

Impact of Subclinical Hypothyroidism on Cardiometabolic Biomarkers in Women

Paulo H. N. Harada,^{1,2,3} Julie E. Buring,^{2,4} Nancy R. Cook,^{2,4} Michael E. Cobble,⁵ Krishnaji R. Kulkarni,⁶ and Samia Mora^{1,2}

¹Center for Lipid Metabolomics, Cardiovascular Division and ²Division of Preventive Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts 02115;

³Center for Clinical and Epidemiologic Research, Hospital Universitário, University of Sao Paulo, Sao Paulo 05508-000, Brazil;

⁴Harvard T. H. Chan School of Public Health, Boston, Massachusetts 02115;

⁵Canyons Medical Center, Sandy, Utah 84094; and

⁶VAP Diagnostics Laboratory, Birmingham, Alabama 35211

Context: Whether subclinical hypothyroidism (SCH) is associated with cardiometabolic abnormalities is uncertain.

Objective: To examine diverse cardiometabolic biomarkers across euthyroid, SCH, and overt hypothyroidism (HT) in women free of cardiovascular disease.

Design: Cross-sectional adjusted associations for lipids, lipoprotein subclasses, lipoprotein insulin resistance score, inflammatory, coagulation, and glycemic biomarkers by analysis of covariance for thyroid categories or thyroid stimulating hormone (TSH) quintiles on a Women's Health Study subcohort.

Setting: Outpatient.

Patients or Other Participants: Randomly sampled 3914 middle-aged and older women for thyroid function analysis (TSH, free T4), of whom 3321 were not on lipid-lowering therapy.

Intervention: None.

Main Outcome Measure: Associations of SCH and HT with cardiometabolic markers.

Results: Going from euthyroid to HT, the lipoprotein subclass profiles were indicative of insulin resistance (respective values and *P* for trend): larger very-low-density lipoprotein size (nm) (51.5 [95% confidence interval (CI), 51.2, 51.8] to 52.9 [51.8, 54.1], *P* = 0.001); higher low-density lipoprotein (LDL) particle concentration (nmol/L) [1283 (95% CI, 1267, 1299) to 1358 (1298, 1418), *P* = 0.004], and smaller LDL size. There was worsening lipoprotein insulin resistance score from euthyroid (49.2; 95% CI, 48.3, 50.2) to SCH (52.1; 95% CI, 50.1, 54.0) and HT (52.1; 95% CI, 48.6, 55.6); *P* for trend of 0.008. Of the other biomarkers, SCH and HT were associated with higher high-sensitivity C-reactive protein and hemoglobin A1c. For increasing TSH quintiles, results were overall similar.

Conclusions: In apparently healthy women, SCH cardiometabolic profiles indicated worsening insulin resistance and higher cardiovascular disease risk markers compared with euthyroid individuals, despite similar LDL and total cholesterol. These findings suggest that cardiometabolic risk may increase early in the progression toward SCH and overt HT.

Copyright © 2017 by the Endocrine Society

This article has been published under the terms of the Creative Commons Attribution License (CC BY-NC-ND; <https://creativecommons.org/licenses/by-nc-nd/4.0/>).

Abbreviations: apo, apolipoprotein; BMI, body mass index; CI, confidence interval; CV, coefficient of variation; CVD, cardiovascular disease; FT4, free thyroxine; GlycA, glycan *N*-acetylglucosamines; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; HT, hypothyroidism; LDL, low-density lipoprotein; Lp(a), lipoprotein(a); LPIR, lipoprotein insulin resistance; NMR, nuclear magnetic resonance; SCH, subclinical hypothyroidism; sICAM-1, soluble intercellular adhesion molecule 1; T2D, type 2 diabetes; TSH, thyroid stimulating hormone; VLDL, very-low-density lipoprotein.

Freeform/Key Words: subclinical hypothyroidism, metabolomics, lipoproteins, inflammation, coagulation, insulin resistance

Subclinical hypothyroidism (SCH) and overt hypothyroidism (HT) are common diseases, affecting, respectively, 9.0% and 0.4% of the United States population [1], predominantly women. As thyroid function has multisystemic effects, its derangement could affect a broad range of cardiometabolic pathways potentially related to clinical manifestations. However, the definition of normal thyroid function has been intensely debated, with some experts advocating for lowering the upper limit of normal for thyroid stimulating hormone (TSH) [2] and others for maintaining the current standard [3]. In this regard, thyroid-related risk for incident type 2 diabetes (T2D) and cardiovascular disease (CVD) may impact the definition of TSH normality. In the one hand, HT has been associated with incident T2D [4]; on the other hand, findings are mixed for CVD, especially in the gray zone of SCH [5–8].

The potential relationship of thyroid hypofunction with T2D and CVD may be mediated by abnormalities in lipids, lipoprotein subclasses, endothelial function, coagulation, inflammatory pathways, and insulin resistance [9, 10]. To date, there have been inconsistent data regarding thyroid function across the spectrum of euthyroid to HT and its potential cardiometabolic mediators, both in direction and magnitude [11–13]. Detailed assessment of thyroid function effects on these mediators/markers may have high population health implications, especially along the milder hypofunction spectrum within euthyroidism and SCH. Understanding the role of thyroid function in cardiometabolic pathways may guide the clinically relevant definition of thyroid function and unveil potential targets for controlling related morbidity.

Therefore, in this study of apparently healthy middle-aged and older women, we examined thyroid function across the spectrum of euthyroid to HT in relationship to cardiometabolic pathways represented by lipids, lipoproteins, inflammation, coagulation, glycemic, and insulin resistance biomarkers.

1. Methods

A. Study Design, Sample, and Exposure Assessment

The sample comprised apparently healthy middle-aged and older women at study entry of an ongoing prospective cohort, the Women's Health Study [14]. At enrollment, women gave written informed consent and completed questionnaires on demographics, anthropometrics, medical history, and lifestyle factors. Race was self-referred, and body mass index (BMI) was measured as weight in kilograms by squared height in meters. The study was approved by the Institutional Review Board of the Brigham and Women's Hospital (Boston, MA).

As our initial inclusion criteria were similar to the original protocol, that is, women without CVD or cancer, 28,024 participants with available stored frozen plasma at baseline were eligible for our study. From those, 3914 women were randomly selected for thyroid function testing, and after excluding those on lipid-lowering therapy and ineligible thyroid categories amounted to 3321 individuals. Eligible categories based on the Roche Cobas assay recommendations by Atherotech Diagnostics Laboratory (Birmingham, AL) were defined as follows: euthyroid [TSH, 0.27 to 4.2 mIU/L; free thyroxine (FT4), 0.93 to 1.7 ng/dL], SCH (TSH > 4.2 mIU/L; FT4, 0.93 to 1.7 ng/dL), and hypothyroid (TSH > 4.2 mIU/L; FT4 < 0.93 ng/dL). Thyroid category distributions were: euthyroidism, 2571 (77.4%); SCH, 573 (17.3%); and HT, 177 (5.3%). We performed additional analyses of TSH quintiles within normal FT4 individuals (0.93 to 1.7 ng/dL), which includes only euthyroid and SCH, 3144 (94.7%).

B. Biomarker Assessment

Samples were obtained from stored blood in vapor-phase liquid nitrogen (–170°C) at the time of enrollment into the Women's Health Study and thawed for the following laboratory

analyses. TSH and FT4 were measured at Atherotech Diagnostics Laboratory using the Roche Cobas e601 analyzer with coefficients of variation (CVs) of $\leq 8.7\%$ and $\leq 6.6\%$, respectively. In a laboratory certified by the National Heart, Lung, and Blood Institute/Centers for Disease Control and Prevention Lipid Standardization program, standard lipids were measured directly with reagents from Roche Diagnostics (Indianapolis, IN) [15] and apolipoprotein (apo) B and A1 were measured with immunoturbidimetric assays (DiaSorin, Stillwater, MN). All measures had CVs of $\leq 5\%$ and comprised total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, apoB, and apoA1. Lipoprotein(a) [Lp(a)] was measured with a well characterized in-house immunoturbidimetric assay using a Technicon Axon autoanalyser (Miles Inc., Tarrytown, NY), rabbit anti-human lipoprotein(a) polyclonal antibodies (Q023, DAKO, Glostrup, Denmark), and a human serum lipoprotein(a) calibrator (DAKO)[16]. Nuclear magnetic resonance (NMR) spectroscopy (LabCorp, previously LipoScience, Raleigh, NC) measured the methyl terminal of lipoprotein subclasses and size of LDL, HDL, and very-low-density lipoprotein (VLDL) particles using a targeted metabolomics platform (NMR LipoProfile analysis by the LP3 algorithm) [17]. This platform comprises concentrations of large, medium, and small VLDL particles; large and small LDL particles; very large, large, medium, small, and very small HDL particles; and average size of VLDL, LDL, and HDL particles. As the distribution of small LDL particles is bimodal in this population, we analyzed this parameter separately in those with concentrations higher or lower than 164 nmol/L, which is the nadir between modes. The group with the higher small LDL concentration is referred to as pattern B; the group with the lower small LDL concentration is referred to as pattern A. The lipoprotein insulin resistance (LPIR) score is part of the same panel, and is a weighted composite score of lipoprotein subclasses independently related to insulin resistance [18]. It includes 6 parameters (respective maximum points and direction for more insulin resistance): VLDL size (32 points, larger size); large VLDL particles (22 points, increasing concentration); LDL size (6 points, smaller size); small LDL particles (8 points, increasing concentration); HDL size (20 points, smaller size); and large HDL particles (12 points, decreasing concentration). The summed score ranges from 0 to 100, and higher values indicate increasing insulin resistance [18].

The inflammation and coagulation biomarkers were: high-sensitivity C-reactive protein (hs-CRP) measured by a high-sensitivity immunoturbidimetric assay on a Hitachi 917 auto-analyzer (Roche Diagnostics), with reagents and calibrators from Denka Seiken [19]; fibrinogen measured by an immunoturbidimetric assay (Kamiya Biomedical, Seattle, WA); homocysteine measured by an enzymatic assay (Catch Inc., Seattle, WA); and soluble intercellular adhesion molecule 1 (sICAM-1) measured by an enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN). Additionally, glycan *N*-acetylglucosamines (GlycA) is a novel composite inflammatory biomarker of acute phase glycoproteins, mostly $\alpha 1$ -acid glycoprotein, haptoglobin, $\alpha 1$ -antitrypsin, $\alpha 1$ -antichymotrypsin, and transferrin [20]. Their *N*-acetyl methyl group protons of the *N*-acetylglucosamine moieties located on their biantennary, triantennary, and tetraantennary branches were measured by magnetic resonance. This signal only arises from the *N*-acetylglucosamine units with $\beta 1 \rightarrow 2$ and $\beta 1 \rightarrow 6$ linkages on preceding mannose residue centered at 2.00 ± 0.01 ppm. GlycA signal was successfully deconvoluted from neighboring lipoprotein, majorly triglyceride concentration in VLDL, with a CV of 4.3% [20]. Hemoglobin A1c (HbA1c) was measured by turbidimetric immunoinhibition on hemolyzed whole blood or packed red cells (Roche Diagnostics). Metabolic syndrome was classified by the presence of at least of 3 of the following: HbA1c ≥ 5.7 or diabetes history; blood pressure $\geq 130/85$, hypertension diagnosis or treatment; BMI > 26.7 kg/m²; triglycerides ≥ 150 mg/dL; or HDL cholesterol < 50 mg/dL. As waist circumference was not measured until year 6 of follow-up, baseline BMI was used instead. BMI baseline cut point was chosen according to the same percentile for BMI at year 6 as for 88-cm waist circumference at that moment.

C. Statistical Analysis

Descriptive demographics of functional thyroid categories were displayed by percentages for categorical variables, and by mean (standard deviation) continuous variables. For univariable

statistical comparisons, we used Cochran-Mantel-Haenszel for categorical variables and linear regression for trend for continuous variables.

We addressed the relationship of thyroid categories with each biomarker by adjusted linear regression analysis, with tests for linear trend, and pairwise comparisons adjusted by Bonferroni in the thyroid categories and TSH quintiles. For TSH quintiles, we tested linear trend across median TSH values within each quintile. For pairwise comparisons, the respective references were euthyroid or the bottom TSH quintiles. The covariables were age, race, household income, current smoking, systolic blood pressure, antihypertensive treatment, menopause status, and hormone replacement therapy. Skewed distributed variables were transformed by the natural logarithm [HDL cholesterol, triglycerides, Lp(a), large and small VLDL particles, small LDL particles (pattern B), hs-CRP, fibrinogen, homocysteine, and HbA1c] or by the square root (very large, large, medium, and small HDL particles) for better model fit, and then back transformed. We also conducted sensitivity analyses adjusted for BMI to address possible mediation of adiposity on thyroid function and metabolic abnormalities.

Finally, to assess the risk of having clinical metabolic syndrome across the thyroid categories or TSH quintiles, we applied logistic regression adjusted for age, race, smoking status, income, menopause status, and hormone replacement therapy. Analyses were performed using SAS version 9.3 (SAS Institute, Cary, NC).

2. Results

Compared with euthyroid women, those with SCH and HT were older and had higher BMI, greater prevalence of postmenopausal status, lower prevalence of current smoking, higher blood pressure, and greater prevalence of metabolic syndrome (Table 1). Noticeably, metabolic syndrome prevalence (25.4% on euthyroid to 39.6% on HT) and smoking (18.2% on euthyroid to 11.3% on HT) were largely different across thyroid categories.

In analyses that adjusted for age, race, household income, current smoking, systolic blood pressure, antihypertensive treatment, menopause status, and hormone replacement therapy, across thyroid categories there were no significant contrasts for total cholesterol, LDL cholesterol, ApoA1, or Lp(a) (Table 2). By contrast, going from euthyroid to SCH and HT, there was a pattern of increasing atherogenic dyslipidemia, namely, lower HDL cholesterol, higher triglycerides, higher ApoB concentrations, as well as higher

Table 1. Baseline Clinical Characteristics by Thyroid Function

Variable	Euthyroid (77.4%; N = 2571)	SCH (17.3%; N = 573)	HT (5.3%; N = 177)	P Value for Trend
Age, y	56.5 (7.8)	58.5 (8.3)	60.4 (8.6)	<0.001
White, N (%)	2451 (96.2)	558 (98.4)	174 (99.4)	<0.001
BMI, kg/m ²	25.8 (5.0)	26.1 (4.9)	26.8 (4.9)	0.005
Income, annual household > 50K USD, N (%)	1208 (50.0)	235 (43.0)	66 (38.6)	<0.001
Postmenopausal, N (%)	1575 (61.4)	402 (70.2)	134 (75.7)	<0.001
Hormone replacement treatment, N (%)	1092 (42.5)	260 (45.6)	66 (37.3)	0.843
Diabetes, N (%)	95 (3.7)	27 (4.7)	10 (5.7)	0.105
HbA1c, %	5.0 (4.8–5.2)	5.0 (4.8–5.2)	5.1 (4.9–5.3)	<0.001
Family history of diabetes, N (%)	672 (26.1)	154 (26.9)	45 (25.4)	0.950
Current smoking, N (%)	496 (18.2)	55 (9.6)	20 (11.3)	<0.001
Systolic blood pressure, mm Hg	125 (14)	126 (14)	128 (14)	<0.001
Diastolic blood pressure, mm Hg	77 (9)	77 (9)	79 (9)	0.017
Hypertension history, N (%)	694 (27.0)	167 (29.1)	56 (31.6)	0.108
Metabolic syndrome, N (%)	650 (25.4)	175 (30.7)	70 (39.6)	<0.001

Values are shown as mean (standard deviation) or number (percentage). For P values, the Cochran–Mantel–Haenszel test for categorical variables and linear regression for linear trend for continuous variables were used.

triglyceride/HDL-c ratios and apoB/ApoA1 ratios. Additional adjustment for BMI did not change the results.

On examining the lipoprotein particle distributions, there was a trend toward increasing insulin resistance going from euthyroid to SCH and HT (Table 3). In this order, there were increasing concentrations of large and medium VLDL particles, reflected by increasing VLDL size. Regarding LDL subclasses, from ET to HT there were increasing concentrations of total LDL particles and particularly of the small LDL (pattern B). This resulted in a smaller average LDL particle size. In contrast to differences seen in LDL particles, there were no HDL particle differences across thyroid categories. The LPIR score reflected the overall profile of the lipoprotein subclasses, as indicated by greater lipoprotein insulin resistance going from a euthyroid mean LPIR score of 49.2 (95% CI, 48.3, 50.2) to 52.1 in SCH (95% CI, 50.1, 54.0) and 52.1 in HT (95% CI, 48.6, 55.6) (P for trend of 0.008). Additional adjustment for BMI neutralized differences for LDL particle size and LPIR signaled (Table 3).

For inflammatory and coagulation biomarkers, there were mixed results across thyroid categories (Table 4). Mean hs-CRP levels increased from 2.0 mg/L (95% CI, 1.9, 2.1) in euthyroid to 2.1 mg/L (95% CI, 1.9, 2.3) in SCH and 2.4 mg/L (95% CI, 2.0, 2.8) in HT (P for trend of 0.028); however, it was null after BMI adjustment. In contrast, no significant differences were noted for the other inflammatory/coagulation biomarkers. HbA1c levels were all below the upper limit of normality across thyroid categories, although a small but statistically significant difference was noted comparing HT with euthyroid.

On analysis restricted to euthyroid and SCH women (Supplemental Tables 1–3), TSH quintile values were mostly within the normal range (*i.e.*, euthyroid). Only the top TSH quintile had individuals in the SCH range, which comprised 91.0% (573 women) of the top quintile. Similar to the analyses for thyroid categories, across increasing TSH quintiles the overall profile was consistent with worsening dyslipidemia and dyslipoproteinemia. In contrast to the analysis according to thyroid categories, we noted that there were decreasing concentrations of large HDL particles and increasing concentrations of small HDL particles from the bottom to the top quintile, reflected in smaller HDL particles size. Of note, increasing TSH quintiles presented quite linear LPIR increments (Supplemental Table 2; Fig. 1).

Regarding metabolic syndrome, there was increasing prevalence from ET to HT ($P < 0.001$). Compared with euthyroid, SCH and HT adjusted odds ratios for metabolic syndrome were, respectively, 1.37 (95% CI, 1.08, 1.73) and 1.88 (95% CI, 1.30, 2.72) (P for linear trend of <0.001). Increasing TSH within euthyroid and SCH range had higher odds ratios for metabolic syndrome in reference to the bottom quintile (P for trend of 0.001) (Supplemental Table 4), in which the top quintile had an odds of 1.49 (95% CI, 1.10, 2.01).

Table 2. Adjusted Standard Lipids and Apolipoprotein According to Functional Thyroid Categories

Variable	Euthyroidism	SCH	HT	P for Linear Trend
Total cholesterol, mg/dL	213 (212, 215)	212 (208, 215)	218 (212, 224)	0.621
LDL cholesterol, mg/dL	126 (124, 127)	124 (121, 127)	128 (123, 133)	0.976
HDL cholesterol, mg/dL	51.6 (51.1, 52.2)	49.3 (48.2, 50.4) ^a	49.4 (47.4, 51.4)	<0.001
Triglycerides, mg/dL	122 (120, 125)	133 (128, 139) ^a	141 (131, 153) ^a	<0.0001
Triglycerides/HDL ratio	2.4 (2.3, 2.4)	2.7 (2.5, 2.9) ^a	2.9 (2.6, 3.2) ^a	<0.0001
ApoB, mg/dL	105 (104, 106)	106 (104, 109)	112 (107, 116) ^a	0.011
ApoA1, mg/dL	150 (149, 151)	148 (146, 150)	147 (143, 151)	0.074
ApoB/ApoA1 ratio	0.73 (0.72, 0.74)	0.74 (0.72, 0.76)	0.80 (0.76, 0.84) ^a	0.001
Lp(a), mg/dL	11.2 (10.6, 11.8)	10.9 (9.7, 12.2)	11.1 (9.1, 13.5)	0.788

For linear regression across categories, adjustment covariates included age, race, household income, current smoking, systolic blood pressure, antihypertensive treatment, menopause status, and hormone replacement therapy. Least square means (95% CI) were determined.

^a $P < 0.050$ for pairwise comparison with euthyroid category (Bonferroni adjusted). Additional adjustment for BMI did not yield a change in P for trend across 0.05 thresholds.

Table 3. Adjusted Analysis for Lipoprotein Particle Concentration and Size According to Functional Thyroid Categories

Variable	Euthyroidism	SCH	HT	<i>P</i> for Linear Trend
VLDL particles				
Total, nmol/L	61.8 (60.7, 62.8)	62.7 (60.6, 64.9)	65.2 (61.4, 69.1)	0.084
Large, nmol/L	2.5 (2.4, 2.6)	2.7 (2.5, 3.0)	3.1 (2.7, 3.6) ^a	0.002
Medium, nmol/L	16.9 (16.4, 17.3)	17.8 (16.8, 18.7)	19 (17.4, 20.7) ^a	0.005
Small, nmol/L	36.1 (35.2, 36.9)	36.0 (34.3, 37.8)	35.6 (32.6, 38.7)	0.804
Average size, nm	51.5 (51.2, 51.8)	52.3 (51.7, 53)	52.9 (51.8, 54.1) ^a	0.001
IDL particles				
Total, nmol/L	179 (175, 183)	176 (167, 184)	188 (174, 203)	0.674
LDL particles				
Total, nmol/L	1283 (1267, 1299)	1319 (1285, 1353)	1358 (1298, 1418)	0.004
Large, nmol/L	575 (564, 586)	552 (529, 576)	562 (520, 604)	0.157
Small pattern B 2225 (67.0%)	668 (653, 684)	734 (699, 770) ^a	829 (759, 906) ^a	<0.001
Small pattern A 1096 (33.0%)	64.8 (63.0, 66.6)	63.8 (60.0, 67.8)	62.5 (56.5, 69.1)	0.456
Average size, nm	21.1 (21.0, 21.1)	21 (20.9, 21.1)	21 (20.9, 21.1)	0.024 ^b
HDL particles				
Total, nmol/L	37.4 (37.2, 37.7)	37.3 (36.8, 37.8)	37.1 (36.1, 38.0)	0.431
Large, μ mol/L	6.3 (6.2, 6.4)	6.1 (5.8, 6.3)	6.4 (5.9, 6.9)	0.433
Medium, μ mol/L	12.5 (12.3, 12.7)	12.6 (12.1, 13)	12.8 (12, 13.6)	0.602
Small, μ mol/L	18.7 (18.5, 18.9)	19.1 (18.6, 19.5)	18.1 (17.4, 18.9)	0.904
Average size, nm	9.2 (9.2, 9.2)	9.2 (9.1, 9.2)	9.3 (9.2, 9.3)	0.987
Lipoprotein insulin resistance index score ^b	49.2 (48.3, 50.2)	52.1 (50.1, 54) ^a	52.1 (48.6, 55.6)	0.008 ^b

For linear regression across categories, adjustment covariates included age, race, household income, current smoking, systolic blood pressure, antihypertensive treatment, menopause status, and hormone replacement therapy. Least square means (95% CI) were determined. The small LDL pattern A group had 1096 individuals and the small LDL pattern B group had 2225 individuals, which corresponds to 33.0% and 67.0% of all those with available small LDL particles.

^a*P* < 0.050 for pairwise comparison with euthyroid category (Bonferroni adjusted).

^bAdditional adjustment for BMI yield change in *P* for trend across 0.05 threshold.

3. Discussion

In this population of apparently healthy middle-aged and older women, individuals with SCH and HT had differences in the lipid and lipoprotein subclass profile that indicated worsening insulin resistance and higher cardiometabolic risk compared with euthyroid individuals, despite having similar LDL cholesterol and total cholesterol. Of the other biomarkers, only hs-CRP and HbA1c were associated with SCH and HT. For TSH quintiles mostly within the

Table 4. Inflammatory, Coagulation, and Metabolism Markers by Thyroid Function Classification

Variable	Euthyroidism	SCH	HT	<i>P</i> for Linear Trend
hs-CRP, mg/L	2.0 (1.9, 2.1)	2.1 (1.9, 2.3)	2.4 (2.0, 2.8)	0.028 ^a
Fibrinogen, mg/dL	361 (358, 364)	362 (356, 369)	357 (345, 369)	0.865
Homocysteine, μ mol/L	10.8 (10.7, 10.9)	10.9 (10.6, 11.2)	10.8 (10.3, 11.4)	0.712
sICAM-1, ng/ml	368 (365, 372)	368 (360, 375)	376 (363, 389)	0.446
GlycA, μ mol/L	381 (378, 384)	380 (375, 386)	381 (371, 391)	0.924
HbA1c, %	5.1 (5.1, 5.1)	5.1 (5.1, 5.2)	5.2 (5.1, 5.3) ^b	0.011

For linear regression across categories, adjustment covariates included age, race, household income, current smoking, systolic blood pressure, antihypertensive treatment, menopause status, and hormone replacement therapy. Least square means (95% CI) were determined.

^aAdditional adjustment for BMI yield change in *P* for trend across 0.05 threshold.

^b*P* < 0.050 for pairwise comparison with euthyroid category (Bonferroni adjusted).

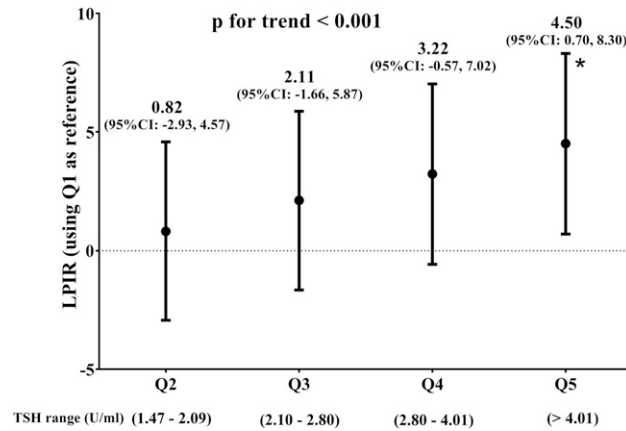


Figure 1. LPIR according to TSH quintiles. LPIR score differences for TSH quintiles (Q) vs the first TSH quintile along euthyroid and SCH are shown. For linear regression for median TSH across categories, adjustment covariates include age, income, current smoking, race, household income, systolic blood pressure, high blood pressure treatment, menopause status, and hormone replacement therapy. The *P* value for the trend for median TSH values within each quintile is shown. *Bonferroni adjusted *P* value = 0.010 for the fifth quintile vs the first quintile.

normal range, lipid and lipoprotein results for TSH quintiles were generally similar but null for other biomarkers. Hence, progressive thyroid hypofunction was associated with insulin-resistant and proatherogenic lipids and lipoproteins profile in a graded manner, with potential clinical consequences.

This study adds to the literature by describing the graded relationship of progressive thyroid hypofunction, even within the “normal” range of euthyroidism, as manifested by increasing lipoprotein insulin resistance. For inflammatory, coagulation, and glucose metabolism biomarkers, only hs-CRP and HbA1c were higher across the euthyroid to HT spectrum, but none of those biomarkers was higher along the TSH spectrum within euthyroid and SCH. Given that, the current classification of euthyroidism, SCH, and HT may be less sensitive to earlier thyroid disturbances within euthyroidism, in particular in relationship to insulin resistance–related dyslipidemia/dyslipoproteinemia.

We studied an apparently healthy population of women who were predominantly insulin sensitive (89% with HbA1c < 5.7%). Should the progressive insulin resistance–related dyslipidemia along thyroid hypofunction have any impact on clinical outcomes as incident T2D or CVD, even small relative risk may have a high population impact. This is due to the very broad generalizability of our findings, as euthyroid and SCT women comprise 97.3% of middle-aged women in the United States [1]. Previous studies have found strong associations for LPIR with incident T2D [21, 22], and also in the current Women’s Health Study population [23]. The robust association between thyroid and LPIR may be a pathophysiological pathway of its hypofunction toward T2D. For CVD, despite neutral risk with increasing TSH levels within the euthyroid range [24], the role of SCH is still open to debate [25]. In these contexts, our findings suggest that lipids and lipoproteins are potentially important mediators for therapeutic targeting beyond TSH replacement.

The literature regarding euthyroid and hypothyroid relationships with the panel of biomarkers that we examined is rather mixed. For standard lipids, the association of thyroid hypofunction with low HDL cholesterol and high triglycerides has been shown in a population study with >30,000 euthyroid people [12]. Alternatively, in the Framingham Offspring Study population, there was no association between TSH and either HDL cholesterol or triglycerides [11]. Additionally, in the 2 studies mentioned previously there was higher total and LDL cholesterol for increasing TSH. However, it is not possible to infer to which extent population profile and adjustment models explain the divergent results.

Regarding lipoprotein subclasses, Hernández-Mijares [26] found, similarly to the current study, an increasing proportion of LDL pattern B profile patients from euthyroid to SCH.

However, they did not address LDL subclasses across the euthyroidism range. In contrast, Pearce *et al.* [11] found lipoprotein subclass profiles totally opposite to the present results, toward insulin sensitivity in analyses adjusted for age, BMI, systolic blood pressure, anti-hypertensive treatment, diabetes, smoking, HDL cholesterol, and triglycerides. Nevertheless, thyroid hypofunction seems to equally affect HDL cholesterol, triglyceride, and lipoprotein subclasses toward insulin resistance profile. After further adjusting our current models for HDL and triglycerides, lipoprotein subclass profiles on SCH and HT were null or compatible with insulin sensitiveness (data not shown), similar to the study by Pearce *et al.* [11]. Thus, the collinear effect of thyroid hypofunction on HDL, triglyceride, and lipoprotein subclasses could justify those paradoxical results.

Regarding inflammation, in our study SCH and HT were associated with high hs-CRP, but not after adjustment for BMI. Lee *et al.* [27] also found no association of thyroid function with hs-CRP adjusted for BMI, which raises the hypothesis of BMI mediation between thyroid and inflammation. Also, Hueston *et al.* [28] did not find association of SCH with hs-CRP or homocysteine levels in a US adult population (National Health and Nutrition Examination Survey). However, the authors did not adjust for smoking, which is positively associated with inflammation [29] and inversely with HT [30]. This is corroborated by the fact that in our study there was no hs-CRP difference for euthyroid, SCH, and HT when smoking was not adjusted for (data not shown). Regarding homocysteine, Zhou *et al.* [13] also did not find an association with SCH, but they did find a positive association with HT. Nevertheless, in that meta-analysis, there was evidence for study heterogeneity and publication bias. Fibrinogen, a marker of hypercoagulation state, was similarly not associated with HT in other studies [31, 32], despite their limited adjustment for confounders. sICAM-1, associated with initiation and formation of atherosclerosis in animal models [33], and GlycA, a novel composite marker of acute phase glycoproteins signaling chronic inflammation [20], were both not associated with thyroid hypofunction in the present study. To the best of our knowledge, the association of euthyroidism, SCH, and HT with sICAM-1 and GlycA has not been reported previously. Finally, reverse causation for thyroid function and inflammation should be considered. The most common cause for thyroid hypofunction is Hashimoto disease [34], which may confound the association by its inflammatory pathophysiology. We could not address this hypothesis due to unavailable thyroid peroxidase antibodies.

A. Underlying Mechanisms

Thyroid hormones act as modulators of cholesterol synthesis and degradation through key enzymes. One of the main mechanisms is the stimulus of thyroid hormones over sterol regulatory element-binding protein 2, which in turn induces LDL receptor gene expression [35]. However, it was shown that the association of HT and higher LDL cholesterol levels is present only in insulin-resistant subjects [36]. Indeed, the lack of LDL cholesterol differences could be explained by our insulin-sensitive study population (low HbA1c levels). HT has also been associated with lower catabolism of lipid-rich lipoproteins by lipoprotein lipase [37], hepatic lipase [38], and decreased activity of cholesterol ester transfer protein [39] that mediates exchanges of cholesteryl esters of HDL particles with triglyceride-rich LDL and VLDL particles. These mechanisms might explain the relationship of thyroid hypofunction with atherogenic and insulin-resistant lipid and lipoprotein abnormalities.

Finally, the milder differences noted in HbA1c compared with LPIR across thyroid categories may be explained by the earlier effects of insulin resistance on lipoprotein metabolism than on glucose metabolism [40].

B. Strengths and Limitations

Our study has a relatively large sample of apparently healthy middle-aged and older women free of CVD, which may limit generalizability to men or higher risk woman. The study design is cross-sectional, which limits any causal inferences. Additionally, there is no information

about duration of thyroid dysfunction as well as thyroid hormone replacement and/or thyroid-modulating drugs. In spite of that, these factors may have limited impact given that our sample is composed of apparently healthy women on enrollment. We had an unprecedented comprehensive set of biomarkers, which offered a broad perspective on pathways between thyroid hypofunction and cardiometabolic risk. Despite the large number of biomarkers examined, insulin resistance is the common biological mechanism that is likely underlying the overall findings. Therefore, it is improbable that our results were due to chance alone.

4. Conclusion

In this large population of apparently healthy women, individuals with SCH had differences in their biomarker profile that indicated worsening lipoprotein insulin resistance and higher cardiometabolic risk compared with euthyroid individuals, despite having similar LDL cholesterol and total cholesterol levels. These findings suggest that cardiometabolic risk may increase early in the progression toward SCH and overt HT.

Acknowledgments

Address all correspondence to: Samia Mora, MD, Center for Lipid Metabolomics, Division of Preventive Medicine, Brigham and Women's Hospital, 900 Commonwealth Avenue, Boston, Massachusetts 02215. E-mail: smora@partners.org.

P.H.N.H. was funded by the Lemann Foundation. The Women's Health Study was funded by National Institutes of Health Grants CA047988, HL043851, HL080467, HL099355, and UM1 CA182913. Additional funding was received from a charitable gift from the Molino Family Trust. Atherotech Diagnostics provided an institutional grant to the Brigham and Women's Hospital for measuring the thyroid assays. S.M. received funding from National Heart, Lung, and Blood Institute of the National Institutes of Health (Grant HL 117861).

Author contributions: S.M. and P.H.N.H., as the guarantors of this manuscript, state that all of the authors approved submission of the manuscript. Both authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the analyses. The views expressed in this manuscript are those of the authors only.

Clinical trial registry: Women's Health Study no. NCT00000479 (registered 27 October 1999) (<http://clinicaltrials.gov/ct/show/NCT00000479>).

Disclosure Summary: M.E.C. was Chief Medical Officer at Atherotech Diagnostics Laboratory and is an Advisor/Researcher/Speaker for Amarin, Amgen, Astra Zeneca, Janssen, Sanofi, and Regeneron. K.R.K. was an employee of Atherotech Diagnostics. S.M. received research grant support from Atherotech Diagnostics and National Heart, Lung, and Blood Institute (Grant HL 117861); is consultant to Lilly, Pfizer, Amgen, Quest Diagnostics, and Cerenis Therapeutics; and is co-inventor on a patent on the use of NMR-measured GlycA for predicting the risk of colorectal cancer. The remaining authors have nothing to disclose.

References and Notes

1. Canaris GJ, Manowitz NR, Mayor G, Ridgway EC. The Colorado thyroid disease prevalence study. *Arch Intern Med*. 2000;**160**(4):526–534.
2. Wartofsky L, Dickey RA. The evidence for a narrower thyrotropin reference range is compelling. *J Clin Endocrinol Metab*. 2005;**90**(9):5483–5488.
3. Surks MI, Goswami G, Daniels GH. The thyrotropin reference range should remain unchanged. *J Clin Endocrinol Metab*. 2005;**90**(9):5489–5496.
4. Gronich N, Deftereos SN, Lavi I, Persidis AS, Abernethy DR, Rennert G. Hypothyroidism is a risk factor for new-onset diabetes: a cohort study. *Diabetes Care*. 2015;**38**(9):1657–1664.
5. Walsh JP, Bremner AP, Bulsara MK, O'Leary P, Leedman PJ, Feddema P, Michelangeli V. Subclinical thyroid dysfunction as a risk factor for cardiovascular disease. *Arch Intern Med*. 2005;**165**(21):2467–2472.
6. Razvi S, Weaver JU, Vanderpump MP, Pearce SH. The incidence of ischemic heart disease and mortality in people with subclinical hypothyroidism: reanalysis of the Whickham Survey cohort. *J Clin Endocrinol Metab*. 2010;**95**(4):1734–1740.

7. LeGrys VA, Funk MJ, Lorenz CE, Giri A, Jackson RD, Manson JE, Schectman R, Edwards TL, Heiss G, Hartmann KE. Subclinical hypothyroidism and risk for incident myocardial infarction among postmenopausal women. *J Clin Endocrinol Metab.* 2013;**98**(6):2308–2317.
8. Cappola AR, Fried LP, Arnold AM, Danese MD, Kuller LH, Burke GL, Tracy RP, Ladenson PW. Thyroid status, cardiovascular risk, and mortality in older adults. *JAMA.* 2006;**295**(9):1033–1041.
9. Biondi B, Klein I. Hypothyroidism as a risk factor for cardiovascular disease. *Endocrine.* 2004;**24**(1):1–13.
10. Maratou E, Hadjidakis DJ, Kollias A, Tsegka K, Peppas M, Alevizaki M, Mitrou P, Lambadiari V, Boutati E, Nikzas D, Tountas N, Economopoulos T, Raptis SA, Dimitriadis G. Studies of insulin resistance in patients with clinical and subclinical hypothyroidism. *Eur J Endocrinol.* 2009;**160**(5):785–790.
11. Pearce EN, Wilson PW, Yang Q, Vasan RS, Braverman LE. Thyroid function and lipid subparticle sizes in patients with short-term hypothyroidism and a population-based cohort. *J Clin Endocrinol Metab.* 2008;**93**(3):888–894.
12. Asvold BO, Vatten LJ, Nilsen TI, Bjørø T. The association between TSH within the reference range and serum lipid concentrations in a population-based study. The HUNT Study. *Eur J Endocrinol.* 2007;**156**(2):181–186.
13. Zhou Y, Chen Y, Cao X, Liu C, Xie Y. Association between plasma homocysteine status and hypothyroidism: a meta-analysis. *Int J Clin Exp Med.* 2014;**7**(11):4544–4553.
14. Lee IM, Cook NR, Gaziano JM, Gordon D, Ridker PM, Manson JE, Hennekens CH, Buring JE. Vitamin E in the primary prevention of cardiovascular disease and cancer: the Women's Health Study: a randomized controlled trial. *JAMA.* 2005;**294**(1):56–65.
15. Mora S, Rifai N, Buring JE, Ridker PM. Comparison of LDL cholesterol concentrations by Friedewald calculation and direct measurement in relation to cardiovascular events in 27,331 women. *Clin Chem.* 2009;**55**(5):888–894.
16. Kamstrup PR, Benn M, Tybjaerg-Hansen A, Nordestgaard BG. Extreme lipoprotein(a) levels and risk of myocardial infarction in the general population: The Copenhagen City Heart Study. *Circulation.* 2008;**117**:176–84.
17. Jeyarajah EJ, Cromwell WC, Otvos JD. Lipoprotein particle analysis by nuclear magnetic resonance spectroscopy. *Clin Lab Med.* 2006;**26**(4):847–870.
18. Shalaurova I, Connelly MA, Garvey WT, Otvos JD. Lipoprotein insulin resistance index: a lipoprotein particle-derived measure of insulin resistance. *Metab Syndr Relat Disord.* 2014;**12**(8):422–429.
19. Ridker PM, Rifai N, Rose L, Buring JE, Cook NR. Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. *N Engl J Med.* 2002;**347**(20):1557–1565.
20. Otvos JD, Shalaurova I, Wolak-Dinsmore J, Connelly MA, Mackey RH, Stein JH, Tracy RP. GlycA: a composite nuclear magnetic resonance biomarker of systemic inflammation. *Clin Chem.* 2015;**61**(5):714–723.
21. Mackey RH, Mora S, Bertoni AG, Wassel CL, Carnethon MR, Sibley CT, Goff DC, Jr. Lipoprotein particles and incident type 2 diabetes in the multi-ethnic study of atherosclerosis. *Diabetes Care.* 2015;**38**(4):628–636.
22. Dugani SB, Akinkuolie AO, Paynter N, Glynn RJ, Ridker PM, Mora S. Association of lipoproteins, insulin resistance, and rosuvastatin with incident type 2 diabetes mellitus: secondary analysis of a randomized clinical trial. *JAMA Cardiol.* 2016;**1**(2):136–145.
23. Harada PH, Demler OV, Dugani S, Akinkuolie A, Moorhty MV, Ridker PR, Cook NR, Pradhan A, Mora S. Lipoprotein Insulin Resistance Score and the Risk of Incident Diabetes: The Women's Health Study. Orlando, FL; American Heart Association Conference; 2015.
24. Åsvold BO, Vatten LJ, Bjørø T, Bauer DC, Bremner A, Cappola AR, Ceresini G, den Elzen WP, Ferrucci L, Franco OH, Franklyn JA, Gussekloo J, Iervasi G, Imaizumi M, Kearney PM, Khaw KT, Maciel RM, Newman AB, Peeters RP, Psaty BM, Razvi S, Sgarbi JA, Stott DJ, Trompet S, Vanderpump MP, Völzke H, Walsh JP, Westendorp RG, Rodondi N; Thyroid Studies Collaboration. Thyroid function within the normal range and risk of coronary heart disease: an individual participant data analysis of 14 cohorts. *JAMA Intern Med.* 2015;**175**(6):1037–1047.
25. Rodondi N, Bauer DC. Subclinical hypothyroidism and cardiovascular risk: how to end the controversy. *J Clin Endocrinol Metab.* 2013;**98**(6):2267–2269.
26. Hernández-Mijares A, Jover A, Bellod L, Bañuls C, Solá E, Veses S, Víctor VM, Rocha M. Relation between lipoprotein subfractions and TSH levels in the cardiovascular risk among women with subclinical hypothyroidism. *Clin Endocrinol (Oxf).* 2013;**78**(5):777–782.

27. Lee WY, Suh JY, Rhee EJ, Park JS, Sung KC, Kim SW. Plasma CRP, apolipoprotein A-1, apolipoprotein B and Lpa levels according to thyroid function status. *Arch Med Res*. 2004;**35**(6):540–545.
28. Hueston WJ, King DE, Geesey ME. Serum biomarkers for cardiovascular inflammation in subclinical hypothyroidism. *Clin Endocrinol (Oxf)*. 2005;**63**(5):582–587.
29. Bermudez EA, Rifai N, Buring JE, Manson JE, Ridker PM. Relation between markers of systemic vascular inflammation and smoking in women. *Am J Cardiol*. 2002;**89**(9):1117–1119.
30. Åsvold BO, Bjørø T, Nilsen TI, Vatten LJ. Tobacco smoking and thyroid function: a population-based study. *Arch Intern Med*. 2007;**167**(13):1428–1432.
31. Mazur P, Sokołowski G, Hubalewska-Dydejczyk A, Płaczkiewicz-Jankowska E, Undas A. Prothrombotic alterations in plasma fibrin clot properties in thyroid disorders and their post-treatment modifications. *Thromb Res*. 2014;**134**(2):510–517.
32. Toruner F, Altinova AE, Karakoc A, Yetkin I, Ayvaz G, Cakir N, Arslan M. Risk factors for cardiovascular disease in patients with subclinical hypothyroidism. *Adv Ther*. 2008;**25**(5):430–437.
33. Nakashima Y, Raines EW, Plump AS, Breslow JL, Ross R. Upregulation of VCAM-1 and ICAM-1 at atherosclerosis-prone sites on the endothelium in the ApoE-deficient mouse. *Arterioscler Thromb Vasc Biol*. 1998;**18**(5):842–851.
34. Pearce EN, Farwell AP, Braverman LE. Thyroiditis. *N Engl J Med*. 2003;**348**(26):2646–2655.
35. Shin DJ, Osborne TF. Thyroid hormone regulation and cholesterol metabolism are connected through sterol regulatory element-binding protein-2 (SREBP-2). *J Biol Chem*. 2003;**278**(36):34114–34118.
36. Bakker SJ, ter Maaten JC, Popp-Snijders C, Slaets JP, Heine RJ, Gans RO. The relationship between thyrotropin and low density lipoprotein cholesterol is modified by insulin sensitivity in healthy euthyroid subjects. *J Clin Endocrinol Metab*. 2001;**86**(3):1206–1211.
37. Lithell H, Boberg J, Hellsing K, Ljunghall S, Lundqvist G, Vessby B, Wide L. Serum lipoprotein and apolipoprotein concentrations and tissue lipoprotein-lipase activity in overt and subclinical hypothyroidism: the effect of substitution therapy. *Eur J Clin Invest*. 1981;**11**(1):3–10.
38. Valdemarsson S, Nilsson-Ehle P. Hepatic lipase and the clearing reaction: studies in euthyroid and hypothyroid subjects. *Horm Metab Res*. 1987;**19**(1):28–30.
39. Tan KC, Shiu SW, Kung AW. Effect of thyroid dysfunction on high-density lipoprotein subfraction metabolism: roles of hepatic lipase and cholesteryl ester transfer protein. *J Clin Endocrinol Metab*. 1998;**83**(8):2921–2924.
40. Sørensen LP, Søndergaard E, Nellemann B, Christiansen JS, Gormsen LC, Nielsen S. Increased VLDL-triglyceride secretion precedes impaired control of endogenous glucose production in obese, normoglycemic men. *Diabetes*. 2011;**60**(9):2257–2264.