Introduction

Autoimmune diseases in general are much more common in females (1). Autoimmune thyroid disease is highly prevalent, with the highest female-to-male ratio among all autoimmune diseases (2). The underlying causes of the female preponderance of autoimmune thyroid disease are likely to be related to sex differences in immunity, which may partly be attributable to estrogen levels as well as certain female-specific genetic factors (3).

Although estrogen may play a major role in the higher susceptibility to autoimmune disease in females when compared with males, it is unclear whether estrogen levels contribute to the differences in susceptibility among males. Estrogen plays an important role, no less than or even more important than that of testosterone, in the attainment of bone mass during adulthood and in bone loss later in life (4). Moreover, males with a nonsense mutation in the genes encoding estrogen receptor or aromatase have delayed bone maturation and low bone mass (5); this abnormality can be reversed by estrogen supplementation in males with aromatase gene mutation (6). There is also evidence that estrogen has a sexual dimorphic effect on energy homeostasis. Loss of estrogen sulfotransferase, an estrogen-inactivating enzyme, improves energy expenditure and insulin sensitivity in female mice whereas it induces the diabetic phenotype in male animals (7).

The biological function of estrogen with regard to the thyroid gland and susceptibility to autoimmune thyroid...
disease in humans is unknown. In this study, we therefore examined whether circulating estradiol (E₂) is related to thyroid autoimmunity in males.

**Subjects and methods**

**Population**

This study used data and blood samples from the Thai 4th National Health Examination Survey (NHES-IV) conducted in 2008–2009 by the National Health Examination Survey Office, Health Systems Research Institute. Subjects aged 15–98 years were randomly selected from 21 provinces in four geographical regions of Thailand as well as the capital city, Bangkok, using stratified, multistage probability sampling of the population aged ≥15 years, with a sample size of 21,960 individuals. Demographic data such as age and sex were included. BMI was measured using standard procedure. Fasting blood samples were obtained and transferred to a freezer at a central laboratory in Ramathibodi Hospital, a university hospital in Bangkok, where they were maintained at −80°C.

This study used a subsample of the NHES-IV serum samples. The subsamples were randomly selected according to age group (15–29, 30–44, 45–59, 60–69, 70–79, and ≥80 years), sex, urban/rural, and region. In each layer of age group, 25 individuals were randomly selected using statistical software. Only male subjects were included, and 1263 serum samples were available. The study was approved by the local Instructional Review Board. Informed consent was obtained from all subjects.

**Hormonal measurements**

Serum levels of E₂, thyroid-stimulating hormone (TSH), free thyroxine (FT₄), TSH receptor antibody (TRAb), thyroid peroxidase antibody (TPOAb), and thyroglobulin antibody (TgAb) were measured by ELISA on a Cobas e 411 analyzer (Roche Diagnostics GmbH). The assays had intra-assay precision of 4.3, 2.4, 1.3, 1.7, 7.0, and 6.1% respectively. The normal TSH and FT₄ ranges are of the order 0.3–4.2 mU/l and 12.0–24.0 pmol/l respectively. Positive TRAb, TPOAb, and TgAb were defined as values ≥1.25, 85, and 120 IU/ml respectively.

**Statistical analysis**

Data were expressed as mean ± s.d. Pairwise correlation was assessed using Pearson or Spearman correlation coefficients. Logistic regression analysis was performed to identify the predictive variables. A P value of <0.05 was considered statistically significant. All analyses were performed using Stata, version 10.1 (StataCorp. LP, College Station, TX, USA) and SPSS Statistical Software, version 16.0 (SPSS, Inc.).

**Results**

Table 1 demonstrates the clinical and laboratory characteristics of the study population. The mean age was 54.9 ± 21.6 (s.d.) (range 15–94) years. E₂ ranged from 18.4 to 403.7 pmol/l, with a mean value of 136.2 ± 51.7 pmol/l. The proportions of subjects positive for TRAb, TPOAb, and TgAb were 6.5, 7.0, and 6.9% respectively. There was a positive association between circulating E₂ and age (r = 0.18, P < 0.001). No relationship between E₂ and BMI was found (r = 0.02, P = 0.40).

To examine the relationship between serum E₂ and detectability of thyroid autoantibodies, subjects were classified into E₂ quartiles. As shown in Table 2, the proportion of subjects positive for TRAb progressively increased from 1.9% in the lowest E₂ quartile to 11.4% in the highest E₂ quartile with the P value for trend (P trend) across all E₂ quartiles <0.001. It is of note that almost 80% of subjects who were TRAb positive were in the upper two quartiles of E₂. On the contrary, there was no association between E₂ and the detectability of either TPOAb or TgAb. When the relationship between age and

**Table 1** Characteristics of the study population.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Mean ± s.d. or n (%)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>54.9 ± 21.6</td>
<td>15–94</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.7 ± 4.1</td>
<td>13.6–43.3</td>
</tr>
<tr>
<td>E₂ (pmol/l)</td>
<td>136.2 ± 51.7</td>
<td>18.4–403.7</td>
</tr>
<tr>
<td>TSH (mIU/l)</td>
<td>2.1 ± 4.1</td>
<td>0–114.3</td>
</tr>
<tr>
<td>FT₄ (pmol/l)</td>
<td>18 ± 3.9</td>
<td>6.4–100.4</td>
</tr>
<tr>
<td>TRAb positive</td>
<td>82 (6.5%)</td>
<td>–</td>
</tr>
<tr>
<td>TPOAb positive</td>
<td>88 (7.0%)</td>
<td>–</td>
</tr>
<tr>
<td>TgAb positive</td>
<td>87 (6.9%)</td>
<td>–</td>
</tr>
</tbody>
</table>

**Table 2** Relationship between detectability of thyroid autoantibodies and circulating estradiol.

<table>
<thead>
<tr>
<th>E₂ quartile</th>
<th>TRAb</th>
<th>TPOAb</th>
<th>TgAb</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (n = 316)</td>
<td>6 (1.9%)</td>
<td>23 (7.3%)</td>
<td>23 (7.3%)</td>
</tr>
<tr>
<td>2 (n = 316)</td>
<td>13 (4.1%)</td>
<td>18 (5.7%)</td>
<td>24 (7.6%)</td>
</tr>
<tr>
<td>3 (n = 316)</td>
<td>27 (8.5%)</td>
<td>21 (6.6%)</td>
<td>18 (5.7%)</td>
</tr>
<tr>
<td>4 (n = 315)</td>
<td>36 (11.4%)</td>
<td>26 (8.3%)</td>
<td>22 (7.0%)</td>
</tr>
</tbody>
</table>

P trend < 0.0001    P trend = 0.64    P trend = 0.80
thyroid autoantibodies was examined, it was found that only TPOAb and TgAb were significantly associated with age \( (r = 0.08, \ P < 0.01 \) and \( r = 0.08, \ P < 0.01 \) respectively). In a logistic regression model for E2, with age and BMI as independent variables and the detectability of TRAb as the dependent variable, it was found that higher E2 was significantly related to positive TRAb, independent of age and BMI (Table 3). It is conceivable that positive TRAb may cause subclinical hyperthyroidism, which leads to increased sex hormone-binding globulin (SHBG) and hence E2. In order to minimize such an effect, the analysis was repeated after 36 subjects with suppressed TSH were excluded. E2 was still significantly related to positive TRAb as shown in Table 4.

Overall, there was no relationship between serum E2 and ln(TSH) (log-transformed TSH) or FT4. However, when stratifying subjects according to the reference range of TSH (i.e. below, within, and above the reference range), it was found that E2 was negatively related to TSH in subjects whose TSH levels were within or above the reference range. For FT4, no relationship between E2 and FT4 was found, regardless of the TSH categories.

### Discussion

In this study, circulating E2 was found to be related to TRAb positivity in males, but there was no relationship between E2 and TPOAb or TgAb. TSH receptor is the main antigenic etiology of Graves’ disease, while TPOAb and TgAb are likely to be non-pathogenic secondary consequences of altered thyroid function by stimulating TRAb in Graves’ disease. Injection of TRAb causes a disorder similar to Graves’ disease in mice (8). Moreover, TRAb has been shown to be a predictor of the severity of Graves’ disease (9). Our finding of an association between E2 levels and TRAb suggests that E2 might play a role in the pathogenesis of Graves’ disease in men. However, association does not indicate causation, and the reverse causation leading from TRAb to E2 or the notion that TRAb and E2 are simply covariates still needs to be excluded. The reverse causation in which TRAb may cause subclinical hyperthyroidism elevated SHBG, and hence, total E2 appears less likely as the relationship between E2 and positive TRAb still remained significant after subjects with suppressed TSH were excluded. Studies to explore the relationship between E2 levels and the future development of TRAb and Graves’ disease are warranted to help further address the issue.

Thyroid autoimmunity as measured by thyroid autoantibodies usually decreases with anti-thyroid drug treatment or thyroid surgery (10, 11), suggesting an intra-thyroidal origin of thyroid autoimmunity. However, a number of patients do not demonstrate decreases in thyroid autoantibodies after medical or surgical treatment, indicating extra-thyroidal influence in thyroid autoimmunity. The nature of the factors external to the thyroid gland is uncertain. Our findings suggest that estrogen may be such a factor. It should be noted that

### Table 3: Determinants of positive serum TRAb in all subjects (\( n = 1263 \)).

<table>
<thead>
<tr>
<th>Determinant</th>
<th>Adjusted OR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>1.01 (1.00–1.02)</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>1.04 (0.98–1.09)</td>
<td>NS</td>
</tr>
<tr>
<td>Serum E2 (pmol/l)</td>
<td>1.17 (1.11–1.23)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

NS, not significant.

### Table 4: Determinants of positive serum TRAb in subjects with non-suppressed TSH (\( n = 1227 \)).

<table>
<thead>
<tr>
<th>Determinant</th>
<th>Adjusted OR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>1.01 (1.00–1.02)</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>1.05 (0.99–1.11)</td>
<td>NS</td>
</tr>
<tr>
<td>Serum E2 (pmol/l)</td>
<td>1.16 (1.10–1.22)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**OR, odds ratio; NS, not significant.**

### Table 5: Spearman’s correlation between serum E2 and TSH according to the TSH values.

<table>
<thead>
<tr>
<th>TSH (mIU/l)</th>
<th>Adjusted OR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.3 ((n=36))</td>
<td>0.3–4.2 ((n=1153))</td>
<td>&gt;4.2 ((n=74))</td>
</tr>
<tr>
<td>E2 vs TSH</td>
<td>−0.45, ( P &lt; 0.01 )</td>
<td>−0.02, ( P = 0.42 )</td>
</tr>
<tr>
<td>E2 vs FT4</td>
<td>0.31, ( P = 0.07 )</td>
<td>−0.01, ( P = 0.79 )</td>
</tr>
</tbody>
</table>

### Table 6: Relationship of serum E2 and TRAb with ln(TSH) in subjects with suppressed TSH (\( n = 36 \)).

<table>
<thead>
<tr>
<th>Determinant</th>
<th>β-Coefficient</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum E2 (pmol/l)</td>
<td>−0.55</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Positive TRAb</td>
<td>−0.11</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS, not significant.
our findings apply to males only. For females, it is less likely that estrogen would play a major role in thyroid autoimmunity, as the severity of autoimmune thyroid disease does not decrease with menopause and the prevalence of thyroid autoimmunity does not differ by menopausal status (12). Nevertheless, it is well known that the severity of autoimmune thyroid disease usually improves during the third trimester of pregnancy (13, 14). This may be due to pregnancy-induced immune tolerance (15), and increased estrogen-modulating Th1/Th2 cytokine balance may also play a role (16). However, the direct effect of estrogen is less likely as estrogen levels rise throughout pregnancy (17).

Besides its relationship with positive TRAb, a negative association was also found between E2 and TSH, but only in those whose TSH levels fell below the reference range. No association between E2 and FT4 was evident. The association between E2 and TSH may suggest the influence of E2 on thyroid function through thyroid autoimmunity. However, the association between E2 and TSH was still significant after positive TRAb was taken into account. It is also possible that clinical or subclinical hyperthyroidism increased total E2 through elevated SHBG in this group of subjects. However, the lack of association between E2 and FT4 makes this explanation less likely. On the other hand, it is conceivable that E2 may affect thyroid function directly. Estrogen receptors are present in both normal and neoplastic thyroid tissues (18, 19). Although estrogen modulates growth of the thyroid gland (20), which may partly be mediated through its effect on iodide uptake by thyroid follicular cells (21), the lack of change in thyroid hormones after administration of gonadotropins to humans (22) and estrogen to animals (23) makes a direct influence of estrogen on the production and secretion of thyroid hormones less likely. The sample size of this group of subjects with suppressed TSH, however, was very small (n=36) and conclusive remarks regarding the underlying basis based on our results still cannot be made.

It is of note that E2 was associated with TRAb but not TPOAb or TgAb in this study. Despite the fact that both Graves’ disease and Hashimoto’s thyroiditis are related to autoimmunity, they are pathogenetically different. There are differences in association within the HLA class II region between Hashimoto’s thyroiditis and Graves’ disease, which may partly contribute to the different immune-pathological processes and clinical presentation of these diseases (24). Unlike Graves’ disease where TRAb is pathogenic, there is no clear pathogenic antigen in Hashimoto’s thyroiditis. Although TRAb with TSH receptor blocking activity has been implicated in Hashimoto’s thyroiditis, immunoglobulin with TSH binding activity is not present in the majority of patients with Hashimoto’s thyroiditis (25). Although both TPOAb and TgAb are commonly present in patients with Hashimoto’s thyroiditis, it is generally believed that they do not play direct pathogenetic roles. In order to elucidate the role of E2 in men with Hashimoto’s thyroiditis, further studies on the effects of E2 directly on components of the immune-regulatory system such as cytotoxic or regulatory T cells are warranted.

There are a number of limitations in this study. We measured E2 by ELISA. At very low concentrations, liquid chromatography and tandem mass spectrometry have been shown to provide a more reliable and accurate result than immunoassay (26). Moreover, the lack of SHBG measurement in this study makes it difficult to make straightforward interpretation of some of the findings in our study.

In conclusion, higher circulating E2 is related to thyroid autoimmunity in males as reflected by TRAb. Studies to further explore the role of estrogen in the pathogenesis of autoimmune thyroid diseases in males are warranted.

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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References


