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Low normal thyroid function enhances plasma cholesteryl ester transfer in Type 2 diabetes mellitus

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ABSTRACT

Background: Plasma cholesteryl ester transfer (CET), reflecting endogenous transfer of cholesteryl esters from HDL to very low and low density lipoproteins, is elevated in Type 2 diabetes mellitus (T2DM), and may predict (subclinical) cardiovascular disease. Low normal thyroid function may adversely affect lipoprotein metabolism and atherosclerosis development. We tested whether plasma CET is related to thyroid function in euthyroid T2DM and non-diabetic subjects.
Subjects and methods: Plasma CET was measured in 74 T2DM and 82 non-diabetic subjects with thyroid-stimulating hormone (TSH) and free thyroxine levels within the reference range.
Results: Plasma CET was 20% higher in T2DM ($P = 0.003$) coinciding higher cholesteryl ester transfer protein (CETP) mass ($P = 0.009$) and triglycerides ($P = 0.02$). In univariate analysis, plasma CET was correlated positively with TSH in T2DM only ($r = 0.330$, $P = 0.004$). Multiple linear regression analysis revealed a positive interaction between the presence of T2DM and TSH on plasma CET after controlling for age, sex, body mass index, non-HDL cholesterol, triglycerides and CETP mass ($\beta = 0.167$, $P = 0.030$). The relationship of plasma CET with TSH was also positively modified by plasma glucose and HbA1c (interaction terms: $\beta = 0.119$, $P = 0.036$, $\beta = 0.170$, $P = 0.001$, respectively). Additionally, plasma triglycerides interacted positively with TSH on plasma CET in T2DM ($\beta = 0.198$, $P = 0.011$).
Conclusions: Low normal thyroid function, as inferred from higher TSH, confers increased plasma CET in the context of chronic hyperglycemia. Effects of thyroid function on plasma CET may be particularly relevant in hypertriglyceridemic T2DM. Low normal thyroid function could influence atherosclerosis susceptibility in T2DM by affecting the plasma cholesteryl ester transfer process.

1. Introduction

The impact of thyroid dysfunction on the development of cardiovascular disease is receiving continued interest [1]. It has been variably documented that subclinical hypothyroidism confers increased atherosclerosis susceptibility [2–5]. In the same vein, the concept is now emerging that higher thyroid-stimulating hormone (TSH) and lower thyroid hormone levels within the euthyroid range may adversely affect (subclinical) atherosclerosis [6–8]. Low normal thyroid function may also relate to higher plasma levels of apolipoprotein B-(apoB)-containing lipoproteins, as well as to their atherogenic potential, as evidenced by higher levels of oxidized low density lipoproteins (LDL) [9–12].

The cholesteryl ester transfer protein (CETP)-mediated process of cholesteryl ester transfer (CET), which enables the transport of cholesteryl esters from high density lipoproteins (HDL) to very low and low density lipoproteins (VLDL and LDL), provides a pathophysiologically relevant metabolic intermediate between a high cholesterol content in VLDL and LDL and a low high density lipoprotein (HDL) cholesterol concentration [13]. Importantly, high plasma CET is a determinant of intima media thickness, an established biomarker of subclinical atherosclerosis [14], predicts incident cardiovascular disease even independent of plasma lipoproteins and CETP mass [15], and relates to young age at presentation of myocardial infarction [16]. Of further note, plasma CET is to an important extent modified by overt thyroid dysfunction [17], as evidenced by decreased plasma CET rates and CETP levels in hypothyroidism [18,19].

It is well recognized that plasma CET is elevated in Type 2 diabetes mellitus (T2DM) [13,20–22]. Abnormalities in the concentration and the composition of VLDL and LDL, i.e. lipoproteins that are able to

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accept cholesteryl esters from HDL, have been repeatedly reported to be primarily responsible for accelerated plasma CET rates in T2DM [13,20]. In line, we and others have documented that elevated plasma CET in T2DM is to a considerable extent attributable to higher triglycerides [21,22]. Despite the relevance of both thyroid dysfunction and T2DM for accelerated plasma CET, no data are available concerning elevated plasma CET in relation to low normal thyroid function among euthyroid subjects with and without T2DM. In view of accelerated plasma CET in T2DM as well as in overt hypothyroidism, we tested the hypothesis that low normal thyroid function would specifically confer higher plasma CET in T2DM.

The present study was, therefore, initiated to determine the extent to which thyroid functional status modifies plasma CET in euthyroid subjects with and without T2DM. Additionally, we assessed the contribution of high plasma triglycerides to accelerated plasma CET in T2DM in relation to low normal thyroid function.

2. Materials and methods

2.1. Subjects

The study was approved by the medical ethics committee of the University Medical Center Groningen, The Netherlands. All participants were aged >18 years, and had a Caucasian background. They were recruited by advertisement in local newspapers. Written informed consent was obtained from all of them. Eligible subjects had a negative history of thyroid disease, did not show a thyroid abnormality on physical examination, had a serum TSH and a serum free thyroxine (T_4) level within the institutional reference range, and did not have elevated anti-thyroid peroxidase (anti-TPO) and anti-thyroglobulin (anti-Tg) auto-antibodies (see below). T2DM had been previously diagnosed by primary care physicians using guidelines from the Dutch College of General Practitioners (fasting plasma glucose ≥7.0 mmol/l and/or non-fasting plasma glucose ≥11.1 mmol/l). T2DM patients did not attend the outpatient clinic or our University Diabetic Clinic, but were treated by their primary care physicians with diet alone or diet in combination with metformin and/or sulfonylurea. The use of anti-hypertensive medication was allowed, but patients using insulin were not eligible. Subjects who used lipid lowering drugs were excluded in order to minimize possible confounding on lipid metabolism. Renal function abnormalities (elevated serum creatinine and/or elevated urinary albumin excretion), liver function abnormalities (transaminase levels >1.5 times the upper normal level), clinically manifest cardiovascular disease and pregnancy were also exclusion criteria. Current smokers and subjects who used >3 alcoholic drinks per day were also not allowed to participate. A fasting plasma triglyceride level ≥1.70 mmol/l was considered elevated applying NCEP-ATPIII criteria [23].

BMI (in kg/m^2) was calculated as weight divided by height squared. After 15 min rest, systolic and diastolic blood pressure was measured 3 times with 5 min intervals with a digital sphygmomanometer in the supine position. The study subjects were evaluated between 0800 and 1000 h after an overnight fast.

2.2. Laboratory analyses

Serum and EDTA-anticoagulated plasma samples were stored at −80 °C until analysis. Plasma glucose was measured shortly after blood collection.

Serum TSH (sandwich principle; Roche Diagnostics GmbH., Mannheim Germany, cat. no. 117314591; reference range 0.5–4.0 mU/l) and free T_4 (competition principle; Roche Diagnostics GmbH., Mannheim Germany, cat. no. 11731297; reference range 11.0–19.5 pmol/l) were measured by electrochemiluminescence immunoassay using a Modular Analytics immunoassay analyzer. Anti-TPO and anti-Tg auto-antibodies were determined using commercially available automated enzyme-linked immunoassays (ImmunoCap cat nos. 14-4508-35 and 14-4507-35, respectively, Phadia, Freiburg, Germany). Thyroid auto-antibodies were considered to be positive using cut-off values as indicated by the supplier (anti-TPO auto-antibodies >60 IU/ml and anti-TG auto-antibodies >289 IU/ml, respectively).

Plasma cholesterol and triglycerides were assayed by enzymatic methods (Roche/Hitachi cat. nos. 11876023 and 11875540 respectively, Roche Diagnostics GmbH, Mannheim, Germany). HDL cholesterol was measured using a homogeneous enzymatic colorimetric test (Roche/Hitachi cat. no. 03030024). Non-HDL cholesterol was calculated as the difference between plasma total cholesterol and HDL cholesterol. LDL cholesterol was calculated by the Friedewald formula (only if plasma triglycerides were <4.5 mmol/l).

Plasma CET was assayed by a radioisotope method exactly as outlined previously [14]. Briefly, [^{3}H] cholesterol was equilibrated for 24 h with plasma cholesterol at 4 °C, followed by incubation at 37 °C for 3 h. ApoB-containing lipoproteins were then precipitated by the addition of phosphotungstate/$MgCl_2$. Lipids were extracted from the precipitate, and the labeled cholesteryl esters were separated from labeled unesterified cholesterol on silica columns. This isotope method is an accurate measure of net mass transfer of cholesteryl esters from HDL to VLDL + LDL [22]. All assays were performed in duplicate. The CET measurements are expressed in nanomoles per ml per hour. Plasma CETP mass was measured with a double-antibody sandwich ELISA [24]. A combination of monoclonal antibodies TP1 and TP2 was employed as coating antibodies, and monoclonal antibody TP20, labeled with digoxigenine, was the secondary antibody. The CETP control samples were validated using a radioimmunoassay (carried out by Dr. R. M. McPherson, Montreal, Canada). The plasma CETP concentration is closely correlated with the CETP activity level measured using an excess exogenous substrate assay [25]. The CET and CETP mass measurements were carried out in duplicate. The intra-assay coefficients of variation of the plasma CET and CETP mass assays were 7.1% and 7.8%, respectively.

2.3. Statistical analysis

SPSS 20 was used for data analysis. Results are expressed as mean ± SD or as median (interquartile range). Differences between diabetic and non-diabetic subjects were determined by unpaired T, Mann–Whitney U and Chi-square tests where appropriate. Differences in variables between diabetic and non-diabetic subjects were also evaluated after adjustment for age and sex. Differences in plasma CET according to tertiles of TSH were determined by analysis of variance with polynomial contrast. Univariate relationships were calculated using Pearson correlation coefficients. Triglycerides were logarithmically transformed because of skewed distribution. Multiple linear regression analyses were carried out to disclose those variables that independently contributed to plasma CET. Interaction terms were calculated as the product terms between diabetes status and TSH, and in alternative analyses between plasma glucose or HbA1c and TSH. Additionally, interaction terms between triglycerides and TSH were calculated. For continuous variables (TSH, plasma glucose, HbA1c and triglycerides) distributions centered to the mean were made by subtracting the individual value of the variable of interest from their group mean values to account for possible outliers. Interaction terms were considered to be statistically significant at two-sided P-values <0.10 [26]. Otherwise, the level of significance was set at two-sided P-values <0.05.

3. Results

The study population consisted of 74 subjects with previously diagnosed T2DM (median diabetes duration: 5.5 (range 0.2–13)