The hypercoagulable state in hyperthyroidism is mediated via the thyroid hormone β receptor pathway

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Abstract

Objective: Hyperthyroidism is associated with a hypercoagulable state, but the underlying mechanism is unknown. Patients with resistance to thyroid hormone (RTH) due to defective thyroid hormone receptor β (THRB or THRβ) exhibit elevated circulating thyroid hormones (TH) with refractoriness to TH action in THRβ-expressing tissues. We tested the hypothesis that the hypercoagulable state in hyperthyroidism is mediated via the THRβ.

Design: We conducted a cross-sectional study from November 2013 to January 2015 in 3 hospitals in the Netherlands and the United Kingdom.

Methods: Patients with RTH due to defective THRβ (n = 18), patients with hyperthyroidism (n = 16) and euthyroid subjects (n = 18) were included. TH concentrations and markers of coagulation and fibrinolysis were measured. Data are expressed as median (interquartile range).

Results: Free thyroxine (FT4) levels were slightly higher in hyperthyroid patients than in RTH patients (53.9 (30.5–70.0) and 34.9 (28.4–42.2) pmol/L, respectively, P = 0.042). Both groups had raised FT4 levels compared with euthyroid subjects (14.0 (13.0–15.8) pmol/L, P ≤ 0.001). Levels of von Willebrand factor (VWF), factor (F) VIII, fibrinogen and d-dimer were significantly higher in hyperthyroid patients than in RTH patients (VWF 231 (195–296) vs 111 (82–140)% P ≤ 0.001, FVIII 215 (192–228) vs 145 (97–158)% P ≤ 0.001, fibrinogen 3.6 (3.0–4.4) vs 2.8 (2.5–3.2) g/L, d-dimer 0.41 (0.31–0.88) vs 0.20 (0.17–0.26) mg/L, respectively, P ≤ 0.001), while there were no differences between RTH patients and euthyroid controls.

Conclusions: Parameters of coagulation and fibrinolysis were elevated in hyperthyroid patients compared with patients with RTH due to defective THRβ, whereas these parameters were not different between euthyroid controls and RTH patients, despite elevated FT4 concentrations in RTH patients. This indicates that the procoagulant effects observed in hyperthyroidism are mediated via the THRβ.
Introduction

Venous thromboembolism (VTE) is considered a multi-factorial disease in which multiple genetic and environmental determinants combine to cross a so-called ‘thrombotic threshold’. Several risk factors for VTE influence haemostatic balance, via increases in coagulation factors such as factor (F) VIII and Von Willebrand Factor (VWF), decreased regulatory proteins and impaired fibrinolysis with d-dimer levels as a major determinant. Recently, it has been shown that hyperthyroidism is associated with several changes in haemostatic factors, resulting in a hypercoagulable state (7) putting patients with hyperthyroidism at increased risk of developing VTE (7). This is of clinical importance because of the high prevalence of hyperthyroidism. However, the underlying mechanism leading to a hypercoagulable state in hyperthyroidism is unknown. The thyroid hormone receptors (TR), encoded by two separate genes (THRA and THRB), exhibit differing tissue distribution (7). THRA is the predominant subtype in brain, skeletal muscle, and the heart, while THRB is most abundant in the liver. Mutations in THRA have been discovered recently, with few cases being resistance to thyroid hormone (RTH) due to defective THRA identified to date (7). By contrast, patients with RTH due to defective THRB have been recognized for two decades, with the disorder having an incidence of about one in 40 000 (8). These patients exhibit elevated circulating thyroid hormones (TH) with refractoriness to TH action in THRB-expressing tissues. As THRB is widely expressed in the liver, which plays a key role in the synthesis of coagulation factors, we hypothesized that the hypercoagulable state in hyperthyroidism results from a THRB-mediated effect of TH on coagulation factors. Therefore, patients with RTH due to defective THRB can serve as a unique human model to investigate the role of THRB in the hypercoagulable state observed in hyperthyroidism. In this study, we compared markers of coagulation and fibrinolysis between patients with RTH due to defective THRB or hyperthyroidism and euthyroid control subjects.

Subjects and methods

Study design

In this cross-sectional study, thyroid hormone concentrations and markers of coagulation and fibrinolysis were measured in patients with RTH due to defective THRB (n=18) and in two control groups: patients with hyperthyroidism (n=16) and euthyroid subjects (n=18). The study was performed between 26 November 2013 and 7 January 2015 at the Department of Internal Medicine of the Medical Center Slotervaart, the Departments of Vascular Medicine and Endocrinology of the Academic Medical Center of the University of Amsterdam, Amsterdam, The Netherlands and the Wellcome Trust-MRC Institute of Metabolic Science, Addenbrooke’s Hospital, Cambridge, UK. The study was approved by the medical ethical committee of all institutions functioning according to the 3rd edition of the Guidelines on the Practice of Ethical Committees in Medical Research issued by the Royal College of Physicians of London, and all participants provided written informed consent after full explanation of the purpose and nature of all procedures used.

Study population

Patients with RTH due to mutations in THRB were recruited at Wellcome Trust-MRC Institute of Metabolic Science, Addenbrooke’s Hospital, Cambridge, UK (n=16) and at the Department of Endocrinology and Metabolism, Academic Medical Center of the University of Amsterdam, The Netherlands (n=2). The diagnosis of RTH was confirmed by sequence analysis of the THRB gene. Genotypes and clinical features are presented in Supplementary Table 1, see section on supplementary data given at the end of this article. Hyperthyroid controls were recruited in both participating centres in The Netherlands. All patients with elevated T3, FT3 and/or FT4 according to the local reference ranges were eligible for participation in the study. Euthyroid controls were recruited by local advertisements. In order to be eligible to participate in this study, participants had to meet the following criteria: patients with RTH due to defective THRB and hyperthyroid patients; elevated plasma T3, FT3 and/or FT4 according to the local reference ranges. Hyperthyroid controls were matched for gender, and for age as much as possible. Inclusion criteria for the euthyroid controls were as follows: general good health, matched for age (± 5 years) and gender, and plasma TSH within the reference range. Subjects who met any of the following criteria were excluded from participation in this study: no informed consent; current use of anti-thyroid drugs, vitamin K antagonists or oral corticosteroid therapy; a history of haemophilia or von Willebrand’s disease; and previous VTE within the last 6 months, previous total thyroidectomy or radio-iodine treatment.
Hyperthyroid patients due to underlying subacute thyroiditis were excluded because of the possibility of the accompanying acute inflammatory state influencing markers of coagulation markers.

**Study procedures**

Study visits were scheduled between 8.00 and 11.00 h, and 30 mL of venous blood sampling was drawn. Additional questions were asked about weight, height, medical history, smoking status and the use of (recently stopped) medication. This information was completed by reviewing the charts of all the patients. Participation in this study did not influence the treatment of the patients.

**Laboratory assays**

Blood samples were collected in 4.5 mL heparin tubes for assessing thyroid function and sex hormone-binding globulin (SHBG, a T3-responsive gene expressed in the liver) levels, and in 2.7 mL 0.109 mol/L sodium citrate tubes for testing coagulation and fibrinolysis parameters (BD Vacutainer, Plymouth, UK). Citrated blood was immediately centrifuged, and the supernatant re-centrifuged, for 15 min at 3000 r.p.m. (1860 g) at 15°C to obtain platelet-poor plasma. Heparin tubes were centrifuged for 15 min. at 3000 r.p.m. (1860 g) at 15°C. Plasma was aliquoted and stored at −80°C until further use. Coagulation and fibrinolysis parameters were measured in one batch at the Department of Experimental Vascular Medicine, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands, after completion of the study. Prothrombin time (PT) was measured by BCS-XP (Siemens), reference values: 10.7–12.9 s. Activated partial thromboplastin time (aPTT) was measured by BCS-XP (Siemens), reference values: 25.0–38.0 s. VWF antigen was measured by a PT-derived measurement (Siemens), reference values: 1.9–4.0 g/L. Factor VIII activity was measured by BCS-XP (Siemens), reference values: 63–173%. Factor IX activity was measured by BCS-XP (Siemens), reference values: 80–145%. Prothrombin fragment 1+2 (F1+2) was measured by ELISA (Siemens), reference values: 53–271 pmol/L. Endogenous thrombin potential (ETP) was measured by CAT (Thrombinoscope BV), reference values: 65–146%. Lagtime was measured by calibrated automated thrombinography (CAT) (Thrombinoscope BV), reference values: 1.5–3.2 min. Peak thrombin was measured by CAT (Thrombinoscope BV), reference values: 1.5–3.2 min. Peak thrombin was measured by CAT (Thrombinoscope BV), reference values: 63–154%.

**Statistical analyses**

The main outcome of this study was comparison of coagulation parameters between patients with RTH due to defective THRβ or hyperthyroidism and euthyroid controls. A previous study of hyperthyroidism showed that compared with pre-treatment values (1.82 ± 0.40 U/mL), highly significant decreases in FVIII activity (0.97 ± 0.32 U/mL) (9) were noted with normalization of thyroid function. To obtain a power of 80% and an alpha error of 0.05%, 4 patients would be needed to have enough power to detect a similar reduction in this study. In another study, a statistically significant difference in FIX was detected in 41 patients with hyperthyroidism and 20 controls (7). The mean value of FIX in the control group was 83.6 ± 16.9% and in the group with hyperthyroidism it was 109.6 ± 27.9%. To obtain a power of 80% and an alpha error of 0.05% at least 8 patients would be needed in this study to detect a similar difference. Because of inter-individual differences in markers of coagulation and to achieve higher statistical power, we sought to recruit at least 16 participants in each study group. The characteristics of the participants are expressed as median (interquartile range) or percentages where appropriate. The parameters of coagulation and fibrinolysis are expressed as median (interquartile range). Numeric data were compared between the three groups by the Kruskal-Wallis test and in case of a P value <0.05 a Mann–Whitney U test was performed to compare each single group with the other two groups separately. Categorical data was
compared between the three groups with a $\chi^2$-test, and in case of statistical significance ($P<0.05$), a $\chi^2$-test or Fisher’s exact test, where appropriate, was performed to compare each single group with the other two groups separately. To investigate the effect of age and smoking status on the parameters of coagulation and fibrinolysis, a multivariate linear regression model was used on the logarithmic data of the parameters with a $P$ value $<0.05$ when comparing the 3 groups, with the variables age and currently smoking added to the model. To rule out that the observed results in VWF, FVIII, fibrinogen and d-dimer were due to the higher percentage of smokers in the hyperthyroid patients, a sensitivity analysis was performed that was restricted to the non-smoking participants. In this analysis, a Mann–Whitney $U$ test was performed to compare each single group with the other two groups separately.

In case of a value of FT$_4$ or FT$_3$$>70$ pmol/L ($n=6, n=1$, respectively), the value was designated as 70 pmol/L. In case of a value of d-dimer $<0.17$ mg/L ($n=15$), the value was designated as 0.17 mg/L. In case of F1 $+2>1200$ ($n=1$) (most likely caused by in vitro activation of coagulation), the value was assumed as missing and the value of d-dimer of the concerning participant was also disqualified. Statistical analysis was performed using SPSS 21 software package (SPSS Inc.).

**Results**

**Participants**

Characteristics of participants are given in Table 1. We enrolled 18 patients with RTH due to defective $THRB$, 16 hyperthyroid cases and 18 euthyroid controls. Although not statistically significant ($P=0.071$), the hyperthyroid patients were older than RTH cases. There were no differences in gender, BMI and oral contraceptive use between the three groups. None of the participants were on hormone replacement therapy during the study period. Ninety-four percent of hyperthyroidism cases were due to Graves’ disease with significantly more smokers in this group. Free thyroxine (FT$_4$) levels were slightly higher in patients with hyperthyroidism than those with RTH (53.9 (30.5–70.0) and 34.9 (28.4–42.2) pmol/L, respectively, $P=0.042$). Both groups had significantly raised FT$_3$ levels compared with the euthyroid subjects (14.0 (13.0–15.8) pmol/L, $P\leq0.001$). As expected, SHBG levels were significantly higher in hyperthyroid patients than in RTH cases and euthyroid controls.

**Coagulation and fibrinolytic parameters**

Parameters of coagulation, thrombin generation and fibrinolysis are given in Table 2. Levels of VWF, FVIII, fibrinogen and d-dimer were higher in hyperthyroid patients than in RTH cases (VWF 231 (195–296) vs 111 (82–140)$\%$, FVIII 215 (192–228) vs 145 (97–158)$\%$, fibrinogen 3.6 (3.0–4.4) vs 2.8 (2.5–3.2) g/L, d-dimer 0.41 (0.31–0.88) vs 0.20 (0.17–0.26) mg/L, respectively, $P\leq0.001$), while there were no differences between RTH patients and euthyroid controls (see Fig. 1 for boxplots of VWF, FVIII, fibrinogen and d-dimer per group and Fig. 2 for their role in the coagulation and fibrinolysis pathways). These differences persisted after correcting for age and smoking status ($P\leq0.010$). When restricting the analyses to the non-smokers, levels of VWF, FVIII, fibrinogen and d-dimer were significantly higher in hyperthyroid patients than in RTH patients and euthyroid controls ($P$ values $\leq0.001$), while there were no differences between

<table>
<thead>
<tr>
<th>Parameter</th>
<th>RTH ($n=18$)</th>
<th>Hyperthyroid controls ($n=16$)</th>
<th>Euthyroid controls ($n=18$)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>31 (25–55)</td>
<td>53 (38–64)</td>
<td>31 (28–57)</td>
<td>0.071</td>
</tr>
<tr>
<td>Gender (% male)</td>
<td>67</td>
<td>67</td>
<td>63</td>
<td>0.958</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>23.5 (21.5–26.5)</td>
<td>23.0 (21.2–25.0)</td>
<td>24.5 (22.5–26.4)</td>
<td>0.001</td>
</tr>
<tr>
<td>Currently smoking (%)</td>
<td>6$^a$</td>
<td>50$^b$</td>
<td>6$^a$</td>
<td>0.762</td>
</tr>
<tr>
<td>Use of oral contraceptives (%)</td>
<td>0</td>
<td>63</td>
<td>56</td>
<td>0.762</td>
</tr>
<tr>
<td>Use of HRT (%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.762</td>
</tr>
<tr>
<td>Aetiology of hyperthyroidism (%)</td>
<td>Graves (94), TMNG (6)</td>
<td>53.9 (30.5–70.0)$^c$</td>
<td>14.0 (13.0–15.8)$^b$</td>
<td>$\leq0.001$</td>
</tr>
<tr>
<td>FT$_4$ (pmol/L)</td>
<td>34.9 (28.4–42.2)$^a$</td>
<td>215 (173–230)$^a$</td>
<td>95 (85–98)$^b$</td>
<td>$\leq0.001$</td>
</tr>
<tr>
<td>Total T$_4$ (nmol/L)</td>
<td>175 (156–200)$^a$</td>
<td>23.8 (12.5–51.3)$^c$</td>
<td>4.0 (3.5–4.9)$^b$</td>
<td>$\leq0.001$</td>
</tr>
<tr>
<td>Total T$_3$ (nmol/L)</td>
<td>2.58 (2.54–3.38)$^a$</td>
<td>5.05 (3.58–6.58)$^c$</td>
<td>1.83 (1.63–1.93)$^b$</td>
<td>$\leq0.001$</td>
</tr>
<tr>
<td>TSH (mU/L)</td>
<td>1.74 (1.26–2.49)$^a$</td>
<td>0.01 (0.01–0.01)$^b$</td>
<td>1.66 (1.33–2.13)$^b$</td>
<td>$\leq0.001$</td>
</tr>
<tr>
<td>SHBG (nmol/L)</td>
<td>28 (21–48)$^a$</td>
<td>179 (82–230)$^b$</td>
<td>44 (22–54)$^a$</td>
<td>$\leq0.001$</td>
</tr>
</tbody>
</table>

RTH, resistance to thyroid hormone; BMI, body-mass index; HRT, hormone replacement therapy; TMNG, toxic multinodular goiter; FT$_4$, free thyroxine; FT$_3$, free tri-iodothyronine; TSH, thyroid-stimulating hormone (thyrotropin); SHBG, sex hormone-binding globulin. Values with different letters are statistically significantly different ($P\leq0.006$). *$P$ value 0.042 for FT$_4$ levels in RTH vs hyperthyroid patients.
Coagulation in hyperthyroidism

RTH patients and euthyroid controls. FIX was higher in hyperthyroid patients compared with euthyroid controls (P = 0.016 when testing 3 groups), but this difference did not persist after correcting for age and smoking status (P = 0.094). ETP was lower in hyperthyroid controls compared with euthyroid controls. No differences were found in PT, aPTT, F1+2, lag time, and peak thrombin between the three groups.

Discussion

The aim of this study was to compare markers of coagulation and fibrinolysis between patients with RTH due to defective THRβ, patients with hyperthyroidism and euthyroid control subjects. We hypothesized that the hypercoagulable state in hyperthyroidism results from a THRβ-mediated effect of TH on coagulation factors. In our study, parameters of coagulation and fibrinolysis were elevated in hyperthyroid patients compared with patients with RTH due to defective THRβ, whereas these parameters were not different between euthyroid controls and RTH patients, despite elevated FT4 concentrations in RTH patients. This indicates that the procoagulant effects observed in hyperthyroidism are mediated via the THRβ pathway. The hyperthyroid patients, but not the RTH cases in our study, had significantly elevated VWF antigen and FVIII levels. Since these factors are known

Table 2 Parameters of coagulation and fibrinolysis. Data are presented as median (interquartile range).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>RTH</th>
<th>Hyperthyroid controls</th>
<th>Euthyroid controls</th>
<th>P value</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coagulation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PT (s)</td>
<td>11.8 (11.4–12.2)</td>
<td>11.7 (11.4–12.8)</td>
<td>12.1 (11.4–12.4)</td>
<td>0.661</td>
<td></td>
</tr>
<tr>
<td>aPTT (s)</td>
<td>30.3 (28.1–32.8)</td>
<td>30.6 (27.5–33.3)</td>
<td>31.1 (28.6–32.0)</td>
<td>0.951</td>
<td></td>
</tr>
<tr>
<td>VWF (%)</td>
<td>111 (82–140)a</td>
<td>231 (195–296)b</td>
<td>104 (78–121)a</td>
<td>≤0.001</td>
<td>≤0.001</td>
</tr>
<tr>
<td>Fibrinogen (g/L)</td>
<td>2.8 (2.5–3.2)a</td>
<td>3.6 (3.0–4.4)b</td>
<td>2.8 (2.3–3.4)a</td>
<td>0.001</td>
<td>0.009</td>
</tr>
<tr>
<td>FIX (%)</td>
<td>145 (97–158)a</td>
<td>215 (192–228)b</td>
<td>125 (107–142)a</td>
<td>≤0.001</td>
<td>≤0.001</td>
</tr>
<tr>
<td></td>
<td>121 (115–125)a,b</td>
<td>123 (112–137)b</td>
<td>112 (100–118)a</td>
<td>0.016</td>
<td>0.094</td>
</tr>
<tr>
<td>Thrombin generation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1+2 (pmol/L)</td>
<td>170 (133–298)</td>
<td>235 (158–287)</td>
<td>160 (110–237)</td>
<td>0.120</td>
<td></td>
</tr>
<tr>
<td>ETP (%)</td>
<td>92 (81–103)a,b</td>
<td>84 (77–89)b</td>
<td>96 (93–104)a</td>
<td>0.004</td>
<td>0.028</td>
</tr>
<tr>
<td>Lagtime (min)</td>
<td>3.3 (3.0–3.5)</td>
<td>3.8 (3.2–4.9)</td>
<td>3.3 (3.0–3.9)</td>
<td>0.138</td>
<td></td>
</tr>
<tr>
<td>Peak thrombin (%)</td>
<td>99 (87–121)</td>
<td>92 (61–114)</td>
<td>88 (70–105)</td>
<td>0.199</td>
<td></td>
</tr>
<tr>
<td>Fibrinolysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d-dimer (mg/L)</td>
<td>0.20 (0.17–0.26)a</td>
<td>0.41 (0.31–0.88)b</td>
<td>0.18 (0.17–0.27)a</td>
<td>≤0.001</td>
<td>0.001</td>
</tr>
</tbody>
</table>

RTH, resistance to thyroid hormone; PT, prothrombin time; aPTT, activated partial thromboplastin time; VWF, Von Willebrand Factor; F, factor; F1+2, prothrombin fragment 1+2; ETP, endogenous thrombin potential. Values with different letters are statistically significantly different (P ≤ 0.005).

*Multivariate linear regression model on logarithmic data with variables age and currently smoking.

RTH patients and euthyroid controls. FIX was higher in hyperthyroid patients compared with euthyroid controls (P = 0.016 when testing 3 groups), but this difference did not persist after correcting for age and smoking status (P = 0.094). ETP was lower in hyperthyroid controls compared with euthyroid controls. No differences were found in PT, aPTT, F1+2, lag time, and peak thrombin between the three groups.

Discussion

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Figure 1

Levels of Von Willebrand Factor, Factor VIII, fibrinogen and d-dimer in patients with resistance to thyroid hormone due to defective thyroid hormone receptor β (THRβ), patients with hyperthyroidism and euthyroid controls. RTH, resistance to thyroid hormone; VWF, Von Willebrand factor; FVIII, factor VIII. Dotted lines are reference values. Numeric data were compared between the three groups by the Kruskal–Wallis test and in case of a P value <0.05 a Mann–Whitney U test was performed to compare each single group to the other two groups separately. Statistical significance was defined as a P<0.05.
European Journal of Endocrinology
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groups had markedly raised FT₄ levels compared with patients with hyperthyroidism than in RTH patients. Both pathways leading to the formation of fibrin. The intrinsic pathway is initiated by exposure to negatively charged molecules such as polyphosphates, neutrophil extracellular traps, DNA or RNA. Factor (F) IX was measured because it is produced in the liver and FVIII because it is produced in (liver) endothelial cells. The extrinsic pathway is initiated by the exposure of tissue factor after injury of the endothelium. F1+2 is the activation peptide that is cleaved of during thrombin formation, and can therefore be considered a marker for thrombin generation. Fibrinogen was measured because it is the precursor of fibrin. Plasmin is the enzyme that degrades fibrin clots. Plasminogen activator inhibitor-1 (PAI1) inhibits tissue-type plasminogen activator (t-PA). D-dimer is a degradation product of fibrin and therefore a marker of both coagulation and fibrinolysis.

to be released primarily from endothelial cells (7), which are also present in the liver, this suggests that thyroid hormones exert this effect on endothelium via a THRB pathway. Consistent with this hypothesis, THRB expression in endothelial cells has been documented (7). Of note, the hyperthyroid patients had only a modest increase in Factor IX, which disappeared after correcting for age and smoking status. As Factor IX is produced by the liver, we cannot ascribe the hypercoagulable state to a specific target organ, but can conclude that it occurs via a THRB-mediated pathway. In our study, FT₄ levels were slightly higher than in RTH patients. Both groups had markedly raised FT₄ levels compared with euthyroid subjects (14.0 (13.0–15.8) pmol/L, P≤0.001). One could argue that this difference in FT₄ levels could account for divergent VWF, FVIII, fibrinogen, and d-dimer levels between the two groups. However, in previous studies, we showed that thyroid hormones affect circulating coagulation factor levels at plasma concentrations far below FT₄ levels documented in the RTH patients, making this explanation unlikely (7). Levels of ETP were lower in hyperthyroid patients vs euthyroid controls, with three possible explanations for this observation. First, this could be due to chance, particularly as there are no differences in the peak thrombin value which is considered a more important marker of thrombin generation. Secondly, since ETP is an in vitro measurement, in contrast to the other markers of coagulation and fibrinolysis measured in this study, this finding could be due to consumption of coagulation factors, although there is no other evidence to support this in this study. A third explanation might be another determinant which lowered ETP in the hyperthyroid controls, which we did not measure in this study. In 94% of our hyperthyroid cases, the underlying aetiology was Graves’ disease, raising the possibility that the observed effects on coagulation and fibrinolysis in this group might be partly due to autoimmune or concomitant inflammatory processes. However, a recent study has ruled out inflammatory processes as a mediator of the effect of thyroid hormones on the coagulation system (7). In further support of this notion, raised plasma levels of FT₄ following administration of pharmacological doses of levothyroxine in healthy volunteers also causes a hypercoagulable state (7). One could argue that the observed increased levels of VWF, FVIII, fibrinogen and d-dimer in the hyperthyroid patients are due to the higher percentage of smokers in this group, e.g., as a result of endothelial damage. In the Münster Arteriosclerosis Study (MAS), a prospective longitudinal epidemiological study on an industrial population in Westfalia including 2880 male and 1306 female persons, a correlation of fibrinogen was found with cigarette smoking (7). In the same study, however, no correlation was found for FVIII. The Atherosclerosis Risk in Communities (ARIC) Study, a prospective study designed to assess risk factors for the development of atherosclerotic diseases in fifteen 800 middle-aged persons, reported no association of VWF with smoking status while FVIII was negatively associated with smoking status (7). The sensitivity analysis in non-smokers in the current study ruled out that the observed results in VWF, FVIII, fibrinogen and d-dimer were due to the higher percentage of smokers in the hyperthyroid patients. In line, the observed differences in these parameters when comparing the hyperthyroid patients with the RTH

Figure 2
Schematic representation of coagulation and fibrinolysis pathways. Parameters measured in this study are indicated in bold. Von Willebrand factor (VWF), present in plasma by constitutive or induced release from endothelial cells, facilitates adhesion and aggregation of platelets to the injured vessel wall (primary haemostasis). The coagulation system (secondary haemostasis) can be activated via two pathways leading to the formation of fibrin. The intrinsic pathway is initiated by exposure to negatively charged molecules such as polyphosphates, neutrophil extracellular traps, DNA or RNA. Factor (F) IX was measured because it is synthesized in the liver and FVIII because it is produced in (liver) endothelial cells. The extrinsic pathway is initiated by the exposure of tissue factor after injury of the endothelium. F1+2 is the activation peptide that is cleaved of during thrombin formation, and can therefore be considered a marker for thrombin generation. Fibrinogen was measured because it is the precursor of fibrin. Plasmin is the enzyme that degrades fibrin clots. Plasminogen activator inhibitor-1 (PAI1) inhibits tissue-type plasminogen activator (t-PA). D-dimer is a degradation product of fibrin and therefore a marker of both coagulation and fibrinolysis.
patients and the euthyroid controls, respectively, persisted after correcting for age and smoking status. Thyroid hormones exert their effects through nuclear receptors with differential tissue distributions (7). A recent study has demonstrated that T₃ administration to hypothyroid mice has widespread effects on the expression of hepatic and vessel wall-associated genes in the coagulation pathway, with both immediate early and late transcriptional changes resulting in altered plasma levels of a panel of coagulation factors (18). As yet, the coagulation pathways have not been investigated in transgenic, mutant THrb mouse models. Our findings of unaltered parameters of coagulation and fibrinolysis despite circulating hyperthyroxinaemia in a cohort of patients with RTH clearly suggests that such procoagulant effects observed in hyperthyroidism are mediated via a THRβ-dependent pathway. Extrapolating from this, it is conceivable that thyromimetics (e.g. eprotirome), developed to lower LDL-cholesterol by stimulating THRβ pathways in the liver, (7) could induce a hypercoagulable state. Whether patients with RTH due to defective TRα (7) treated with levothyroxine in supraphysiological dosage to overcome hormone resistance in TRα-expressing tissues, who are also at risk of hypercoagulability, remains to be determined.

Supplementary data
This is linked to the online version of the paper at http://dx.doi.org/10.1530/EJE-15-1249.

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 the SKWOSZ (Foundation for Clinical Scientific Research Medical Center Slotervaart), the Wellcome Trust (095564/Z/11/Z to K C) and the National Institute for Health Research Cambridge Biomedical Research Centre (C M, K C).

The funding source had no involvement in the collection, analysis and interpretation of the data, in the writing of the report or the decision to submit the paper for publication.

Author contribution statement
Laura P B Elbers and Carla Moran contributed to the literature search, study design, data collection, data analysis, data interpretation and writing of the manuscript (including figures). Victor E A Gerdes contributed to the study design, data collection, data analysis, data interpretation and writing of the manuscript (including figures). Bregje van Zaane contributed to the study design, data interpretation and writing of the manuscript. Joost C M Meijers contributed to the literature search, data analysis, data interpretation and writing of the manuscript (including figures). Erik Endert contributed to the data analysis and writing of the manuscript. Greta Lyons contributed to the data collection and writing of the manuscript. Krishna Chatterjee contributed to the study design, data collection, data interpretation and writing of the manuscript (including figures). Peter H Bischof and Eric Fliers contributed to the literature search, study design, data analysis, data interpretation and writing of the manuscript (including figures).

Acknowledgements
The authors thank all participants for their willingness to undertake his study. They thank Huib Bout, Ismael Derraz and the internists of the Medical Center Slotervaart for their help in identifying patients with hyperthyroidism, the co-workers of the Laboratory of Endocrinology and Radiochemistry at the Academic Medical Center for their help in identifying patients with hyperthyroidism and laboratory procedures, Maaike Gerards for her help in including participants and Wil Kopatz for her help in laboratory procedures.

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