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Clinical Research Article

Higher Free Triiodothyronine Is Associated With Higher HDL Particle Concentration and Smaller HDL Particle Size

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Abstract

Context: Thyroid function status has effects on the development of atherosclerotic cardiovascular disease by affecting lipid metabolism, but associations of high-density lipoprotein (HDL) particle concentrations and subfractions with thyroid hormone levels within the reference range remain elusive.

Objective: The aim of the present study was to determine the associations of free triiodothyronine (FT3), free thyroxine (FT4) and thyroid-stimulating hormone (TSH) levels with HDL particle characteristics in euthyroid individuals.

Methods: This cross-sectional study on the associations of thyroid hormones with HDL particle concentrations, HDL subfractions, and HDL particle size included 5844 euthyroid individuals (FT3, FT4, and TSH levels within the reference range and no medication use affecting thyroid function), participating in the Prevention of REnal and Vascular ENd-stage Disease (PREVEND) study. HDL particles and subfractions were measured by nuclear magnetic resonance using an optimized version of the NMR LipoProfile Test (LP4).

Results: In multivariable linear regression analyses, FT3 was positively associated with total HDL particle concentration (std.β = 0.14; $P < 0.001$) and with small (std.β = 0.13; $P < 0.001$) and medium-sized HDL particles (std.β = 0.05; $P = 0.001$). Conversely, FT3 was inversely associated with large HDL particles (std.β = −0.07; $P < 0.001$) and with HDL particle size (std.β = −0.08; $P < 0.001$). Such associations with FT4 or reciprocally with TSH were less pronounced or nonsignificant.

Conclusion: In euthyroid individuals, higher FT3 is cross-sectionally associated with higher total HDL particle concentration and with lower HDL particle size. These associations may be relevant to better understand the role of HDL in thyroid function–associated atherosclerotic cardiovascular disease.

Key Words: thyroid hormones, HDL cholesterol, HDL particles, general population

Abbreviations: ApoA1, apolipoprotein A1; ApoB, apolipoprotein B; BMI, body mass index; CETP, cholesterol ester transfer protein; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; FT3, free triiodothyronine; FT4, free thyroxine; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NMR, nuclear magnetic resonance; PREVEND, Prevention of REnal and Vascular ENd-stage Disease; T3, triiodothyronine; T4, thyroxine; TPO, thyroid peroxidase; TSH, thyrotropin (thyroid-stimulating hormone); VLDL, very-low-density lipoprotein.

The importance of thyroid hormones in maintaining cardiovascular homeostasis is inferred from clinical and experimental studies demonstrating that even subtle changes in thyroid hormone concentrations can predispose to major health issues, including an increased risk of cardiovascular disease (CVD) (1). Variation in thyroid hormones, and even variation of these hormones within the reference range, is known to have multiple effects on lipid metabolism, including (apo)lipoprotein synthesis, lipoprotein catabolism, and lipid mobilization from fat tissue (2-12). For example, low-normal thyroid function as determined by thyrotropin (thyroid-stimulating hormone [TSH]) levels in the upper reference range and free thyroxine (FT4) levels in the lower reference range are associated with higher plasma concentrations of total cholesterol and low-density lipoprotein (LDL) cholesterol (13, 14). Remarkably, the association between thyroid hormones and the plasma high-density lipoprotein (HDL) cholesterol concentration remains elusive, with increased, normal, and decreased HDL cholesterol having been described in both hypothyroidism and hyperthyroidism (15-18). In euthyroid Chinese individuals, higher free triiodothyronine (FT3) was related to lower HDL cholesterol (19).

Notably, using novel techniques to measure HDL particle characteristics, evidence is accumulating that the inverse association of the plasma HDL particle concentration with incident atherosclerotic CVD is stronger than that of HDL cholesterol (20). Importantly, HDL particles vary considerably in size and cholesterol content (21-23). Moreover, profound short-term hypothyroidism results in increased HDL cholesterol and larger HDL particles, without an effect on the total HDL particle concentration, corroborating the observation that the cholesterol content of HDL particles may change drastically in the context of thyroid dysfunction (24).

The association of variations in thyroid hormone status within the reference range with HDL subfractions and HDL size, as determined by novel nuclear magnetic resonance

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(NMR)-based techniques, is currently unknown. It is well established that thyroid function status profoundly affects the metabolism of apolipoprotein A1 (ApoA1), the major apolipoprotein constituent of HDL, with increased (hepatic) synthesis being observed in response to triiodothyronine (T3) administration (25, 26). We, therefore, hypothesized that high normal thyroid function, in particular evidenced by FT3 levels in the upper reference range, may be associated with higher HDL particle concentrations and alterations in HDL subfraction distribution beyond possible changes in HDL cholesterol concentration per se. The aim of the present study was to cross-sectionally determine the associations of thyroid function status with HDL particle concentrations, HDL subfractions, and HDL size in euthyroid individuals recruited from the general population.

Methods

Study Design and Participants

This cross-sectional study was conducted as part of the Prevention of REnal and Vascular ENd-stage Disease (PREVEND) study, a prospective Dutch cohort study among inhabitants, aged from 28 to 75 years, of the city Groningen, The Netherlands. The details of the study design and recruitment have been described before (27). Based on the importance of variations in thyroid function in the euthyroid range for a variety of health issues, including (subclinical) atherosclerosis and altered levels of atherogenic biochemical markers (11), our study only included euthyroid individuals, defined as plasma concentrations of free triiodothyronine (FT3, reference range, 3.1-6.8 pmol/L), free thyroxine (FT4, reference range, 12-22 pmol/L) and thyroid-stimulating hormone (TSH, reference range, 0.27-4.20 mIU/L) that were all within their respective reference ranges. We excluded noneuthyroid individuals and individuals using thyroid hormones, antithyroid drugs, amiodarone, and lithium carbonate. Consequently, a total of 5844 individuals were included for the current study. Detailed information on the flow of participants through the study is provided in Supplemental Figure S1 (28). The PREVEND study has been approved by the local medical ethics committee (approval number: MEC96/01/022) and was undertaken in accordance with the Declaration of Helsinki. All participants provided written informed consent.

Self-administered questionnaires concerning demographics, alcohol intake, smoking habits, and medication use were provided by all participants prior to the baseline visit. History of previous cardiovascular disease (CVD) (hospitalization for myocardial infarction, revascularization procedures, or obstructive coronary artery disease) was acquired from the questionnaires. Information on medication use was combined with information from IADB.nl, a database containing information of prescribed medication in public pharmacies in The Netherlands since 1999 (http://www.iadb.nl/). Height and weight were measured with the participants standing without shoes and heavy outer garments. Body mass index (BMI) was calculated by dividing weight (in kilograms) by height (in meters, squared). Type 2 diabetes was defined as a fasting serum glucose concentration > 7.0 mmol/L, a nonfasting serum glucose concentration > 11.1 mmol/L, a self-report of a physician diagnosis, or the use of glucose-lowering drugs.

Laboratory Measurements

Baseline venous blood samples were drawn between 8:00 and 10:00 am from all participants after an instructed overnight fast. Aliquots of ethylenediaminetetraacetic acid (EDTA) plasma were obtained by centrifugation, and samples were immediately frozen at −80 °C until analysis.

Lipoprotein parameters were measured by NMR spectroscopy at Labcorp on plasma EDTA specimens (22, 29). Total cholesterol, very-low-density (VLDL) cholesterol, HDL cholesterol, triglycerides, apolipoprotein B (ApoB) and ApoA1 were measured using an optimized version of the NMR LipoProfile Test, named LP4 (23, 30). LDL cholesterol (LDL-C) was calculated using the Friedewald equation. Non-HDL cholesterol was calculated as total cholesterol minus HDL cholesterol. For quantification of HDL particles, each subfraction NMR signal comes from the aggregate number of terminal methyl groups of lipids within the particles (ie, cholesteryl esters and triglycerides in the particle core, and phospholipids and unesterified cholesterol in the surface shell). To a close approximation, the number of methyl groups in a particle of a given size is unaffected by the lipid compositional variation. Thus, the signal amplitude can provide subclass particle quantification. The value for total HDL particles (HDL-P) was calculated by the sum of the concentrations of small, medium, and large HDL particles. Mean HDL size was calculated using the weighted averages derived from the sum of the diameters of small, medium, and large HDL particles multiplied by its relative mass percentage. Estimated ranges of particle diameter for the subclasses were as follows: small HDL, 7.4 to 8.0 nm; medium HDL, 8.1 to 9.5 nm; and large HDL, 9.6 to 13 nm. Mean diameter for the HDL subfractions were as follows: H1P, 7.4 nm; H2P, 7.8 nm; H3P, 8.7 nm; H4P, 9.5 nm; H5P, 10.3 nm; H6P, 10.8 nm; and H7P, 12.0 nm. Small HDL comprises H1 and H2, medium HDL, H3 and H4, and large HDL, H5 to H7.

FT3, FT4, TSH, and antithyroid peroxidase measurements were performed in baseline fasting serum samples stockpiled at −80 °C using the Roche Modular E170 Analyzer electrochemiluminescent immunoassays (Roche Diagnostics, Mannheim, Germany). Samples had not been thawed before. An antithyroid peroxidase titer of > 34 kIU/L was considered positive. Fasting plasma glucose was measured in fresh blood specimens by dry chemistry (Eastman Kodak, Rochester, NY, USA). Serum creatinine was measured with an enzymatic method on a Roche Modular analyzer, using reagents and calibrators from Roche (Roche Diagnostics, Mannheim, Germany). Serum cystatin C was measured by Gentian Cystatin C Immunoassay (Gentian AS, Moss, Norway) on a Modular analyzer (Roche Diagnostics). Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) combined creatinine–cystatin C equation (31). Urinary albumin was measured by nephelometry (Dade Behring Diagnostic, Marburg, Germany).

Statistical Analyses

Statistical analyses were performed with R version 3.6.2 (Vienna, Austria) (http://cran.r-project.org/). Results were expressed as mean ± SD, median [interquartile range], or number (percentage) for normally distributed, skewed, and categorical data, respectively. A 2-sided P value < 0.05 was considered to indicate statistical significance. Comparisons

of baseline characteristics among sex-stratified tertiles of FT3, FT4, TSH, and total HDL particles concentrations were tested using the Chi-square test for categorical variables and 1-way ANOVA for continuous data. Linear regression analyses were used to investigate the associations of FT3, FT4, and TSH with lipoproteins, HDL particle concentrations, and HDL subfractions. The assumption of normally distributed error terms was validated by inspection of Q-Q plots of the residuals. Regression coefficients were presented as standardized beta values (Std. β), referring to the number of standard deviations a dependent variable changes per standard deviation increase in the independent variable. Adjustments were made for a priori selected potential confounders, including age, sex, waist circumference, alcohol intake, smoking status, type 2 diabetes, urinary albumin excretion, eGFR, triglycerides, usage of antihypertensives, usage of lipid-lowering drugs, usage of glucose-lowering drugs, and history of CVD. Furthermore, we also adjusted for baseline characteristics that significantly differed across sex-stratified tertiles FT3, namely, positive anti–thyroid peroxidase (anti-TPO) antibodies and LDL cholesterol. The variance inflation factor was assessed in all models to investigate multicollinearity among covariables. The maximal variance inflation factor was 2, indicative of a low degree of multicollinearity. Sensitivity analyses were performed by excluding participants with type 2 diabetes, usage of antihypertensives, usage of lipid-lowering drugs, usage of glucose-lowering drugs, and participants with a history of CVD. Lastly, a sensitivity analysis was performed in which we did not exclude participants with FT3, FT4, or TSH outside of the euthyroid range but still excluding participants using thyroid hormones or thyroid function affecting medication.

The associations of FT3 with HDL cholesterol, total HDL particle concentration, and HDL particle size was graphically represented using both linear models and nonlinear models. The nonlinear models were based on a natural spline with 3 degrees of freedom. The reporting of the current study adheres to the STROBE guidelines.

Results

Baseline Characteristics

Among the 5844 participants, aged 56 ± 12 years, mean FT3, FT4, and TSH were 4.87 ± 0.52 pmol/L, 15.8 ± 1.87 pmol/L, and 1.72 ± 0.81 mIU/L, respectively. Mean HDL cholesterol was 1.32 ± 0.32 mmol/L, mean HDL particle concentration was 21.1 ± 2.73 μmol/L, and the mean HDL particle size was 8.96 ± 0.45 nm. Large, medium, and small HDL particle concentrations were 1.80 ± 1.28 μmol/L, 5.12 ± 2.18 μmol/L, and 14.20 ± 2.85 μmol/L, respectively. An overview of baseline characteristics according to sex-stratified tertiles of FT3 is shown in Table 1. Among sex-stratified tertiles of FT3, there were significant differences in age, BMI, alcohol intake, smoking status, type 2 diabetes, eGFR, history of CVD, and usage of antihypertensive drugs but not of lipid-lowering drugs. Overviews of baseline characteristics according to sex-stratified tertiles of FT4, TSH, and the total HDL particle concentration are shown in Supplemental Tables S1-S3 (28). Notably in the highest tertile of total HDL particle concentration, FT3 was higher but FT4 and TSH levels were not different. Furthermore, in the highest tertile of HDL particle concentration, waist circumference and BMI were the lowest.

Linear Regression Analyses of Thyroid Hormones With Conventional Lipids and (Apo)Lipoproteins

An overview of linear regression analyses of thyroid hormones with conventional lipids and lipoproteins is shown in Table 2. After adjusting for potential confounders, including age, sex, waist circumference, alcohol intake, smoking status, type 2 diabetes, urinary albumin excretion, eGFR, triglycerides (except for analyses with VLDL cholesterol and triglycerides as outcome variable), LDL cholesterol, usage of antihypertensives, usage of lipid-lowering drugs, usage of glucose-lowering drugs, history of CVD, and anti-TPO antibodies, higher FT3 levels were associated with lower total cholesterol, LDL cholesterol, and ApoB, and with higher triglycerides and ApoA1 (all $P < 0.05$). Notably, the association between FT3 and HDL cholesterol was nonsignificant after adjusting for potential confounders. In fully adjusted linear regression analyses, higher FT4 was associated with lower total cholesterol, VLDL cholesterol, and HDL cholesterol. TSH was positively associated with VLDL cholesterol, HDL cholesterol, triglycerides, and ApoA1.

Linear Regression Analyses of Thyroid Hormones With HDL Particles, HDL Subfractions, and HDL Size

An overview of linear regression analyses of thyroid hormones with HDL particle concentrations and HDL particle size is shown in Table 3. After adjusting for potential confounders (as done for conventional lipids and lipoproteins; Table 2), FT3 was positively associated with total HDL particle concentration and with small- and medium-sized HDL particles, but inversely with large HDL particles and with HDL particle size. These adjusted associations were similar when waist circumference as a measure of central obesity was replaced by BMI (data not shown). The robustness of these associations was demonstrated in several sensitivity analyses, where we excluded participants with type 2 diabetes, usage of antihypertensives, usage of lipid-lowering drugs, usage of glucose-lowering drugs and participants with a history of CVD (Supplemental Table S5 (28)). In each of the aforementioned sensitivity analyses, the associations of FT3 with HDL particle concentration and particle size remained materially unchanged.

A graphical representation of the linear associations of FT3 with HDL cholesterol, HDL particle concentration, and HDL particle size is shown in Fig. 1. A similar graphical representation using nonlinear terms (natural spline with 3 degrees of freedom) is demonstrated in Supplemental Figure S2 (28). There was no significant effect modification of age on the association between FT3 and HDL particle concentrations or HDL particle size ($P_{interaction}$ all > 0.05).

In fully adjusted linear regression analyses, FT4 was positively associated with small HDL particles, and inversely associated with medium HDL particles. FT4 was not significantly associated with total HDL particle concentration, large HDL particle concentration, and HDL particle size. In fully adjusted linear regression analyses, TSH was positively associated with large HDL particles and HDL particle size. TSH was not significantly associated with total HDL particle concentration. As inferred from the standardized beta coefficients in the fully adjusted analyses, the associations of FT3 with HDL particle characteristics were all stronger than those with FT4 and TSH.

Table 1. Clinical and laboratory characteristics according to sex-stratified tertiles of FT3 (n = 5844)

Variable	All participants	Tertile 1	Tertile 2	Tertile 3	*P* value
General characteristics					
Sex, n male (%)	3049 (52)	1016 (52)	1016 (52)	1017 (52)	1.000
Age, years	53.2 ± 12.0	55.9 ± 12.3	52.8 ± 11.7	51.0 ± 11.3	<0.001
Waist circumference, cm	92.1 ± 12.8	91.5 ± 13.0	92.0 ± 12.7	92.9 ± 12.7	0.003
BMI, kg/m^2	26.62 ± 4.33	26.30 ± 4.23	26.61 ± 4.29	26.95 ± 4.44	<0.001
Alcohol consumption					
No alcohol consumption	1413 (24)	433 (23)	495 (26)	485 (25)	0.001
Up to 1 consumption per day	2819 (49)	910 (47)	946 (49)	963 (50)	
More than 1 consumption per day	1555 (27)	583 (30)	488 (25)	484 (25)	
Current smoker, n (%)	1660 (29)	474 (25)	537 (28)	649 (34)	<0.001
Impaired fasting glucose, n (%)	657 (11)	201 (10)	221 (11)	235 (12)	0.23
Type 2 diabetes, n (%)	346 (6)	135 (7)	114 (6)	97 (5)	0.04
eGFR, mL/min/1.73m^2	92.7 ± 16.9	90.7 ± 18.3	93.2 ± 16.5	94.3 ± 15.6	<0.001
Urinary albumin excretion, mg/24h	8.6 [6.1, 15.7]	8.6 [6.0, 17.6]	8.4 [6.0, 15.0]	8.9 [6.3, 15.4]	0.19
Usage of lipid-lowering drugs, n (%)	445 (9)	143 (8)	153 (9)	149 (9)	0.78
Usage of antihypertensives, n (%)	1110 (22)	409 (24)	369 (22)	332 (20)	0.01
Use glucose-lowering drugs, n (%)	193 (3)	89 (5)	61 (3)	43 (2)	<0.001
History of cardiovascular events, n (%)	575 (10)	232 (12)	199 (10)	144 (7)	<0.001
Thyroid hormones					
TSH, mU/L	1.72 ± 0.81	1.73 ± 0.82	1.72 ± 0.81	1.70 ± 0.80	0.56
FT4, pmol/L	15.75 ± 1.87	15.21 ± 1.77	15.77 ± 1.83	16.28 ± 1.86	<0.001
FT3, pmol/L	4.87 ± 0.52	4.34 ± 0.28	4.85 ± 0.19	5.42 ± 0.34	<0.001
Positive anti-TPO antibodies, n (%)	450(8)	159 (8)	170 (9)	121 (6)	0.01
Conventional lipids and lipoprotein					
Total cholesterol, mmol/L	4.99 ± 0.90	5.04 ± 0.87	4.98 ± 0.91	4.96 ± 0.92	0.01
Non-HDL cholesterol, mmol/L	3.67 ± 0.93	3.70 ± 0.92	3.66 ± 0.94	3.65 ± 0.94	0.23
LDL cholesterol, mmol/L	2.94 ± 0.76	2.98 ± 0.75	2.92 ± 0.77	2.92 ± 0.76	0.01
VLDL cholesterol, mmol/L	0.73 ± 0.33	0.72 ± 0.32	0.74 ± 0.34	0.73 ± 0.33	0.07
HDL cholesterol, mmol/L	1.32 ± 0.32	1.34 ± 0.33	1.32 ± 0.31	1.31 ± 0.31	0.003
Triglycerides, mmol/L	1.07 [0.75, 1.58]	1.00 [0.72, 1.46]	1.08 [0.76, 1.63]	1.12 [0.79, 1.67]	<0.001
ApoB, g/L	0.91 ± 0.23	0.91 ± 0.23	0.90 ± 0.24	0.90 ± 0.23	0.39
ApoA1, g/L	1.31 ± 0.23	1.31 ± 0.23	1.30 ± 0.22	1.30 ± 0.22	0.98
HDL particles concentrations					
HDL particle concentration, µmol/L	21.13 ± 2.73	20.80 ± 2.79	21.19 ± 2.68	21.39 ± 2.69	<0.001
Large HDL, µmol/L	1.80 ± 1.28	2.01 ± 1.39	1.78 ± 1.24	1.60 ± 1.16	<0.001
Medium HDL, µmol/L	5.12 ± 2.18	5.07 ± 2.13	5.08 ± 2.19	5.20 ± 2.23	0.13
Small HDL, µmol/L	14.21 ± 2.85	13.72 ± 2.87	14.33 ± 2.82	14.59 ± 2.78	<0.001
HDL size, nm	8.96 ± 0.45	9.04 ± 0.48	8.95 ± 0.44	8.89 ± 0.42	<0.001
HDL particle subfractions					
H1P, µmol/L	3.47 [2.29, 4.67]	3.40 [2.20, 4.54]	3.54 [2.39, 4.77]	3.46 [2.28, 4.74]	0.001
H2P, µmol/L	10.59 [9.02, 12.22]	10.26 [8.71, 11.88]	10.57 [9.03, 12.25]	10.92 [9.46, 12.55]	<0.001
H3P, µmol/L	3.14 [1.93, 4.39]	3.12 [1.92, 4.38]	3.14 [1.90, 4.36]	3.15 [1.96, 4.45]	0.55
H4P, µmol/L	1.69 [1.11, 2.44]	1.67 [1.08, 2.42]	1.69 [1.12, 2.41]	1.71 [1.12, 2.52]	0.39
H5P, µmol/L	0.29 [0.05, 0.62]	0.29 [0.03, 0.64]	0.32 [0.06, 0.64]	0.28 [0.06, 0.58]	0.06
H6P, µmol/L	0.61 [0.25, 1.34]	0.73 [0.30, 1.58]	0.61 [0.25, 1.33]	0.50 [0.21, 1.15]	<0.001
H7P, µmol/L	0.33 [0.13, 0.62]	0.37 [0.15, 0.69]	0.31 [0.13, 0.61]	0.30 [0.11, 0.58]	<0.001

Comparisons of baseline characteristics among sex-stratified tertiles of FT3 were tested using the Chi-square test for categorical variables and one-way ANOVA for continuous data.
Abbreviations: ApoA1, apolipoprotein A1; ApoB, apolipoprotein B; BMI, body mass index; eGFR, estimated glomerular filtration rate; FT3, free triiodothyronine; FT4, free thyroxine; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein.

Furthermore, an overview of linear regression analyses of thyroid hormones with the 7 HDL subfractions is demonstrated in Supplemental Table S4 (28). Finally, as an additional sensitivity analyses, linear regression analyses of FT3, FT4, and TSH with HDL particle characteristics were performed after including participants with FT3,

Table 2. Linear regression analyses of thyroid hormones with conventional lipids and(apo) lipoprotein measures (n = 5844)

	Free triiodothyronine		Free thyroxine		Thyroid-stimulating hormone	
	Std. β (95% CI)	*P* value	Std. β (95% CI)	*P* value	Std. β (95% CI)	*P* value
Total cholesterol						
Model 1	-0.06 (-0.08; -0.03)	<0.001	-0.07 (-0.10; -0.05)	<0.001	0.03 (0.01; 0.06)	0.03
Model 2	-0.01 (-0.04; 0.02)	0.48	-0.06 (-0.08; -0.03)	<0.001	0.02 (-0.01; 0.05)	0.11
Model 3	-0.02 (-0.05; 0.01)	0.09	-0.06 (-0.08; -0.03)	<0.001	0.03 (0.01; 0.06)	0.01
Model 4	-0.06 (-0.08; -0.03	<0.001	-0.03 (-0.06; -0.01)	0.04	0.01 (-0.02; 0.03)	0.85
Non-HDL cholesterol						
Model 1	0.01 (-0.03; 0.03)	0.99	-0.06 (-0.08; -0.03)	0.001	-0.01 (-0.02; 0.04)	0.49
Model 2	0.01 (-0.02; 0.04)	0.47	-0.06 (-0.08; -0.03)	<0.001	0.02 (-0.01; 0.04)	0.22
Model 3	-0.02 (-0.05; 0.01)	0.14	-0.05 (-0.08; -0.03)	<0.001	0.03 (0.01; 0.06)	0.02
Model 4	-0.06 (-0.09; -0.04)	<0.001	-0.02 (-0.04; 0.01)	0.13	-0.01 (-0.03; 0.02)	0.47
LDL cholesterol						
Model 1	-0.03 (-0.06; -0.01)	0.01	-0.04 (-0.07; -0.01)	0.004	0.01 (-0.02; 0.03)	0.84
Model 2	-0.01 (-0.03; 0.02)	0.62	-0.03 (-0.06; -0.01)	0.01	0.01 (-0.02; 0.03)	0.84
Model 3	-0.03 (-0.06; -0.01)	0.04	-0.03 (-0.06; -0.01)	0.02	0.02(-0.01; 0.04)	0.21
Model 4	-0.05 (-0.08; -0.02)	0.001	-0.02 (-0.05; 0.01)	0.25	-0.01 (-0.03; 0.02)	0.70
VLDL cholesterol*						
Model 1	0.08 (0.06; 0.11)	<0.001	-0.06 (-0.09; -0.03)	<0.001	0.02 (-0.01; 0.05)	0.08
Model 2	0.05 (0.02; 0.08)	<0.001	-0.08 (-0.11; -0.05)	<0.001	0.05 (0.02; 0.07)	0.001
Model 3	0.01 (-0.01; 0.04)	0.32	-0.07 (-0.09; -0.04)	<0.001	0.05 (0.03; 0.08)	<0.001
Model 4	0.01 (-0.01; 0.04	0.31	-0.06 (-0.09; -0.04)	<0.001	0.05 (0.02; 0.07)	<0.001
HDL cholesterol						
Model 1	-0.17 (-0.19; -0.14)	<0.001	-0.04 (-0.07; -0.01)	0.004	0.05 (0.03; 0.08)	<0.001
Model 2	-0.06 (-0.08; -0.03)	<0.001	0.01 (-0.02; 0.03)	0.46	0.01 (-0.01; 0.04)	0.32
Model 3	-0.01 (-0.04; 0.01)	0.28	-0.01 (-0.03; 0.01)	0.44	0.01 (-0.02; 0.03)	0.56
Model 4	0.02 (-0.01; 0.04)	0.15	-0.03(-0.05; 0.01)	0.04	0.03 (0.01; 0.06)	0.005
Triglycerides*						
Model 1	0.16 (0.13; 0.18)	<0.001	-0.06 (-0.08; -0.03)	<0.001	0.02 (-0.01; 0.05)	0.07
Model 2	0.12 (0.09; 0.14)	<0.001	-0.08 (-0.11; -0.06)	<0.001	0.05 (0.03; 0.08)	<0.001
Model 3	0.07 (0.04; 0.09)	<0.001	-0.07 (-0.09; -0.04)	<0.001	0.06 (0.04; 0.09)	<0.001
Model 4	0.07 (0.04; 0. 09)	<0.001	-0.07 (-0.09; -0.04)	<0.001	0.06 (0.04; 0.09)	<0.001
ApoB						
Model 1	0.01 (-0.01; 0.04)	0.28	-0.05 (-0.07; -0.02)	0.001	0.01 (-0.02; 0.03)	0.62
Model 2	0.02 (-0.01; 0.04)	0.22	-0.05 (-0.08; -0.03)	<0.001	0.02 (-0.01; 0.04)	0.21
Model 3	-0.02 (-0.04; 0.01)	0.28	-0.04 (-0.07; -0.02)	0.001	0.03 (0.01; 0.06)	0.02
Model 4	-0.01 (-0.02; -0.01)	0.001	0.01 (-0.01; 0.01)	0.48	-0.01 (-0.01; 0.02)	0.23
ApoA1						
Model 1	-0.11 (-0.13; -0.08)	<0.001	-0.05 (-0.07; -0.02)	0.001	0.05 (-0.03; 0.03)	0.99
Model 2	-0.01 (-0.03; 0.02)	0.51	-0.01 (-0.03; 0.02)	0.81	0.02 (-0.01; 0.04)	0.15
Model 3	0.04 (0.01; 0.06)	0.003	-0.02 (-0.04; 0.01)	0.17	0.01 (-0.01; 0.03)	0.42
Model 4	0.06 (0.03; 0.08)	<0.001	-0.02 (-0.05; 0.01)	0.07	0.03 (0.01; 0.06)	0.02

Model 1: Crude. Model 2: Adjusted for age and sex. Model 3: As model 2, additionally adjusted for waist circumference, alcohol, smoking, and type 2 diabetes. Model 4: As model 3, additionally adjusted for urinary albumin excretion, eGFR, triglycerides (except for models with triglycerides and VLDL cholesterol as outcome variable), LDL cholesterol (except for models with total cholesterol non-HDL cholesterol and LDL cholesterol as outcome variable), usage of antihypertensives, usage of glucose-lowering drugs, usage of lipid-lowering drugs, history of cardiovascular disease and anti-TPO antibodies.
* VLDL and triglycerides are $\log_2$ transformed before analysis.
Abbreviations: ApoA1, apolipoprotein A1; ApoB, apolipoprotein B; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein.

FT4, or TSH outside of the euthyroid range, who were not using thyroid function affecting medication (Supplemental Table S6 (28)). In these analyses, both FT3 and FT4 were positively associated with HDL particle concentration and inversely associated with HDL particle size. In these analyses, TSH was also inversely associated with HDL particle concentration and positively associated with HDL particle size.

Table 3. Linear regression analyses of thyroid hormones with high-density lipoprotein (HDL) particle characteristics (n = 5844)

	Free triiodothyronine		Free thyroxine		Thyroid-stimulating hormone	
	Std. β (95% CI)	*P* value	Std. β (95% CI)	*P* value	Std. β (95% CI)	*P* value
Total HDL particle concentration						
Model 1	0.05 (0.02; 0.08)	<0.001	-0.01 (-0.03; 0.02)	0.83	0.03 (0.01; 0.05)	0.04
Model 2	0.09 (0.07; 0.12)	<0.001	0.01 (-0.01; 0.04)	0.32	0.01 (-0.01; 0.04)	0.34
Model 3	0.12 (0.09; 0.15)	<0.001	0.01 (-0.02; 0.03)	0.67	0.01 (-0.02; 0.03)	0.55
Model 4	0.14 (0.11; 0.17)	<0.001	0.01 (-0.02; 0.04)	0.50	0.02 (-0.01; 0.05)	0.22
Large HDL particle concentration						
Model 1	-0.24 (-0.27; -0.22)	<0.001	-0.02 (-0.05; 0.01)	0.10	0.06 (0.03; 0.08)	<0.001
Model 2	-0.15 (-0.17; -0.12)	<0.001	0.02 (-0.01; 0.05)	0.06	0.02 (-0.01; 0.04)	0.12
Model 3	-0.09 (-0.12; -0.07)	<0.001	0.01 (-0.02; 0.03)	0.69	0.01 (-0.01; 0.04)	0.25
Model 4	-0.07 (-0.09; -0.05)	<0.001	-0.01 (-0.03; 0.01)	0.40	0.04 (0.02; 0.06)	0.001
Medium HDL particle concentration						
Model 1	-0.07 (-0.10; -0.05)	<0.001	-0.05 (-0.08; -0.02)	<0.001	0.05 (0.02; 0.07)	0.001
Model 2	-0.01 (-0.03; 0.02)	0.89	-0.02 (-0.04; 0.01)	0.19	0.01 (-0.01; 0.04)	0.27
Model 3	0.03 (0.01; 0.05)	0.03	-0.02 (-0.05; 0.01)	0.07	0.01 (-0.02; 0.03)	0.64
Model 4	0.05 (0.02; 0.07)	0.001	-0.04 (-0.06; -0.01)	0.008	0.02 (-0.01; 0.05)	0.13
Small HDL particle concentration						
Model 1	0.21 (0.19; 0.24)	<0.001	0.05 (0.02; 0.07)	0.001	-0.03 (-0.06; -0.01)	0.01
Model 2	0.16 (0.13; 0.18)	<0.001	0.02 (-0.01; 0.04)	0.25	-0.01 (-0.03; 0.02)	0.57
Model 3	0.13 (0.11; 0.16)	<0.001	0.02 (-0.01; 0.05)	0.12	-0.01 (-0.03; 0.02)	0.82
Model 4	0.13 (0.10; 0.16)	<0.001	0.04 (0.02; 0.07)	0.002	-0.02 (-0.04; 0.01)	0.25
Mean HDL size						
Model 1	-0.26 (-0.29; -0.24)	<0.001	-0.02 (-0.05; 0.01)	0.09	0.04 (0.02; 0.07)	0.001
Model 2	-0.15 (-0.18; -0.13)	<0.001	0.03 (0.01; 0.05)	0.02	-0.01 (-0.03; 0.02)	0.93
Model 3	-0.10 (-0.12; -0.08)	<0.001	0.01 (-0.01; 0.03)	0.47	-0.01 (-0.03; 0.02)	0.70
Model 4	-0.08 (-0.10; -0.06)	<0.001	-0.02 (-0.04; 0.01)	0.07	0.03 (0.01; 0.05)	0.01

Model 1: Crude. Model 2: Adjusted for age and sex. Model 3: As model 2, additionally adjusted for waist circumference, alcohol, smoking, and type 2 diabetes. Model 4: As model 3, additionally adjusted for urinary albumin excretion, eGFR, triglycerides, LDL cholesterol, usage of antihypertensives, usage of glucose-lowering drugs, usage of lipid-lowering drugs, history of cardiovascular disease, and anti-TPO antibodies.

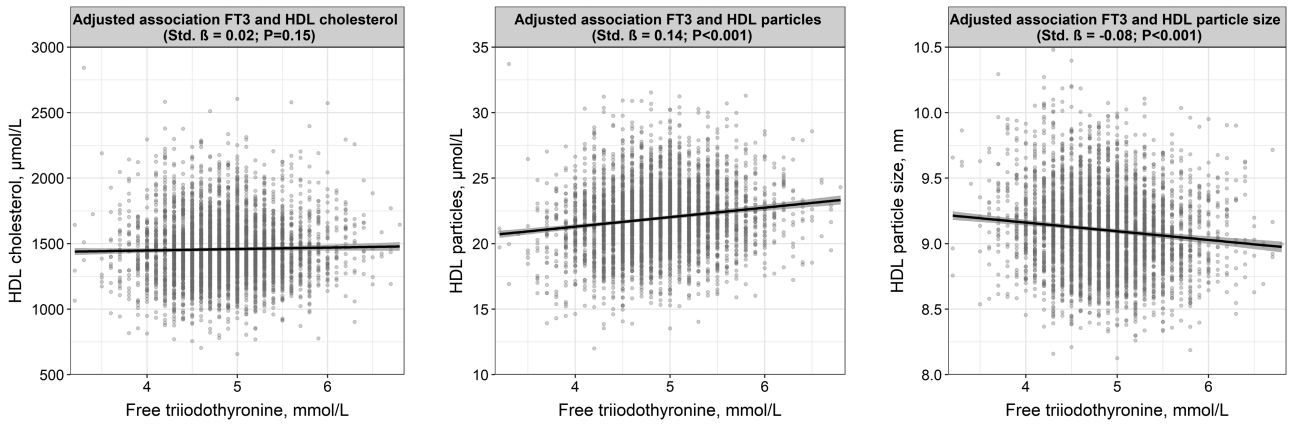

Figure 1. Graphical representations of the associations of triiodothyronine with HDL cholesterol, HDL particles concentration, and HDL particle size. Linear regression analyses are adjusted for age, sex, waist circumference, alcohol consumption, smoking status, type 2 diabetes, urinary albumin excretion, eGFR, triglycerides, LDL cholesterol, usage of antihypertensives, usage of glucose-lowering drugs, usage of lipid-lowering drugs, history of cardiovascular disease, and anti-TPO antibodies.

Discussion

The most salient findings of the current cross-sectional study are: (i) the robust and independent, positive association of FT3 with total HDL particle concentration in fully adjusted analyses; and (ii) the positive association of FT3 with fewer large and more small HDL particles. Consequently, there was a shift toward a smaller HDL size with higher FT3. In general, such associations with FT4 or reciprocally with TSH were less pronounced or nonsignificant. Analyses according to the total HDL particle concentration confirmed

higher FT3 levels in the highest HDL particle tertile, without a significant difference in FT4 or TSH. Taken together, our current findings agree with the notion that higher FT3 levels within the reference range are associated with pronounced changes in HDL particle concentration and HDL subfraction distribution.

Thyroid hormones are critical regulators of many metabolic processes and have well-established effects on cholesterol and fatty acid synthesis and metabolism (32-34). Effects of thyroid hormones on lipid homeostasis are attributed to both transcriptional and posttranscriptional regulation of target genes involved in hepatic lipid homeostasis (35, 36). In overt hypothyroidism there is an increase in plasma total cholesterol and LDL cholesterol (8-12). In general, our results are in line with previous findings in euthyroid individuals (11, 14), as higher levels of TSH and lower levels of FT3 and FT4 within the reference range were indeed independently associated with higher plasma total cholesterol. Higher FT3 concentrations were also associated with lower LDL cholesterol. A discrepancy between FT3 and FT4 worth noting is the association with plasma triglycerides, which were positively associated with FT3 and inversely with FT4. However, we are not the first to report on this differential association. An observational study in 2524 generally healthy individuals from the Asklepios Study previously demonstrated that FT3 and FT4 are differentially associated with adiposity-related cardiovascular risk markers, of which triglycerides were positively associated with FT3 and inversely with FT4, similar to our findings (37). As a potential underlying mechanism, the authors speculated that a higher fat mass and/or central obesity may lead to changes in deiodinase 1 and 2, thereby enhancing thyroxine (T4) to triiodothyronine (T3) conversion (38, 39).

T3 is commonly believed to be more biologically active as a regulator of metabolic processes than its prohormone, T4 (33, 40). This suggests that associations of FT3 with circulating (apo)lipoproteins and lipids could be more relevant than associations with FT4. In multivariable linear regression analyses we found no significant association between FT3 and HDL cholesterol, while we did find an association between FT3 and the total HDL particle concentration. This apparent discrepancy may be explained by the fact that circulating HDL cholesterol concentrations are primarily a reflection of larger, cholesterol loaded HDL particles which were found to be inversely associated with FT3 (30, 41).

Regarding the strong association of FT3 with the total HDL particle concentration, we hypothesize that higher FT3 may lead to an increased synthesis of HDL particles, which is also supported by a positive association between FT3 and ApoA1, the major HDL-associated apolipoprotein (42). Importantly, hypothyroidism is associated with both reduced transcription and abundance of ApoA1 in rat liver, and administration of T3 to euthyroid rats directly increases ApoA1 transcription (26). Besides, low-normal thyroid function was found to be associated with decreased formation of preβ HDL, a precursor of mature discoidal HDL particles (43). On the other hand, T3 may stimulate renal function, as evidenced from the positive relationship of eGFR with FT3 (44). In turn, plasma ApoA1 is inversely related to eGFR, probably as a consequence of enhanced renal degradation (45-47). This highlights the complex and partly opposing effects of thyroid function on ApoA1 metabolism, with the net effect being increased plasma HDL particle concentration and ApoA1 consequent to higher T3.

A second potentially important finding of the current study is the inverse association of FT3 with HDL size, as a result of fewer large and more small HDL particles. While these associations have not been reported before in euthyroid individuals, a comparable trend has been observed in subclinical thyroid dysfunction (48). In a Brazilian adult population, subclinical hypothyroidism was found to be associated with lower levels of small HDL particles, while subclinical hyperthyroidism was associated with lower levels of large HDL particles (48). Among other factors, potential mechanisms for the inverse association of FT3 with larger HDL particles and HDL size may be explained by effects of thyroid function status on cholesteryl ester transfer protein (CETP)– and hepatic lipase–mediated processes (5, 19, 34, 49, 50). CETP is able to transfer cholesteryl esters from larger HDL particles to triglyceride-rich lipoproteins while simultaneously transferring triglycerides to larger HDL particles (5, 34, 49). Subsequently, triglyceride hydrolysis by hepatic lipase results in smaller HDL particles. The stimulation of both CETP and hepatic lipase by thyroid hormones may thus induce a shift toward smaller and fewer larger HDL particles. In line with this, overt hypothyroidism decreases CETP activity, thereby elevating HDL cholesterol, while low-normal thyroid function in turn may affect the CETP-mediated the cholesteryl ester transfer process in plasma (10, 51-53). Conversely, overt hyperthyroidism confers higher CETP and hepatic lipase activities, changes which are reversed after treatment (52). Such CETP- and hepatic lipase-mediated effects on HDL remodeling are likely to be enhanced in the context of higher plasma triglycerides (5, 49, 50). Hence, we adjusted for triglycerides when evaluating the association of FT3 with HDL subfraction distribution and size and found that these associations were attenuated in fully adjusted analyses.

Previous studies have clearly demonstrated that cardioprotective activities of HDL, such as cholesterol efflux capacity, endothelial protective capabilities, and anti-inflammatory effects, depend on specific HDL particle characteristics that are poorly reflected by the HDL cholesterol concentration (41, 54, 55). A recent meta-analysis demonstrated that the HDL particle concentration is inversely associated with atherosclerotic cardiovascular events (both myocardial infarction and ischemic stroke) even when adjusting for HDL cholesterol (20). In accordance with this, our study, although not prospective in design, demonstrated the highest history of cardiovascular events in the lowest tertile of HDL particle concentration.

Furthermore, observational studies of patients with acute heart failure (41) and coronary heart disease (56), demonstrated that a higher total HDL particle concentration, as well as a higher small HDL particle concentration, was associated with a lower risk of all-cause mortality, while HDL cholesterol concentration was not associated with all-cause mortality (41, 56). Possible pathophysiological mechanisms underlying a protective effect of small HDL particles, especially, on the risk of CVD may be the effective cholesterol efflux from macrophages, the anti-inflammatory properties by reducing of VCAM-1 expression at the surface of endothelial cells, or the effective protection of LDL from oxidation by removing phospholipid hydroperoxides (56). In comparison, large HDL particles may be less effective than small HDL particles in atheroprotective functions, such as the cholesterol efflux, cytoprotective effects, and antioxidative effects (56).

We propose that our current findings regarding the positive association of HDL particle concentration with FT3 may help to better understand the potential adverse effect of low-normal thyroid function on the development of atherosclerotic CVD.

Noteworthy strengths of this study were the size of the study and the extensive data collection, allowing for the adjustment for a large set of potential confounders. Several limitations of this study need to be addressed as well. First and most importantly, due to the observational design of this study, it impossible to determine whether the observed associations are causal or associative. Furthermore, despite adjusting for many potential confounders, the possibility of residual confounding cannot be fully excluded. For example, we did not have data on physical activity of participants. It cannot be excluded that the observed associations may be confounded by physical activity, as physical activity can affect both thyroid hormones and lipoprotein particle concentrations (57). Third, the current study was carried out in a predominantly White population, limiting our findings to individuals with different ethnicities. Fourth, we used single euthyroid reference ranges for TSH, FT4, and FT3 across the whole age range of the population studied. Hence, it is relevant to note that the associations of FT3 with HDL variables did not interact with age. Furthermore, considering that the selection of participants into the PREVEND cohort was based on urinary albumin concentration (with enrichment for participants with a urinary albumin concentration >10 mg/L), some prudence is required before extrapolating the results to the general population.

In conclusion, in a large population cohort of euthyroid individuals, multivariable linear regression analyses demonstrated a strong and independent positive association of FT3 with total HDL particle concentration and an inverse association with HDL particle size. These findings may be relevant to better understand the role of HDL in thyroid function–associated atherosclerotic CVD.

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Author Contributions

All authors have substantially contributed to the manuscript design and/or revision and have approved this final version of the work. The authors have agreed to take accountability for all aspects of this study. The authors' responsibilities were as follows: conceptualization, A.P. and R.P.F.D.; data curation, E.G., E.G.G., M.A.C., and E.G.G.; formal analysis, A.P., and R.P.F.D.; investigation, A.P. and R.P.F.D.; methodology A.P. and R.P.F.D.; supervision, S.J.L.B. and R.P.F.D.; visualization, A.P. and R.P.F.D.; writing—original draft, A.P. and R.P.F.D.; writing—review and editing, E.G., E.G.G., D.K., M.A.C., and S.J.L.B.

Disclosures

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E. Garcia and M.A. Connelly are employees of LabCorp. A. Post, E.G. Gruppen, and D. Kremer have nothing to declare.

Data Availability

Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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