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Low normal thyroid function as a determinant of increased large very low density lipoprotein particles

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ABSTRACT

Objectives: Low-normal thyroid function may relate to increases in plasma cholesterol and triglycerides, but effects on lipoprotein subfractions are largely unknown. Associations of alterations in lipoprotein metabolism and functionality with low-normal thyroid function could be more pronounced in Type 2 diabetes mellitus (T2DM). We determined relationships of plasma lipids and lipoprotein subfractions with thyroid-stimulating hormone (TSH) and free thyroxine (free T_4) in euthyroid subjects, and assessed whether such relationships are modified in the context of T2DM.

Design and methods: TSH, free T_4, (apo)lipoproteins and lipoprotein subfractions (nuclear magnetic resonance spectroscopy) were measured after an overnight fast in 61 T2DM subjects and 52 non-diabetic subjects.

Results: TSH and free T_4 were similar in T2DM and non-diabetic subjects. Plasma triglycerides, large very low density (VLDL) particles, VLDL size and small low density lipoprotein (LDL) particles were increased, whereas high density lipoprotein (HDL) cholesterol was decreased in T2DM subjects ($p \leq 0.05$ for each). Age-, sex-, and diabetes status-adjusted multivariable linear regression analysis demonstrated that plasma triglycerides were associated positively with TSH ($\beta = 0.196$, $p = 0.039$). Large VLDL particles ($\beta = -0.215$, $p = 0.020$) and VLDL size were inversely associated with free T_4 ($\beta = -0.285$, $p < 0.001$). These relationships were not significantly modified by diabetes status (interaction terms: $p > 0.10$ for each). In all subjects combined, LDL and HDL subfraction characteristics were not significantly related to thyroid function status.

Conclusions: Low-normal thyroid function may confer increased plasma triglycerides, large VLDL particles and increased VLDL particle size. These relationships are not to a major extent modified in the context of T2DM.

Introduction

The high prevalence of thyroid function abnormalities in the general population provides a rationale to determine the consequences of mild thyroid dysfunction for a number of health issues including cardio-metabolic disorders [1–4]. Each person probably has a rather narrow individual set-point of thyroid function status [5]. It is, therefore, likely that single measurements of circulating thyroid-stimulating hormone (TSH) and thyroid hormones provide relevant information regarding the relationship of thyroid function with cardiovascular and metabolic biomarkers [3].

It is important that low-normal thyroid function, as reflected by higher TSH and/or lower thyroid hormone levels within the euthyroid range, probably confers higher plasma total cholesterol, triglycerides and apolipoprotein B (apoB) concentrations [6–10]. The concept is also emerging that low-normal thyroid function could adversely affect atherosclerosis susceptibility [11–13], although this issue has not been unequivocally settled at present.

In subclinical hypothyroidism the secretion of large very low density lipoprotein (VLDL) particles by the liver has been reported to be increased [14], whereas plasma triglyceride clearance is likely to be unaltered [14,15]. Little is currently known about the effect of low-normal thyroid function on lipoprotein subfraction levels. All major lipoprotein fractions are highly heterogeneous in size, structure and composition, which may have implications of their measurement for improved prediction of cardio-metabolic disorders [16–18]. We have recently observed that the putative effects low-normal thyroid function on several cardio-metabolic biomarkers, i.e., an increase in the plasma cholesteryl ester transfer process by which cholesteryl esters are transferred from HDL towards triglyceride-rich lipoproteins and a decreased ability of high density lipoproteins (HDL) to protect oxidative modification of LDL in vitro are more pronounced in the context of Type 2 diabetes mellitus (T2DM) [19,20]. Additionally, low-normal thyroid function may confer decreased circulating levels of the natural anti-oxidant, bilirubin in T2DM and in insulin resistant individuals [21,22]. Such possible effect modification of chronic hyperglycemia or insulin resistance on the relationship of a number of cardio-metabolic biomarkers with low-

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normal thyroid function makes it relevant to assess whether the association of lipoprotein subfraction distribution with thyroid function varies according to diabetes status.

The present study was initiated to i) evaluate in subjects with and without T2DM whether low-normal thyroid function confers altered lipoprotein subfraction levels, measured by nuclear magnetic resonance (NMR) spectroscopy, and ii) to determine the extent to which such possible relationships are modified in T2DM.

Materials and methods

Subjects

The study was performed in a University Hospital setting, and was approved by the medical ethics committee of the University Medical Center Groningen, The Netherlands. Caucasian participants (aged > 18 years) were recruited by advertisement, and provided written informed consent. T2DM was 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 had been given dietary advice. T2DM patients who were treated with metformin and/or sulfonylurea were eligible, but patients using other glucose lowering drugs and/or insulin were not allowed to participate. The use of anti-hypertensive medication was allowed. Eligible subjects had a serum TSH as well as a serum free thyroxine (free T_4) level within the institutional reference range (see below). Additional exclusion criteria were clinically manifest cardiovascular disease, renal insufficiency (elevated serum creatinine and/or urinary albumin > 20 mg/L), liver disease (serum transaminase levels > 2 times the upper reference limit), pregnancy and use of lipid lowering drugs. Subjects who used other medications (except for oral contraceptives), current smokers and subjects who used >3 alcoholic drinks daily (one drink was assumed to contain 10 g of alcohol) were also excluded.

Physical examination did not reveal pulmonary or cardiac abnormalities. Body mass index was calculated as weight (kg) divided by height (m) squared. Waist circumference was measured on the bare skin between the 10th rib and iliac crest. Blood pressure was measured after 15 min of rest at the left arm using a sphygmomanometer. The participants were evaluated between 0800 and 1000 h after an overnight fast.

Laboratory analyses

Serum and EDTA-anticoagulated plasma samples were stored at −80 °C until analysis. Plasma glucose and glycated hemoglobin (HbA1c) levels were 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 thyroxine (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.

Plasma total cholesterol and triglycerides were assayed by routine enzymatic methods (Roche/Hitachi cat nos 11875540 and 11876023, respectively; Roche Diagnostics GmbH, Mannheim, Germany). HDL cholesterol was measured with a homogeneous enzymatic colorimetric test (Roche/Hitachi, cat no 04713214; Roche Diagnostics GmbH, Mannheim, Germany). Non-HDL cholesterol was calculated as the difference between total cholesterol and HDL cholesterol. Low density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula if the triglyceride concentration was <4.5 mmol/L. ApoA-I and apoB were assayed by immunoturbidimetry (Roche/Cobas Integra Tina-quant catalog no. 03032566 and 033032574, respectively, Roche Diagnostics).

VLDL, LDL and HDL particle profiles were measured by nuclear magnetic resonance (NMR) spectroscopy with the LipoProfile-3 algorithm (LipoScience Inc., Raleigh, North Carolina, USA), as described [28]. VLDL, LDL and HDL subclasses were quantified from the amplitudes of their spectroscopically distinct lipid methyl group NMR signals, and were expressed in concentration units, i.e., μmol/L or nmol/L. The lipoprotein subfraction particle concentrations are considered to represent an estimate of the respective lipoprotein particle numbers [21]. Diameter range estimates were for VLDL: large VLDL (including chylomicrons if present; >60 nm), medium VLDL (35 to 60 nm) and small VLDL (27 to 35 nm), for LDL: IDL (23 to 27 nm), large LDL (21.2 to 23 nm) and small LDL (18 to 21.2 nm), and for HDL: large HDL particles: 9.4 to 14 nm; medium HDL particles: 8.2 to 9.4 nm; small HDL particles: 7.3–8.2 nm. The VLDL, LDL and HDL particle concentrations were calculated as the sum of the respective lipoprotein subclasses. Weighted-average VLDL, LDL and HDL sizes were derived from the sum of the diameter of each subclass multiplied by its relative mass percentage based on the amplitude of its methyl NMR signal [23].

Serum aminotransferase (ALT) was measured with pyridoxal phosphate activation (Merck MEGA, Darmstadt, Germany). Standardization was performed according to International Federation of Clinical Chemistry guidelines. The upper reference value applied for ALT was 30 U/L. Glucose was analyzed with an APEC glucose analyzer (APEC Inc., Danvers, MA). HbA1c was measured by high-performance liquid chromatography (Bio-Rad, Veenendaal, the Netherlands; normal range: 27–43 mmol/mol). Plasma non-esterified fatty acids (NEFA) were measured using an enzymatic colorimetric method (Wako Chemicals, Neuss, Germany, cat no 43691995).

The intra-assay and inter-assay coefficients of variation of TSH, free T_4, ALT, NEFA, lipids, (apo)lipoproteins, and VLDL, LDL and HDL subfraction measurements were ≤7% and ≤8%, respectively.

Statistical analysis

SPSS 20 (version 20.0, SPSS Inc., Chicago, IL, USA) was used for data analysis. Data are expressed as means ± SD, medians (interquartile ranges) or in numbers. Differences between subjects with and without T2DM were determined by unpaired T-tests, Mann–Whitney U tests or Chi-square tests where appropriate.

Serum TSH and free T_4 levels were normally distributed (Kolgomorov–Smirnov test: $p = 0.74$ and $p = 0.31$, respectively). Since triglycerides, several lipoprotein subfraction characteristics and ALT were not parametrically distributed, these variables were logarithmically transformed for correlation analyses. Univariate relationships were calculated using Pearson correlation coefficients.

Multivariable linear regression analyses were carried out to disclose the independent relationships of plasma (apo)lipoproteins and lipoprotein subfraction characteristics with thyroid function parameters. In addition, multivariable linear regression analyses were performed to determine interactions of diabetes status with thyroid function parameters impacting on plasma (apo)lipoproteins and lipoprotein subfraction characteristics. Interaction terms were calculated as the product terms of TSH or free T_4 with the presence of T2DM. To account for possible outliers the distributions of TSH and free T_4 were centered to their mean value by subtracting the individual value from the group mean value. Interaction terms were considered to be statistically significant at two-sided p-values < 0.10 [24,25]. Otherwise, the level of significance was set at two-sided p-values < 0.05.

Results

Out of 133 potentially eligible subjects, 61 T2DM patients and 52 non-diabetic control subjects were included in the study (Table 1). Ten subjects were excluded because of either a TSH or a free T_4 outside the reference range. In T2DM subjects diabetes duration was 5.0 (4.0–6.4) years. Fourteen T2DM patients used metformin and 11 patients