Relationships between plasma thyrotropin receptor antibodies and lipid or lipoprotein parameters in Graves’ disease

Max Rieu, Eric Revue, Rémi Bonete and Sébastien Nunez

Department of Endocrinology, and Biochemistry Laboratory, Saint Michel Hospital Paris, France


Functional thyrotropin receptors (TSH-R) have recently been detected in fat cells but not in liver cells from rat, and it seems that in infant adipocytes stimulatory TSH-R antibodies (TSH-R-ab) act through this receptor pathway, resulting in increased triglyceride catabolism. We investigated the relationships between plasma TSH-R-ab and free thyroxine (FT4) levels and plasma lipid or lipoprotein values in 49 untreated adult women with Graves’ disease, all positive for these antibodies. A simple positive correlation (p < 0.01) was found between TSH-R-ab levels and FT4 values (r = 0.40). Simple positive correlations (p < 0.001) were found between triglyceride levels and FT4 (r = 0.51) or TSH-R-ab (r = 0.52) values. Multiple regression analysis confirmed that both FT4 and TSH-R-ab are strong (p < 0.005) predictors of triglyceride (FT4; partial r = 0.40; TSH-R-ab; partial r = 0.39). Simple negative correlations (p < 0.05, at least) were found between FT4 levels and total cholesterol (TC) (r = −0.45), low-density lipoprotein (LDL)-C (r = −0.46), apoprotein (apo)-B (r = −0.31) or high-density lipoprotein (HDL)-C (r = −0.55) values. Among these lipid parameters, only HDL-C levels (r = −0.31, p < 0.05) correlated to TSH-R-ab values. However, multiple regression analysis revealed that while FT4 is a strong predictor (p < 0.005) of TC (partial r = −0.42), LDL-C (partial r = −0.43) or HDL-C (partial r = −0.47), TSH-R-ab are not. Thus, the apparent positive relationship between TSH-R-ab and HDL-C results from the positive correlation between TSH-R-ab and FT4. In conclusion, this study suggests that stimulating TSH-R-ab are involved in triglyceride metabolism. In contrast to thyroid hormones, these antibodies seem not to be related to cholesterol metabolism.

Max Rieu, Hôpital Saint Michel, 33 rue Olivier de Serres, 75015 Paris, France

It has been clearly established that thyroid hormones are involved in triglyceride (TG) metabolism in adult human (1, 2). Thus, it has been demonstrated repeatedly that in patients with Graves’ disease or in experimental hyperthyroidism the production and plasma concentration of free fatty acids (FFA) and glycerol are both markedly increased, due to stimulation of lipolysis in adipose tissue by thyroid hormone excess (3–5). Thyroid hormones act mainly on β-adrenoceptors but also, to a lesser extent, through their own receptors in adult human adipocytes (6). High plasma concentrations of both FFA and glycerol, on the other hand, are effective stimulators of hepatic very low-density lipoprotein (VLDL)-TG synthesis and release (1). A second factor acting in the same direction is enhancement of hepatic fatty acid synthesis by thyroid hormones through their own receptors (7). Furthermore, thyroid hormone excess increases the fractional removal of endogenous TG (1). Finally, plasma TG levels have been shown to be low (8), normal (9, 10) or high (1) in patients with Graves’ disease. These discrepant results might be due to the fact that factors such as alcohol consumption, body mass index or blood glucose levels have not been strictly considered. Furthermore, cholesterol (C) metabolism is also influenced by thyroid hormones (11). Thyroid hormones seem to increase low-density lipoprotein (LDL) receptor-mediated clearance of LDL (12), especially at the hepatic level, thus lowering plasma LDL-C concentrations, and the post-heparin plasma hepatic lipase activity is increased leading to decreased plasma high-density lipoprotein (HDL)-C levels in hyperthyroid patients (13). Finally, synthesis of apoprotein (apo)-B seems to be decrease in thyrotoxicosis (14) and thyroid hormones reduce plasma total cholesterol (TC) concentrations through increased hepatic catabolism of cholesterol (15).

In patients with Graves’ disease, thyrotropin receptor antibodies (TSH-R-ab) interact with the TSH-R and mimic the action of TSH, leading to thyroid hyperfunction (16). Furthermore, it has been demonstrated recently that fat cells but not liver cells (BRL-3A cells) from rat express high levels of TSH-R whose function is indistinguishable from that in the thyroid gland (17). These findings are in good agreement with previous reports (18–21). Finally, it has been reported in human and rat adipocytes that TSH or stimulating TSH-R-ab induce lipolysis through the TSH-R pathway (22–24). To our knowledge, plasma TSH-R-ab levels have not
been taken into account in interpreting the plasma lipid or lipoprotein changes in patients with Graves’ disease. We, therefore, studied the relationships between plasma TSH-R-ab, free thyroxine (FT₄), lipid and lipoprotein levels in women with Graves’ disease.

Subjects and methods

Subjects

This prospective study included 49 untreated consecutive women (age range 20–81 years, mean 43 ± 2 years) with Graves’ disease diagnosed on the basis of decreased TSH levels (< 0.04 mU/l), increased FT₄ values (> 21 pmol/l) and positive TSH-R-ab levels (> 7%). Plasma TSH, FT₄, TSH-R-ab, TC, HDL-C, apo-B and TG were measured after an overnight fast in all patients. Plasma LDL-C levels were then calculated. In all patients, 24 h urinary iodine excretion (ranging between 60 and 180 µg) and liver and renal function tests were within the reference range. None of the patients had increased alcohol consumption (< 6 g per day), none had body mass index (BMI) above 30 (mean = 20.5 ± 0.6), none has fasting blood glucose level above 5.5 mmol/l (mean = 4.8 ± 0.1 mmol/l), none had any known family history of hyperlipoproteinemia, none were taking any medication known to affect TSH secretion or lipid metabolism and none had undergone thyroid surgery. Thirty-five healthy adult women of comparable (p > 0.05) age (mean = 40 ± 3 years), BMI (mean = 21.6 ± 0.9), fasting blood glucose level (mean = 4.7 ± 0.1 mmol/l) and alcohol consumption (< 6 gr per day) were used as the reference population for plasma FT₄, TSH, lipid and lipoprotein concentrations. They were negative for plasma TSH-R-ab and were taking no medication.

Methods

Plasma FT₄ levels were measured by RIA using T₄ analogue (Bio Merieux Laboratories, France). Plasma TSH values were determined in duplicate by IRMA (Bio Merieux Laboratories, France) with a detection limit of 0.04 mU/l and an interassay coefficient of variation of 5% or less in the range studied. The levels of plasma TSH-R-ab were determined by radioreceptor assay using a commercially available kit (Henning Laboratories, Berlin, Germany). A plasma sample was considered as positive when the level of TSH-R-ab was > 7%, as reported previously (25). Plasma TC and TG levels were determined enzymatically (Boehringer Mannheim, Meylan, France) on a Hitachi 717 analyzer. Plasma HDL-C values were qualified by the same enzymatic method after precipitation of VLDL and LDL with phosphotungstic acid. Plasma LDL-C levels were calculated according to the Friedewald equation (26). Plasma apo-B values were determined by rate nephelometric immunoassay with the Behring Array system (Behring Diagnostic, Rueil-Malmaison, France).

Statistics

Statistical analysis was done using the Statview Statistical Software Program (Abacus Concepts, Calabasas, CA). Statistics were performed using one-way analysis of variance. Data were logarithmically (log) transformed where necessary to achieve homogeneity in the variance. Results were expressed as the mean ± SEM. Statistical differences were determined by applying Students t-test. The relationships between variables were evaluated by simple correlation coefficients and multiple linear regression analysis. The level of significance was taken as p < 0.05 for simple regression analysis and as p < 0.005 for multiple regression analysis according to Bonferroni’s correction.

Results

Plasma thyroid factors and lipid or lipoprotein parameters in patients with Graves’ disease and in controls (Table 1)

The mean TC, LDL-C, apo-B and HDL-C levels in patients with Graves’ disease were lower (p < 0.001) than those observed in controls. In contrast, the mean TG level in patients with Graves’ disease was higher (p < 0.05) than that obtained in controls.

Relations between plasma thyroid factors and lipid or lipoprotein parameters in patients with Graves’ disease (Table 2 and Fig. 1)

A positive (r = 0.40, p < 0.01) correlation was found between TSH-R-ab levels and FT₄ values.

Table 1. Means (± sas) of plasma thyroid, lipid and lipoprotein parameters in untreated adult women with Graves’ disease and positive for TSH-R-ab and in controls.

<table>
<thead>
<tr>
<th></th>
<th>FT₄ (pmol/l)</th>
<th>TSH-R-ab (%)</th>
<th>TC (mmol/l)</th>
<th>LDL-C (mmol/l)</th>
<th>Apo-B (g/l)</th>
<th>HDL-C (mmol/l)</th>
<th>TG (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>55.80 ± 3.31***</td>
<td>22.13 ± 1.41***</td>
<td>4.26 ± 0.14***</td>
<td>2.42 ± 0.11***</td>
<td>0.80 ± 0.03***</td>
<td>1.22 ± 0.05***</td>
<td>0.96 ± 0.04*</td>
</tr>
<tr>
<td>Controls</td>
<td>14.23 ± 1.32</td>
<td>&lt; 7</td>
<td>5.12 ± 0.11</td>
<td>3.20 ± 0.12</td>
<td>1.09 ± 0.04</td>
<td>1.45 ± 0.06</td>
<td>0.83 ± 0.04</td>
</tr>
</tbody>
</table>

*p = total cholesterol: TG = triglycerides. * p < 0.05 and *** p < 0.001 vs values in controls.
Table 2. Simple (R) and partial (r) correlation coefficients between plasma lipid levels and plasma FT4 or TSH-R-ab values in adult women with Graves' disease and positive for TSH-R-ab.

<table>
<thead>
<tr>
<th></th>
<th>FT4</th>
<th>TSH-R-ab</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>-0.45***</td>
<td>-0.16</td>
</tr>
<tr>
<td>R</td>
<td>-0.42**</td>
<td>-0.01</td>
</tr>
<tr>
<td>LDL-C</td>
<td>-0.46***</td>
<td>-0.25</td>
</tr>
<tr>
<td>R</td>
<td>-0.43**</td>
<td>-0.07</td>
</tr>
<tr>
<td>Apo-B</td>
<td>-0.31*</td>
<td>-0.03</td>
</tr>
<tr>
<td>R</td>
<td>-0.32*</td>
<td>0.08</td>
</tr>
<tr>
<td>HDL-C</td>
<td>-0.55***</td>
<td>-0.31*</td>
</tr>
<tr>
<td>R</td>
<td>-0.47**</td>
<td>-0.13</td>
</tr>
<tr>
<td>TG</td>
<td>0.51***</td>
<td>0.52***</td>
</tr>
<tr>
<td>R</td>
<td>0.40***</td>
<td>0.39***</td>
</tr>
</tbody>
</table>

*The model used in multiple regression analysis contained both FT4 and TSH-R-ab as independent variables. TC = total cholesterol; TG = triglycerides.
*p < 0.05. **p < 0.005 and ***p < 0.001.

To assess which thyroid factors (i.e. FT4 or TSH-R-ab) were related to TG, TC, LDL-C, apo-B or HDL-C, we first carried out several simple correlation analyses between FT4 or TSH-R-ab and each of these lipid parameters. The level of significance was taken as p < 0.05. Next, we performed several multiple linear regression analysis using thyroid factors (i.e. FT4 and TSH-R-ab) as independent variables and each of the lipid parameters as a dependent variable. The level of significance was taken as p < 0.005, according to Bonferroni's correction. A simple positive correlation was found between FT4 levels and TG values (r = 0.51, p < 0.001) but also between TSH-R-ab levels and TG values (r = 0.52, p < 0.001). The correlation between TSH-R-ab levels and FT4 values made it difficult to assess the independent relationship of each of these two thyroid factors to TG. Multiple regression analysis revealed that the relation between FT4 and TG was independent of TSH-R-ab (r = 0.40, p < 0.004, when controlling for the influence of TSH-R-ab). Likewise, this procedure indicated also that the relation between TSH-R-ab and TG was independent of FT4 (r = 0.39, p < 0.004, when controlling for the influence of FT4).

Thus, both FT4 and TSH-R-ab are related independently of TG. Furthermore, simple negative correlations (p < 0.05) were found between FT4 levels and TC, LDL-C, apo-B or HDL-C values. The TSH-R-ab levels correlated (p < 0.05) negatively only to HDL-C values. Multiple regression analysis revealed that while FT4 is a strong predictor (p < 0.005) of TC, LDL-C or HDL-C, TSH-R-ab are not. FT4 is a poor predictor (p < 0.05) of apo-B whereas TSH-R-ab are not associated with this apoprotein. The apparent simple positive relationship between TSH-R-ab and HDL-C results from the positive correlation between TSH-R-ab and FT4. Thus, in contrast

![Graph 1](image1.png)
![Graph 2](image2.png)

**Fig. 1.** Simple correlations between plasma TSH-R-ab levels and plasma FT4 values (r = 0.40, p < 0.01) (upper panel) and between plasma triglyceride (TG) levels and FT4 (r = 0.51, p < 0.001) (lower left panel) or TSH-R-ab (r = 0.52, p < 0.001) (lower right panel) values in adult women with Graves' disease and positive for TSH-R-ab. For partial correlations between these parameters, see Table 2.
to TSH-R-ab, FT₄ is directly and independently related to TC, LDL-C and HDL-C.

Discussion
Untreated patients with Graves' disease provide a study model of the effects of factors other than TSH on lipolysis. Our in vivo study has been performed in adult women with normal BMI and blood glucose level. Their liver and renal functions were also normal. None had increased alcohol consumption or a family history of hyperlipoproteinemia. Thus, the main factors which may induce dyslipoproteinemia were excluded, except from thyroid hormone excess. It is well known that thyroid hormone excess increases TG lipolysis in adipose tissue, leading to increased hepatic VLDL-TG synthesis and release (1–5). On the other hand, TSH receptors have been detected in fat cells but not in liver cells from rats (17). Stimulation of adipocytes with TSH or stimulating TSH-R-ab increase the cAMP production through enhanced TSH-R activity (17), leading to TG catabolism (23, 24). In contrast, blocking TSH-R-ab decrease the TSH-induced lipolysis in a dose-dependent manner (24). Our data show that the mean TG level is slightly increased in untreated Graves' patients positive for TSH-R-ab, as reported previously (1). However, individual plasma TG levels remained within the normal range in most patients. This result may be related to the increase in both hepatic TG synthesis and removal, as reported previously in hyperthyroid patients (1). Increased TG removal could be proportionally lower than enhanced TG synthesis when FT₄ levels are sharply increasing. Furthermore, our study indicates that both FT₄ and TSH-R-ab are independently associated with TG, suggesting that FT₄ but also TSH-R-ab play a role in TG metabolism. Indeed, when plasma FT₄ levels but also TSH-R-ab values were enhanced, plasma TG levels gradually increased. Stimulating TSH-R-ab effect on the serum TG level may be related to their action through the TSH-R pathway on adipose tissue (17). However, the presence of functional TSH-R on hepatic cells remains unknown in humans. Interestingly, it has been reported that serum IgG fractions from patients with Graves' disease induce a rise in fat cell cAMP production that is 20-fold higher than that observed with IgG from controls (17). Further studies are necessary to learn whether increased TSH levels act on TG metabolism in patients with primary hypothyroidism and negative for blocking TSH-R-ab.

Finally, our data show that the mean TC, LDL-C, apo-B and HDL-C levels are decreased in hyperthyroid patients positive for TSH-R-ab, in agreement with previous reports (9, 10, 13–15). Furthermore, our results confirm that thyroid hormones are involved in cholesterol metabolism. Indeed, negative correlations were found between FT₄ and TC, LDL-C or HDL-C. Thyroid hormones have been reported to increase LDL receptor-mediated clearance of LDL, especially at the hepatic level, thus lowering plasma LDL-C concentrations (12). Furthermore, it has been shown that the post-heparin plasma lipoprotein hepatic lipase activity is increased in hyperthyroidism, leading to decreased plasma (HDL₂-C) levels (13). Moreover, thyroid hormones reduce plasma TC concentrations through increased hepatic catabolism of cholesterol (15). In contrast to thyroid hormones, stimulating TSH-R-ab seem not to play a role in cholesterol metabolism. Indeed, no correlation was found between TSH-R-ab and TC or LDL-C. Moreover, the apparent relationship between TSH-R-ab and HDL-C results from the significant correlation between TSH-R-ab and FT₄. Interestingly, functional TSH receptors have not been detected in rat liver tissue (17). Further studies in humans are necessary to learn whether functional TSH receptors are present in hepatic and other tissues related to cholesterol metabolism.

In summary, this study suggest that stimulating TSH-R-ab are involved in TG metabolism. In contrast to thyroid hormones, these antibodies seem not to be related to cholesterol metabolism. Direct evidence for relationships between stimulating TSH-R-ab and lipids or lipoproteins requires further studies.

References

Received January 4th, 1996
Accepted March 25th, 1996