

Hyperhomocysteinemia and hypercholesterolemia associated with hypothyroidism in the third US National Health and Nutrition Examination Survey

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Abstract

Hypothyroid (thyroid stimulating hormone (TSH) ≥ 20 mIU/l; $N = 32$) participants in the third National Health and Nutrition Examination Survey, Phase 2 (1991–1994) were compared with non-hypothyroid subjects (0.5 mIU/l $<$ TSH < 20 mIU/l; $N = 6490$) to examine the relationship between hypothyroidism and hyperhomocysteinemia (serum total homocysteine > 12 μ mol/l) and hypercholesterolemia (serum total cholesterol > 6.2 mmol/l). After controlling for age, gender, and race ethnicity, the odds ratios (95% confidence interval (CI)) relating hypothyroidism to hyperhomocysteinemia and high total cholesterol were 4.9 (1.8–14.0) and 8.0 (2.9–21.9), respectively. Based on 26 hypothyroid and 5811 non-hypothyroid subjects with triglyceride concentration ≤ 2.82 mmol/l, the odds ratio for the relationship between hypothyroidism and high low-density lipoprotein (LDL)-cholesterol (> 4.6 mmol/l by the Friedewald equation) was 5.3 (95% CI, 1.3–20.9). Adding additional terms to the multivariate logistic regression model had little effect on the odds ratios relating hypothyroidism to high total or LDL-cholesterol, but adding terms for serum creatinine concentration > 123.8 μ mol/l and for red blood cell folate and serum vitamin B-12 concentrations resulted in an attenuated, but still significant ($P < 0.05$), odds ratio relating hypothyroidism to hyperhomocysteinemia (2.5; 95% CI, 1.0–6.1). Controlling for cigarette smoking, heart attack/stroke history, body mass index, and serum albumin concentration did not affect the odds ratios. Hyperhomocysteinemia and hypercholesterolemia could help to explain the increased risk for arteriosclerotic coronary artery disease in hypothyroidism. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

Hypothyroidism has been linked to an increased risk for arteriosclerotic coronary artery disease [1]. This relationship may be due in part to proatherogenic changes in lipoprotein metabolism accompanying the

hypothyroid state [1]. Recently, Nedrebø et al. [2] reported elevated concentrations of plasma total homocysteine (tHcy), a putative arteriosclerotic risk factor [3], in two groups of hypothyroid patients referred for determination of thyroid stimulating hormone levels, as compared with controls drawn from an independent study of middle-aged Norwegians.

We sought to determine whether relationships between hypothyroidism and hypercholesterolemia and hyperhomocysteinemia could be observed in a general-population sample, in the third US National Health and Nutrition Examination Survey (NHANES III).

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2. Methods

2.1. Subjects

Subjects were participants in the NHANES III, Phase 2 (1991–1994), conducted by the National Center for Health Statistics (NCHS), Centers for Disease Control and Prevention (CDC), to obtain nationally representative data on the health and nutritional status of the civilian, non-institutionalized US population through interviews and direct physical examinations [4]. All respondents gave their informed consent, and the NHANES III protocol was reviewed and approved by the NCHS NHANES Institutional Review Board.

2.2. Blood collection

Total Hcy concentrations were measured in surplus sera from Phase 2 participants aged ≥ 12 years. Of 13 635 interviewed subjects, 10 280 agreed to be physically examined. Blood was drawn across a range of fasting states and processed according to a standard protocol [4]. Whole blood was collected in serum separator tubes and held at room temperature for 30–60 min before centrifugation; no anticoagulant was used. Sera were separated, frozen at -20°C , and transferred on dry ice to the CDC central laboratory for priority analyses.

2.3. Laboratory determinations

Concentrations of total cholesterol, high-density lipoprotein (HDL)-cholesterol, triglycerides, and thyroid stimulating hormone (TSH) were measured in sera according to standardized protocols [4]. Surplus sera were stored at -70°C for up to 3 years and underwent one to four freeze–thaw cycles before tHcy analysis. Long-frozen plasma samples have been shown to be acceptable for characterizing plasma tHcy concentration at the time of phlebotomy [5]. After approval by the New England Medical Center Human Investigations Review Committee and the Surplus Sera Bank Steering Committee, tHcy concentration was measured at the US Department of Agriculture Human Nutrition Research Center on Aging by the high-performance liquid chromatography method of Araki and Sako [6]. Hcy measurements were obtained for 8585 (64%) physically examined Phase 2 participants aged ≥ 12 years. Missing tHcy values for examinees are explained by lack of either blood samples or surplus sera.

2.4. Classification of hypothyroidism

TSH concentrations and data on prescription drug use were used to classify subjects as hypothyroid

(TSH ≥ 20 mIU/l) [7] or non-hypothyroid (not using L-thyroxine, TSH ≥ 0.5 mIU/l and TSH < 20 mIU/l).

2.5. Statistical methods

All hypothyroid subjects were ≥ 15 years old, and none were using cholesterol-lowering medications. We thus restricted entry into the non-hypothyroid category to subjects aged ≥ 15 years who were not on cholesterol-lowering drugs.

The NHANES III oversampled young children, older persons, blacks, and Mexican Americans. To account for this and other design complexities, we used SUDAAN statistical software [8] and sample-weighted analytic procedures [4]. Data files were created and manipulated with SAS [9]. $P < 0.05$ was considered statistically significant.

We first described subjects stratified by hypothyroid status relative to tHcy, total cholesterol, low-density lipoprotein (LDL)-cholesterol, triglycerides, HDL-cholesterol, and potential confounders of relationships between hypothyroidism and hyperhomocysteinemia and hypercholesterolemia. Least-squares means and proportions were generated by linear regression and cross-tabulation procedures. Geometric means were reported for tissue constituents whose distributions were highly skewed (i.e. red blood cell folate, vitamin B-12, triglycerides, creatinine, and tHcy). We used linear regression analysis to identify those factors among the potential confounders that were related to serum tHcy concentration and serum total cholesterol concentration.

Odds ratios and 95% CIs were estimated via multiple logistic regression analyses for associations between hypothyroid status and three dichotomous response variables: high tHcy, > 12 $\mu\text{mol/l}$; high total cholesterol, > 6.2 mmol/l; and high LDL-cholesterol, > 4.5 mmol/l. Referent categories comprised subjects with values up to and including the cut-off value. We calculated LDL-cholesterol (mmol/l) by the Friedewald equation (total cholesterol – HDL-cholesterol – triglycerides/2.2) for subjects ($N = 5811$) with triglyceride concentrations ≤ 2.82 mmol/l — a cut-off point chosen to minimize LDL-cholesterol underestimation [10].

We built four multivariate models including terms for potential confounders as follows: (1) basic — gender, age, race ethnicity; (2) basic-plus — demographic factors plus pack-years of cigarette smoking, heart attack/stroke history, serum albumin concentration, and, depending on the relationship being evaluated, hyperhomocysteinemia or hypercholesterolemia; (3) intermediate — basic-plus model factors plus frankly abnormal serum creatinine concentration (i.e. > 123.8 $\mu\text{mol/l}$); (4) full — intermediate model variables plus red blood cell folate concentration and serum vitamin B-12 concentration. We also used the full logistic re-

gression model to compare the non-hypothyroid group with L-thyroxine-treated subjects with serum TSH concentration between 0.5 and 20 mIU/l.

We used multiple linear regression analysis to examine possible linear relationships between serum TSH concentration and serum concentrations of tHcy (log transformation) and total cholesterol. Because serum creatinine concentration might be decreased among subjects with TSH concentrations in the hyperthyroid range, serum creatinine concentration was controlled for in these analyses by a continuously scaled term (log-transformed serum creatinine concentration). We also used linear regression modeling to estimate least-squares geometric mean serum tHcy concentration and least-squares mean total cholesterol concentration for six TSH categories. The cut-off points between the categories were selected to form the following groups: hyperthyroid subjects, TSH undetectable ($N = 7$); possibly subclinically hyperthyroid subjects, TSH < 0.5

mIU/l ($N = 396$); reference range, TSH ≥ 0.5 mIU/l and TSH < 10 mIU/l ($N = 6547$); possibly subclinically hypothyroid subjects, TSH ≥ 10 mIU/l and TSH < 20 mIU/l ($N = 52$); and two subgroups of hypothyroid subjects, TSH ≥ 20 mIU/l and TSH ≤ 40 mIU/l ($N = 21$), TSH > 40 mIU/l ($N = 11$).

3. Results

Of 7034 subjects with known values for tHcy, total cholesterol, and potential confounders of the relationships of interest, 120 lacked a TSH value. Of the remainder, 32 were classified as hypothyroid and 6490 were classified as non-hypothyroid. Subjects were excluded from comparisons between hypothyroid and non-hypothyroid groups if they had TSH concentration < 0.5 ($N = 403$) or if they were non-hypothyroid under thyroid medication use ($N = 109$). Group sizes for LDL-cholesterol analyses were 26 hypothyroid and 5811 non-hypothyroid.

Hypothyroid subjects were older than non-hypothyroid subjects, and they were somewhat more likely to be female and non-Hispanic white (Table 1). With these differences controlled, the serum tHcy and total cholesterol concentrations of hypothyroid subjects were significantly higher than those of non-hypothyroid subjects, and their LDL-cholesterol and triglyceride concentrations were somewhat higher. Hypothyroid subjects were also significantly more likely than non-hypothyroid subjects to have frankly abnormal serum creatinine concentrations. Groups did not differ significantly relative to any other examined factors, but hypothyroid subjects were somewhat more likely than non-hypothyroid subjects to have had heart attacks or strokes, and they also showed some tendency towards longer smoking histories, higher body mass indices, and lower red blood cell folate and serum vitamin B-12 concentrations.

Of the potential confounders of relationships between hypothyroidism and hypercholesterolemia and hyperhomocysteinemia listed in Table 1, age, female gender, body mass index, and non-Hispanic white race ethnicity were positively related to serum total cholesterol concentration. Marginally significant ($P < 0.1$) positive relationships were found between cholesterol level and serum vitamin B-12 concentration and pack-years of cigarette smoking, and a marginally significant inverse relationship was found for frankly abnormal serum creatinine concentration.

Age, male gender, heart attack/stroke history, frankly abnormal serum creatinine concentration, pack-years of cigarette smoking, and serum albumin concentration were positively related to serum total homocysteine concentration. Significant inverse relationships were found between homocysteine concentra-

Table 1

Characteristics of NHANES III, Phase 2 (1991–1994), participants, by hypothyroid status

Characteristic	Hypothyroid ($N = 32$)	Non-hypothyroid ($N = 6490$)
Gender (% female)	70.3	50.0
Race ethnicity (% white)	84.1	74.2
Age (mean) ^a	52.1	42.1
Smoking (mean, pack-years) ^b	15.6	10.0
Previous heart attack or stroke (%) ^b	12.4	3.8
Body mass index (mean) ^b	29.9	26.6
Serum creatinine (geometric mean, $\mu\text{mol/l}$) ^b	102.5	92.8
Serum creatinine > 123.8 $\mu\text{mol/l}$ (%) ^c	21.5	3.0
Red blood cell folate (geometric mean, nmol/l) ^b	308.0	403.4
Serum vitamin B-12 (geometric mean, pmol/l) ^b	265.1	320.5
Serum albumin (mean, g/l) ^b	41.6	41.2
Serum tHcy (geometric mean, $\mu\text{mol/l}$) ^{b,c}	12.4	8.8
Serum triglycerides (geometric mean, mmol/l) ^b	1.6	1.3
Serum total cholesterol (mean, mmol/l) ^{b,c}	6.1	5.2
Serum HDL-cholesterol (mean, mmol/l) ^b	1.3	1.3
Serum LDL-cholesterol (mean, mmol/l) ^{b,d}	3.9	3.2

^a $P < 0.05$ for H_0 : the hypothyroid categories have the same mean or proportion.

^b Means are adjusted for differences between groups in gender, age, and race ethnicity.

^c $P < 0.05$ for H_0 : the two groups have the same mean or proportion (controlled for gender, age, race ethnicity).

^d Subjects with valid LDL-cholesterol values only; hypothyroid, $N = 26$; non-hypothyroid, $N = 5811$.

Table 2
Odds ratios and 95% CIs relating hypothyroid status to hyperhomocysteinemia and hypercholesterolemia in NHANES III, Phase 2 (1991–1994)

Model	Response variable	Hypothyroid status [<i>N</i> (% ^a)]		OR	95% CI
		Yes	No		
Basic ^b	THcy > 12 μmol/l ^c	14 (63.7)	1096 (17.7)	7.9	4.5–13.9
	THcy > 12 μmol/l ^d	17 (53.7)	1235 (17.8)	4.9	1.8–14.0
	LDL-cholesterol > 4.4 mmol/l ^c	9 (41.5)	548 (9.0)	5.3	1.3–20.9
	TC > 6.2 mmol/l ^d	18 (66.8)	1130 (16.8)	8.0	2.9–21.9
Intermediate ^c	THcy > 12 μmol/l ^c	14 (63.7)	1096 (17.7)	5.4	2.9–10.3
	THcy > 12 μmol/l ^d	17 (53.7)	1235 (17.8)	3.6	1.4–9.2
	LDL-cholesterol > 4.4 mmol/l ^c	9 (41.5)	548 (9.0)	5.0	1.2–21.4
	TC > 6.2 mmol/l ^d	18 (66.8)	1130 (16.8)	7.9	2.6–23.4
Full ^f	THcy > 12 μmol/l ^c	14 (63.7)	1096 (17.7)	4.0	2.4–6.6
	THcy > 12 μmol/l ^d	17 (53.7)	1235 (17.8)	2.5	1.0–6.1
	LDL-cholesterol > 4.4 mmol/l ^c	9 (41.5)	548 (9.0)	5.2	1.1–24.9
	TC > 6.2 mmol/l ^d	18 (66.8)	1130 (16.8)	8.1	3.0–21.8

^a Percentages are sample-weighted percentages generated with SUDAAN PROC CROSSTAB.

^b The basic model included terms for gender, age, and race ethnicity.

^c Analyses involving subjects qualifying for LDL-cholesterol estimation.

^d Analyses involving all hypothyroid and non-hypothyroid subjects.

^e The intermediate model included terms for basic model variables plus terms for body mass index, pack-years of cigarette smoking, heart attack/stroke history, high serum creatinine concentration, serum albumin concentration, and either hypercholesterolemia or hyperhomocysteinemia.

^f The full model included terms for intermediate model variables plus terms for serum vitamin B-12 concentration and red blood cell folate concentration.

tion and Mexican–American race ethnicity, red blood cell folate concentration, and serum vitamin B-12 concentration.

About 18% of non-hypothyroid subjects were hyperhomocysteinemic, and about 17% were hypercholesterolemic (Table 2). In contrast to this, more than one-half of the hypothyroid subjects were hyperhomocysteinemic, and about two-thirds were hypercholesterolemic. Furthermore, almost 17% of hypothyroid subjects had serum tHcy concentrations > 20 μmol/l — levels observed in only about 2% of the non-hypothyroid subjects (data not shown).

With demographic characteristics controlled (Table 2), odds ratios (ORs) comparing hypothyroid subjects with non-hypothyroid subjects were as follows: high tHcy, 4.9 (95% CI, 1.8–14.0); high total cholesterol, 8.0 (95% CI, 2.9–21.9); high LDL-cholesterol, 5.3 (95% CI, 1.3–20.9). Adding terms for body mass index, serum albumin concentration, pack-years of cigarette smoking, and heart attack/stroke history attenuated the odds ratios only slightly, and results obtained using the basic-plus model are not shown. The addition of a term for frankly abnormal serum creatinine concentration to the basic-plus model resulted in a markedly weaker but still strong and significant relationship (OR, 3.6; 95% CI, 1.4–9.2) between hypothyroidism and hyperhomocysteinemia. Control for this factor, however, had little effect on the odds ratios relating hypothyroidism to hypercholesterolemia. Addition of terms for red blood cell folate concentration and serum vitamin B-12 concentration caused a further reduction in the odds ratio

relating hypothyroidism to hyperhomocysteinemia (OR, 2.5; 95% CI, 1.0–6.1), but the relationship remained statistically significant. Once again, odds ratios relating hypothyroidism to hypercholesterolemia were unaffected.

Regardless of the multivariate model used, L-thyroxine-treated non-hypothyroid subjects (*N* = 109) were not more likely than other non-hypothyroid subjects to be hyperhomocysteinemic or hypercholesterolemic. Odds ratios (95% CI) estimated from the full multivariate model were 1.1 (0.6–1.9), 1.3 (0.5–3.5), and 1.6 (0.4–6.0) for high tHcy concentration, high total cholesterol concentration, and high LDL-cholesterol concentration, respectively (data not shown). It is also noteworthy that the odds ratio (95% CI) from the full multivariate model comparing thyroxine-using non-hypothyroid subjects with non-thyroxine-using non-hypothyroid subjects for frankly abnormal serum creatinine concentration was 1.1 (0.5–2.3), as compared with 9.9 (3.1–31.0) for hypothyroid subjects versus non-hypothyroid subjects.

When serum TSH concentration was modeled as a single continuously scaled term in linear regression models controlled only for demographic factors, it was positively related to both serum total cholesterol concentration (*P* = 0.006) and serum tHcy concentration (*P* = 0.03). With the full multiple linear regression model, TSH remained significantly related to serum total cholesterol concentration (*P* = 0.011) but not to serum tHcy concentration (*P* = 0.230). LDL-cholesterol was not significantly related to TSH in either model.

Fig. 1 shows the results of modeling (full multivariate model) serum total cholesterol concentration and serum tHcy concentration on TSH categories. The trend of increasing serum total cholesterol concentration across the six categories from the hyperthyroid range, through the normal range, to the hypothyroid range was highly statistically significant ($P < 0.001$). Although mean total cholesterol concentration of the seven clearly hyperthyroid subjects was not significantly lower than that of subjects with TSH values between 0.5 and 10 mIU/l (referent category), the mean for subjects with detectable TSH concentrations below 0.5 mIU/l was significantly lower than the mean for subjects in the reference category. Furthermore, mean total cholesterol concentration for subjects with TSH concentration > 40 mIU/l was significantly higher than that of subjects in the referent category. LDL-cholesterol concentration did not increase significantly over the six categories ($P = 0.127$), and the only significant difference from the referent group was a highly significantly decreased mean LDL-cholesterol concentration for the small hyperthyroid group (data not shown).

In analyses not described in figures or tables, geometric mean serum creatinine concentration was 92.8 $\mu\text{mol/l}$ for subjects in all TSH categories except the highest, for whom it was increased, and the two lowest, for whom it was decreased. The seven hyperthyroid subjects and the 11 severely hypothyroid subjects differed from subjects in the referent category in one other respect per group: Hyperthyroid subjects had significantly decreased serum albumin concentrations, and severely hypothyroid subjects had significantly decreased red blood cell folate concentrations.

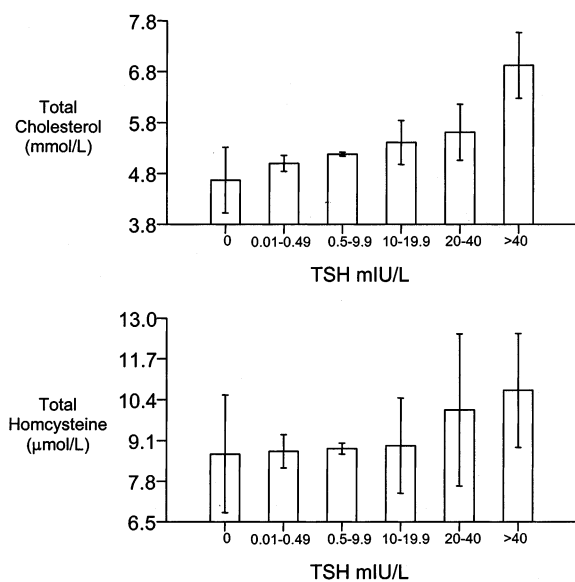


Fig. 1. Mean serum total cholesterol concentration and mean serum total homocysteine concentration by serum TSH category from the hyperthyroid through the hypothyroid range.

A multiple linear regression model without a term for serum creatinine concentration yielded mean total cholesterol concentrations for the six TSH categories that were nearly identical to those shown in the figure from the full multivariate model. The two models, however, produced somewhat different results for serum tHcy concentration. Specifically, when serum creatinine concentration was not controlled for, geometric mean serum tHcy for the hyperthyroid subgroup was non-significantly lower, at 7.8 $\mu\text{mol/l}$, than the mean for the referent category (8.8 $\mu\text{mol/l}$). When serum creatinine concentration was controlled for, however, geometric mean serum tHcy for the hyperthyroid group was only slightly lower than that of the referent group. Also, when serum creatinine concentration was not controlled for, geometric mean serum tHcy for severely hypothyroid subjects was 12.4 $\mu\text{mol/l}$, as compared with 10.7 $\mu\text{mol/l}$ from the model containing the term for serum creatinine concentration. The difference between the severely hypothyroid group and the referent group was statistically significant with both models, however, and, with both models, serum tHcy concentration increased significantly across the six TSH categories ($P = 0.012$; full model).

4. Discussion

This study of the general US population revealed positive relationships between hypothyroidism and both hypercholesterolemia and hyperhomocysteinemia. These findings are consistent with results of several other recent investigations [2,11–13].

With only seven definitely hyperthyroid subjects, we had limited ability to evaluate possible effects of hyperthyroidism on homocysteine and lipid profiles. However, our results were consistent with those of Canaris et al. [12] who observed a graded increase in lipid levels as TSH concentration increased from the hyperthyroid to the hypothyroid range. Our findings also corroborate the report of Nedrebø et al. [2] of no difference in serum total homocysteine concentrations between hyperthyroid and euthyroid subjects upon multivariate analysis. Our results further suggested that non-significantly decreased homocysteine concentrations in hyperthyroidism were due to low creatinine concentrations, possibly reflective of increased glomerular filtration rate (GFR) [14].

Hypercholesterolemia in hypothyroidism may result from reduced catabolism of lipoproteins [15,16] — a phenomenon that may be explained by a reversible decreased expression of lipoprotein receptors [15]. The pathogenesis of mild hyperhomocysteinemia in hypothyroidism likely involves a reduction in GFR, given the strong, independent association between homocysteine concentrations and GFR throughout the norma-

tive range of renal function [17], and the well-established effects of hypothyroidism on the kidney [18]. Mean serum creatinine concentration was not higher in hypothyroid versus non-hypothyroid subjects in this study. However, having a frankly abnormal serum creatinine concentration, which was strongly related to serum total homocysteine concentration, was ten times as common among the hypothyroid NHANES III participants as it was among the non-hypothyroid subjects. Controlling for high serum creatinine concentration reduced but did not eliminate the relationship between hypothyroidism and hyperhomocysteinemia. The remaining association could also be due to reduced GFR, since serum creatinine concentration is an inaccurate indicator of mildly impaired GFR [19,20]. Circulating homocysteine concentrations can also rise through reduced activity of the flavoprotein methylenetetrahydrofolate reductase (MTHFR) [21], an enzyme involved in the catalysis of homocysteine and its remethylation to methionine. Hypothyroid individuals can be defective in converting riboflavin to the co-enzyme flavin-adenine dinucleotide [22,23] and, consequently, deficient in MTHFR activity [24].

Almost 90% of hypothyroid subjects in the US population were either hypercholesterolemic or hyperhomocysteinemic, as compared with only 31% of non-hypothyroid subjects (data not shown). The high prevalence of hypercholesterolemia and hyperhomocysteinemia in hypothyroidism may contribute to the association between hypothyroidism and arteriosclerotic vascular disease [1].

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