quartile have a higher prevalence of metabolic syndrome as compared with subjects with a serum TSH in the lowest quartile.

Introduction

Serum TSH has been associated with components of the metabolic syndrome. In studies comparing euthyroid subjects with subclinical hypothyroid subjects, people in the subclinical hypothyroid group have a higher prevalence of high blood pressure and dyslipidemia (1, 2). Also in the euthyroid range, serum TSH is positively associated with blood pressure, total cholesterol (TC), triglycerides, and LDL, and negatively associated with HDL (3, 4).

In a population-based study that examined the association of different components of the metabolic syndrome between subjects with euthyroid and hypo- or hyperthyroidism, a higher prevalence of hypertension, higher triglycerides, and lower HDL in subjects with subclinical hypothyroidism was observed. In addition, obesity was significantly correlated with serum TSH (5). In two cross-sectional studies performed in euthyroid people, serum TSH was associated with higher triglycerides and an increased risk of the metabolic syndrome (6, 7). Another study with a follow-up period of 3 years found that serum TSH was associated with increased triglycerides, TC, waist circumference, and blood pressure, and decreased HDL, TC, waist circumference, and blood pressure and that subjects with metabolic syndrome had an increased serum TSH over time (8).

In general, these studies have been performed in younger populations. The association between serum TSH and the metabolic syndrome may differ when examined in an older population and also may have other implications. With the increasing number of elderly people, it is important to examine the association between serum TSH and metabolic syndrome specifically in the elderly.

In Taiwanese older persons, a higher prevalence of the metabolic syndrome in persons with subclinical hypothyroidism when compared with persons with subclinical hyperthyroidism was found (9). In the same population, TSH was positively associated with triglycerides and blood pressure and an increased prevalence of metabolic syndrome (10). Recently, Waring et al. found that higher serum TSH was associated with an increase in the odds of prevalent...
metabolic syndrome and this association was even stronger for TSH within the normal range. Furthermore, subclinical hypothyroidism with a TSH > 10 mU/l was significantly associated with increased odds of prevalent metabolic syndrome (11). This study used inclusion criteria like the ability to walk 4 miles, up 10 stairs without rest, and perform basic activities of daily living independently. Also, subjects with diabetes were excluded from the study. Waring et al.’s study population was relatively healthy.

The aim of our study is to examine whether there is an association between serum TSH and the metabolic syndrome within a representative sample of Dutch older persons, without excluding subjects with restricted daily functions and the presence of diabetes. We will examine subjects in a defined serum TSH range, within which no treatment for thyroid dysfunction would be started. This is one of the few studies that focus on an older population, looking at the link between TSH and the metabolic syndrome and its components.

**Materials and methods**

**Subjects**

Data were collected within the Longitudinal Aging Study Amsterdam (LASA), an ongoing interdisciplinary cohort study on predictors and consequences of changes in autonomy and well-being in the aging population in The Netherlands at the VU University and VU University Medical Centre. This cohort study was initiated by the Ministry of Health to determine predictors and consequences of aging.

A random sample of men and women aged 55 years and older, stratified by age, sex, urbanization grade, and expected 5-year mortality rate was drawn from the population registers of 11 municipalities in three regions of The Netherlands, being a representative sample of the Dutch older population. In total, 3107 predominantly Caucasian (≥ 99%) respondents were enrolled in the baseline examination 1992/1993. The present study was performed in a subgroup of the LASA population, including persons who participated in the medical interview of the second data collection (1995/1996), which was restricted to subjects who were aged ≥ 65 years (n = 1509). Blood samples were obtained from 1352 subjects. Informed consent was obtained from all respondents and the study was approved by the Ethical Review Board of the VU University Medical Center. After exclusion of subjects with no data on TSH and subjects with clinical hypo- or hyperthyroidism (free thyroxin (FT₄) lower than 11 pmol/l or higher than 22 pmol/l respectively), 1251 subjects remained.

In older adults, the target serum TSH might be higher and an upper limit of 4–7 mU/l should be maintained (12). In subclinical hypothyroidism with a serum TSH above 10 mU/l, it is recommended to start treatment. We selected those persons who did not receive treatment in the TSH range of 0.3 and 10 mU/l, leaving 1187 subjects to analyze.

**Biochemical data of TSH**

During the medical in-home interview, blood samples were taken. Subjects were allowed to have tea and toast but no dairy products. Thyroid hormones were measured in frozen serum samples. TSH was measured in all persons. When TSH was < 0.3 or > 4.5 mU/l then T₄ was measured. When T₄ was normal, then triiodothyronine (T₃) was measured. TSH was measured by radio-immunometric assay (Centaur, Bayer Diagnostics) with an interassay CV of 6%; FT₄ was measured by a competitive immunoassay (Centaur, Bayer Diagnostics) with an interassay CV of 7%; free T₃ was also measured by a competitive immunoassay.

**Assessment of components of the metabolic syndrome**

Blood pressure was measured with an Omron 706 automatic device while the subject was sitting down. Waist circumference is the average of two measurements calculated to the nearest 0.1 cm midway between the lower rib margin and the iliac crest after normal expiration. Medication use was assessed by recording the medications of the participant directly from the containers and by questionnaires. Fructosamine was determined by a colorimetric test, and HDL and triglycerides were determined by an enzymatic colorimetric test (Roche Diagnostics). The interassay coefficient of variation was < 2.8% for fructosamine and triglycerides and < 6.4% for HDL.

**Metabolic syndrome**

Metabolic syndrome was defined as the presence of three or more of the following criteria: triglycerides ≥ 1.7 mmol/l; HDL < 1.0 mmol/l for men and < 1.3 mmol/l for women; blood pressure ≥ 160/90 mmHg or antihypertensive medication; waist circumference > 102 cm for men and > 88 cm for women; and fructosamine ≥ 0.247 mmol/l or antidiabetic medication. Fructosamine reflects the average glucose concentration over the past 2–3 weeks and correlates with HbA1c and mean blood glucose (13). In a study in The Netherlands, reliability of several biomarkers, including fructosamine, was assessed. Fructosamine was found to be a reliable biomarker for the use of studying an exposure–disease relationship (14). Furthermore, the cutoff of 0.247 mmol/l for fructosamine corresponds to the cutoff of 6.1 mmol/l for fasting plasma glucose in terms of sensitivity and specificity in discriminating subjects with glucose intolerance from those with normal glucose tolerance. Fructosamine was used instead of glucose because a fasting state...
was not required when blood samples were obtained and fructosamine is little affected by eating (15). This definition is in agreement with the US National Cholesterol Education Program (NCEP) Adult Treatment Panel III, with an increased cut-off for blood pressure, adjusted for an older population (16).

In addition, BMI, LDL, and the ratio of TC to HDL (TC/HDL) were analyzed. The ratio of HDL to TC gives a better insight into the risk of cardiovascular disease and insulin resistance syndrome than HDL or TC alone (17, 18).

**Effect modifiers**

Sex was considered to be a potential effect modifier. Women have a higher prevalence of the metabolic syndrome and in some studies concerning the metabolic syndrome the population is stratified according to gender (19).

**Confounders**

Potential confounders were sex, age, alcohol use, smoking and physical activity. The prevalence of the metabolic syndrome increases greatly with age (20). ‘Responsible’ alcohol intake may reduce the prevalence of the metabolic syndrome (21). The Netherlands Economic Institute (NEI) has developed a standard to categorize alcohol use. This standard has been used to compare percentages of alcohol use across surveys (e.g. Central office of Statistics) in The Netherlands. Alcohol was self-reported and, according to the NEI, was divided into no alcohol use, moderate drinking, between moderate and excessive drinking and excessive drinking (22). Smoking has a reversible effect on the thyroid function. Smoking was self-reported and the variable was divided into smoking, used to smoke, and never smoked (23). Subjects who stopped smoking 15 or more years ago were also put in the never smoked group. Physical activity reduces the risk of developing metabolic syndrome and decreases insulin resistance, the underlying problem of the metabolic syndrome (24, 25). Physical activity included walking, bicycling, sports, gardening, and both light and heavy household chores. Information on physical activity was obtained with the LASA Physical Activity Questionnaire (LAPAQ) (26). Physical activity was calculated as time spent on the activity in minutes per day.

BMI may also be a confounder because obesity elevates serum TSH levels (27). Also BMI can be both a confounder and a mediator. Therefore, we included BMI, as a continuous variable, and tested this in a separate model.

Metformin has potential serum TSH-lowering effects (28) but only two persons in our population took metformin. Therefore, it was decided not to adjust the analyses for metformin use.

**Statistical analyses**

Data were analyzed using SPSS version 18. Characteristics of the study sample were compared with ANOVA for normally distributed continuous variables, Kruskal–Wallis test for nonparametric data, and Pearson $\chi^2$ tests for dichotomous variables.

To test the association between TSH and the metabolic syndrome, logistic regression analysis was used. TSH was analyzed in quartiles with the lowest quartile as the reference category. The interaction effect between serum TSH in quartiles and sex in the association between TSH and the metabolic syndrome was tested in the univariable logistic regression model ($P < 0.10$).

To test the association between quartiles of TSH and the different components of the metabolic syndrome, linear regression was used. First, univariable models were performed. Second, the models were adjusted for age, sex, alcohol use, smoking, and exercise.

A $P$ value of 0.05 or less was considered statistically significant.

**Results**

**Population baseline characteristics**

In the TSH range of 0.3–10, there were 590 men and 597 women. Serum TSH was divided in the ranges 0.3–1.04, 1.05–1.53, 1.54–2.28, and 2.29–10 mU/l, with 297 subjects in the first three quartiles and 296 subjects in the upper quartile. In the lower and upper quartiles of serum TSH, there were more women than men. The mean age was 75.44 ($\pm$ 6.56) years. Both the prevalence of metabolic syndrome and obesity was significantly higher in the upper quartile. For the baseline characteristics, see Table 1.

**Serum TSH and the metabolic syndrome**

In the lowest quartile, 28.3% of the subjects had metabolic syndrome and in the upper quartile 39.9% of the subjects had metabolic syndrome.

There was a significant association between metabolic syndrome and serum TSH. Subjects in the upper quartile had a higher risk for the metabolic syndrome (odds ratio (OR) = 1.68; 95% confidence interval (CI) = 1.19–2.37). After adjustment for sex, age, alcohol use, smoking, and exercise, the association between serum TSH and metabolic syndrome was very similar (OR = 1.64; 95% CI 1.15–2.32), see Table 2. Including BMI as a confounding factor next to sex, age, alcohol use, smoking, and exercise, subjects in the upper quartile still had a higher risk for the metabolic syndrome (OR = 1.57; 95% CI 1.08–2.29, see Table 2). No major change was seen in the association between serum TSH and the metabolic syndrome after excluding subjects taking thyroid medication.
Serum TSH-associated components of metabolic syndrome

There was no statistically significant association between serum TSH and systolic or diastolic blood pressure, pulse pressure, waist circumference, TC or triglycerides, both in the unadjusted as well as in adjusted models. For results see Table 3.

Serum TSH was significantly associated with HDL. In subjects in the upper quartile, the HDL was 0.08 lower (95% CI −0.15 to −0.01) than the lowest quartile. After adjustment for confounding, the association did not change and subjects in the upper quartile had a HDL that was 0.08 lower (95% CI 0.01 to 0.05) than the lowest quartile. However, the significance of the association between HDL and serum TSH disappeared when including BMI as a confounding factor. Subjects in the upper quartile had a HDL that was 0.07 lower (95% CI 0.13 to 0.00) than subjects in the lowest quartile.

When calculating the TC/HDL, a significant association was observed with serum TSH. After adjustment, the TC/HDL was 0.39 higher (95% CI 0.06–0.78) in subjects in the upper quartile than in the lowest quartile. For results see Table 3.

Discussion

In this study, a significant association between serum TSH and the metabolic syndrome was observed in older persons. Subjects in the upper quartile with a serum TSH above 2.28 mU/l had a higher prevalence of metabolic syndrome than subjects in the lowest quartile. HDL was lower when serum TSH was higher, with a significant association in the upper quartile. In addition, serum TSH was also positively associated with the ratio of TC to HDL. There was no association found between serum TSH and triglycerides, blood pressure, or fructosamine.

In our study, the only component of the metabolic syndrome that was associated with serum TSH was HDL. In younger populations, associations with other components of the metabolic syndrome such as TC, triglycerides, blood pressure, and waist circumference also change with age, and the association between serum TSH and the metabolic syndrome is weaker in younger populations. Therefore, it is important to consider the age of the population when interpreting the results of this study.

Table 1 Baseline characteristics of the study sample by TSH quartiles.

<table>
<thead>
<tr>
<th>TSH</th>
<th>0.3–10</th>
<th>0.3–1.04</th>
<th>1.05–1.53</th>
<th>1.54–2.28</th>
<th>2.28–10</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>1187</td>
<td>297</td>
<td>297</td>
<td>297</td>
<td>297</td>
<td>−</td>
</tr>
<tr>
<td>Age (years)</td>
<td>75.44</td>
<td>75.34</td>
<td>75.38</td>
<td>75.48</td>
<td>75.55</td>
<td>0.09</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female (%)</td>
<td>50.29</td>
<td>53.53</td>
<td>43.43</td>
<td>49.49</td>
<td>54.73</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Free T4 (pmol/l)</td>
<td>14.00 ± 2.12</td>
<td>16.25 ± 0.96</td>
<td>13.84 ± 2.09</td>
<td>13.84 ± 2.09</td>
<td>13.84 ± 2.09</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Blood pressure (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>153.15 ± 24.02</td>
<td>152.98 ± 23.75</td>
<td>154.20 ± 23.11</td>
<td>153.10 ± 24.36</td>
<td>152.33 ± 24.89</td>
<td>0.83</td>
</tr>
<tr>
<td>Diastolic</td>
<td>81.28 ± 12.25</td>
<td>81.27 ± 12.50</td>
<td>81.41 ± 10.54</td>
<td>81.02 ± 12.10</td>
<td>81.41 ± 13.73</td>
<td>0.98</td>
</tr>
<tr>
<td>Pulse pressure (mmHg)</td>
<td>71.89 ± 18.59</td>
<td>71.70 ± 17.91</td>
<td>72.79 ± 18.80</td>
<td>72.08 ± 18.64</td>
<td>70.93 ± 19.01</td>
<td>0.79</td>
</tr>
<tr>
<td>BMI</td>
<td>26.64 ± 4.21</td>
<td>26.65 ± 4.64</td>
<td>26.49 ± 3.86</td>
<td>27.15 ± 4.13</td>
<td>27.07 ± 4.18</td>
<td>0.10</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>95.75 ± 11.37</td>
<td>95.27 ± 11.91</td>
<td>95.88 ± 10.36</td>
<td>95.86 ± 12.04</td>
<td>95.97 ± 11.16</td>
<td>0.88</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>5.95 ± 1.50</td>
<td>5.99 ± 2.32</td>
<td>5.84 ± 1.08</td>
<td>6.02 ± 1.07</td>
<td>5.96 ± 1.15</td>
<td>0.12</td>
</tr>
<tr>
<td>HDL</td>
<td>1.34 ± 0.43</td>
<td>1.38 ± 0.44</td>
<td>1.34 ± 0.43</td>
<td>1.35 ± 0.44</td>
<td>1.30 ± 0.41</td>
<td>0.14</td>
</tr>
<tr>
<td>LDL</td>
<td>3.68 ± 0.94</td>
<td>3.61 ± 0.93</td>
<td>3.63 ± 0.93</td>
<td>3.77 ± 0.90</td>
<td>3.70 ± 0.97</td>
<td>0.07</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>1.30 (0.8)</td>
<td>1.3 (0.8)</td>
<td>1.2 (0.9)</td>
<td>1.4 (0.8)</td>
<td>1.4 (1.0)</td>
<td>0.06</td>
</tr>
<tr>
<td>Fructosamine (mg/dl)</td>
<td>235.62 (33)</td>
<td>232.77 (32)</td>
<td>236.43 (35)</td>
<td>234.77 (30)</td>
<td>238.52 (34)</td>
<td>0.61</td>
</tr>
<tr>
<td>Prevalence of metabolic syndrome (%)</td>
<td>34.20</td>
<td>28.28</td>
<td>35.35</td>
<td>33.33</td>
<td>39.86</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Prevalence of obesity (BMI ≥ 30) (%)</td>
<td>19.81</td>
<td>19.45</td>
<td>14.24</td>
<td>22.03</td>
<td>23.55</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Model 1: logistic regression analyses for metabolic syndrome in TSH range 0.3–10. Quartiles compared with the lowest quartiles (TSH 0.3–1.04). Results shown as odds ratio (OR) with the 95% confidence interval (95% CI). Model 2: logistic regression analyses adjusted for sex, age, alcohol use, smoking, and physical activity. Model 3: regression analyses adjusted for sex, age, alcohol use, smoking, physical activity, and BMI. Values in bold are statistically significant.
Table 3  Regression analyses of the association of serum TSH with the components of the metabolic syndrome. Linear regression analyses of the association between the components of the metabolic syndrome and the quartiles of serum TSH with the lowest quartile (TSH 0.3–1.04) as reference group. Results shown as β coefficient (β) with S.E.M. and significance (P). Analyses adjusted for sex, age, alcohol use, smoking, and physical activity.

<table>
<thead>
<tr>
<th>Component</th>
<th>Quartile 2</th>
<th>Quartile 3</th>
<th>Quartile 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>S.E.M.</td>
<td>P</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>1.33</td>
<td>2.01</td>
<td>0.51</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>0.02</td>
<td>1.04</td>
<td>0.99</td>
</tr>
<tr>
<td>Pulse pressure (mmHg)</td>
<td>1.31</td>
<td>1.52</td>
<td>0.39</td>
</tr>
<tr>
<td>BMI</td>
<td>0.03</td>
<td>0.34</td>
<td>0.92</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>−0.07</td>
<td>0.92</td>
<td>0.94</td>
</tr>
<tr>
<td>TC (mmol/l)</td>
<td>−0.08</td>
<td>0.12</td>
<td>0.53</td>
</tr>
<tr>
<td>LDL (mmol/l)</td>
<td>0.06</td>
<td>0.08</td>
<td>0.4</td>
</tr>
<tr>
<td>HDL (mmol/l)</td>
<td>−0.02</td>
<td>0.03</td>
<td>0.56</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>−0.05</td>
<td>0.07</td>
<td>0.42</td>
</tr>
<tr>
<td>Fructosamine (μmol/l)</td>
<td>3.57</td>
<td>3.14</td>
<td>0.26</td>
</tr>
<tr>
<td>Total cholesterol/HDL</td>
<td>0.02</td>
<td>0.18</td>
<td>0.91</td>
</tr>
</tbody>
</table>

Values in bold are statistically significant.

were observed (5, 6, 7, 8, 9). In subgroup analyses of persons 70 years and older, Asvold et al. found a significant association between serum TSH and blood pressure (diastolic blood pressure in men and women, and systolic blood pressure only in women). They also found a significant association between lipids and triglycerides and serum TSH in subjects 50 years and older (3, 4). A recent prospective cohort study performed in an older population in the US found the same association between serum TSH and metabolic syndrome and a low HDL. They also found an association between serum TSH and high triglycerides (11). We found similar results in our study with a higher OR for the association between serum TSH and metabolic syndrome in a serum TSH range of 0.3–10 mU/l. The results of both our studies suggest that in older persons, the association between TSH and the metabolic syndrome is based on the association with a worse lipid profile, and that the association between TSH and the other components of the metabolic syndrome is no longer present at an older age.

Thyroid dysfunction has an effect on the activity of cholesteryl ester transfer protein (CETP) and the hepatic lipase (HL). Hypothyroidism is associated with decreased activity of CETP and HL which alters the HDL metabolism (29). Both hypo- and hyperthyroidism is associated with changes in glucose transport rates and insulin resistance (30, 31). These associations between thyroid dysfunction and different metabolic pathways may explain the association between serum TSH and metabolic syndrome we found in our study.

Research has been done to examine the association between serum TSH and mortality. In persons aged 60 years and older, a low serum TSH was associated with increased mortality (32). In a Dutch prospective study in persons aged 85 years, participants with a low serum TSH had highest mortality rate, whereas a high serum TSH was associated with a higher life expectancy (33).

The higher prevalence of metabolic syndrome and lower HDL in the upper quartile of serum TSH seems to contradict the hypothesis that higher TSH levels correspond with better life expectancy in older adults (33). In older persons, a so-called ‘reverse metabolic syndrome’ is present where low BMI, low diastolic blood pressure, and low TC predict mortality. However, a low HDL remains a risk factor for mortality even in older persons (34), especially for cardiovascular mortality (35). Sattar et al. (36) found that the presence of metabolic syndrome in older persons is associated with type 2 diabetes but not with cardiovascular disease. The findings of our study suggest that a high serum TSH level in the elderly is associated with an increased risk for the metabolic syndrome but not necessarily with a higher mortality (37). de Jongh et al. found in the same population we used that subclinical thyroid disorder does not correspond with disadvantageous effects on the physical and cognitive functions of the elderly. However, the group of subclinical hypo- and hyperthyroid persons was relatively small in comparison with the euthyroid group (37).

The strength of this study is that it is carried out in a large population-based sample of older subjects. There are some limitations of our study. LASA has no data on physical activity. Furthermore, due to the individual variation of physical and cognitive functions of the elderly. However, because we had no data on iodine intake, we could not take this into account. Fructosamine may be affected by serum albumin concentrations and we have not adjusted for that possible confounding factor (13). Because this is a cross-sectional study, no conclusion can be made about the possible cause–effect relationship.
In the current study a high serum TSH suggests a disadvantageous effect because of its association with HDL. This association is found in subjects with a serum TSH within the normal range. Therefore, older persons with high normal serum TSH and subclinical hypothyroidism are at increased risk for metabolic syndrome and have lower HDL. Further research should be conducted to examine the association between serum TSH and the metabolic syndrome, cardiovascular disease, diabetes and mortality in a longitudinal study in older persons.

In conclusion, a high serum TSH in older persons is associated with the metabolic syndrome and HDL, and further research should be performed to examine the consequences of this association and to determine the most beneficial serum TSH level for older people.

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.

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