THYROID
Volume 24, Number 8, 2014

DOI: 10.1089/thy.2014.0106

Subclinical Hypothyroidism and Risk for Incident Ischemic Stroke Among Postmenopausal Women

Ayush Giri,[1] Todd L. Edwards,[1] Vicky A. LeGrys,[2] Carol E. Lorenz,[3] Michele Jonsson Funk,[4] Robin Schectman,[5] Gerardo Heiss,[4] Jennifer G. Robinson,[6] and Katherine E. Hartmann[1,7]

Background: Subclinical hypothyroidism (SCH) is postulated to increase stroke risk via atherogenic changes associated with abnormal thyroid function. However, the direct relationship of SCH with subsequent stroke is poorly studied.
Methods: In this nested case–cohort study, we prospectively evaluated the association between any SCH and severity of SCH in relation to incident ischemic stroke risk among postmenopausal women in the Women's Health Initiative Observational Study. Trained Women's Health Initiative staff, masked to thyroid status, adjudicated stroke cases. We assessed thyroid function using baseline blood specimens. Women with normal free thyroxine levels and thyrotropin (TSH) levels ≥4.69 mU/L were considered to have SCH. Primary analysis included 639 ischemic stroke cases and 2927 randomly selected subcohort members with an average of seven years of follow-up.
Results: The multivariable adjusted hazard ratios (HR) from weighted Cox models were 1.06 (95% confidence interval [CI]: 0.77, 1.46) and 0.99 (95% CI: 0.67, 1.47) for women with any SCH and with mild SCH (TSH 4.69 to 6.99 mU/L), when compared with women with normal thyroid function. The HR for moderate/severe SCH (TSH ≥7.00 mU/L) was modestly elevated (HR: 1.22; 95% CI: 0.73, 2.05).
Conclusions: We found no evidence to suggest an association between SCH and ischemic stroke among healthy postmenopausal women.

Introduction

Subclinical hypothyroidism (SCH) is a condition in which thyroid hormone levels are within the normal range but thyrotropin (TSH) levels are elevated, and may be indicative of deteriorating thyroid function (1). This pre-clinical condition is more common with advancing age and may affect 10–15% of postmenopausal women (2–4). Screening for SCH has been a persistent area of discussion for nearly two decades. Whether screening for SCH is warranted, for which population, and at what age, remain major points of contention. The United States Preventive Services Task Force and the Institute of Medicine Medicare reports concluded there is insufficient evidence to endorse screening or treatment of SCH in part because of scant scientific literature specifically focused on the potential value of screening for or treatment of SCH (5,6). No clinical trials have been completed to determine whether identification and treatment of SCH prevents cardiovascular outcomes.

Indirect evidence for or against screening can be drawn from observational studies that evaluate the relationship between SCH and cardiovascular diseases. SCH has been reported to be associated with the grouping of conditions loosely termed coronary heart disease (7,8), ischemic heart disease (9), or cardiovascular disease (10), with definitions including but not limited to myocardial infarction (MI), angina, heart failure, stroke, and other cerebrovascular conditions. However, evidence for an association is not consistent and ranges from strong effects (two-fold increased risk) to null (8). Discrepancies likely arise from the heterogeneity of the composite outcomes, varying follow-up time, and differing population characteristics such as age range, sex, and varied cardiovascular disease status at baseline. Mixed findings extend to the relationship

[1]Institute for Medicine and Public Health, Vanderbilt Epidemiology Center, Vanderbilt University, Nashville, Tennessee.
[2]Division of Clinical Laboratory Science, School of Medicine, University of North Carolina, Chapel Hill, North Carolina.
[3]Department of Epidemiology, Center for Women's Health Research, [4]Gillings School of Global Public Health, and [5]Cecil G. Sheps Center for Health Services Research, University of North Carolina, Chapel Hill, North Carolina.
[6]Departments of Epidemiology and Medicine, College of Public Health, University of Iowa, Iowa City, Iowa.
[7]Department of Obstetrics and Gynecology, Vanderbilt University Medical School, Nashville, Tennessee.

between SCH and intermediate endpoints such as atherosclerotic disease and lipid profiles (11,12).

Because the goals of screening for primary prevention are to identify individuals in a preclinical phase in order to reduce morbidity and mortality, motivation to screen for SCH must include specific outcomes that could be prevented if SCH were identified and treated. Using Women's Health Initiative Observational Study (WHI-OS) data comparable to that for this investigation, we have previously reported that an association of SCH with incident myocardial infarction is not supported by prospective data in postmenopausal women (13). The WHI-OS (14) is a well-characterized cohort of ethnically diverse postmenopausal women with biological samples available at baseline, a high follow-up rate, and a rigorous adjudication process for outcomes.

To round out examination of the association of SCH with cardiovascular disease by investigation of specific outcomes, our research team focused on whether there is evidence to support a link between SCH and incident ischemic stroke among healthy postmenopausal women, a population with high prevalence of SCH. Only two other studies, which were underpowered, have reported on the association between SCH and stroke (15,16). Our investigation improves on these previous studies. We employed an efficient nested case–cohort study design to reach sufficient power in the largest prospective study to date that can examine for main effects as well as for analyses by severity of SCH as it relates to incident ischemic stroke.

Methods

Study population

Women in this study were participants in the WHI-OS, a cohort of postmenopausal women between 50 and 79 years of age enrolled at 40 clinical centers across the United States between October 1993 and December 1998 (17). Inclusion/exclusion criteria for this ancillary analysis have been detailed previously (13). Briefly, from 93,676 women in the WHI-OS, women with anticipated residence in the local area for at least three years with baseline serum available in the specimen bank were eligible for inclusion. Exclusion criteria were self-reported history of current or previous thyroid disease, use of medications that may alter thyroid hormones, history of MI or stroke, history of coronary artery bypass grafting or carotid endarterectomy, and missing race/ethnicity data.

Ischemic stroke cases

WHI investigators conducted ascertainment of incident ischemic stroke cases every 12 months via review of self-reported annual medical follow-up updates (18). Ischemic stroke was defined as the rapid onset of persistent neurologic deficit lasting for 24 hours or more due to an obstruction, fatal or nonfatal, or detection of lesion through computed tomography or magnetic resonance imaging scan. Fatal and nonfatal locally adjudicated ischemic stroke cases through December 15, 2004 that were additionally confirmed by central adjudication by trained neurologists were considered for inclusion. Adjudicators were masked to participants' thyroid status. Reports of transient ischemic attacks and hemorrhagic strokes were not considered in this study. A total of 1050 cases were identified. After applying our inclusion/exclusion criteria, 675 cases were eligible for this analysis. Figure 1 provides details of exclusions. Because our goal was to examine the effects of SCH at baseline on future ischemic stroke risk, we further excluded observations that our laboratory analyses identified as having overt thyroid disease ($n=9$) or subclinical hyperthyroidism ($n=16$). Participants who had negative follow-up time ($n=6$) or for whom laboratory measures were unavailable ($n=5$) were also excluded. A total of 639 incident ischemic stroke cases were included in the primary unadjusted analysis.

Comparison subcohort

By design, the subcohort for this study was generated to serve as a common comparison group for two separate analyses— one that evaluates SCH and risk for MI (13) and the present analysis. The same inclusion/exclusion criteria used for selection of MI and stroke cases were also applied to the entire WHI-OS cohort. We used random sampling and density matching within five-year age strata to match the age distribution of the 800 MI and 675 stroke cases, identifying 3200 members for the comparison subcohort (Fig. 1). We obtained stored specimens for the subcohort and conducted the same laboratory analyses as for case groups; observations in the subcohort with subclinical hyperthyroidism, with overt thyroid disease, and with no laboratory measures were excluded. After exclusions, the subcohort consisted of 2927 observations available for primary unadjusted analysis.

Thyroid hormone measurement

Laboratory procedures for thyroid measurements have been described in detail previously (13). Briefly, specimens were analyzed between March 2005 and December 2006.

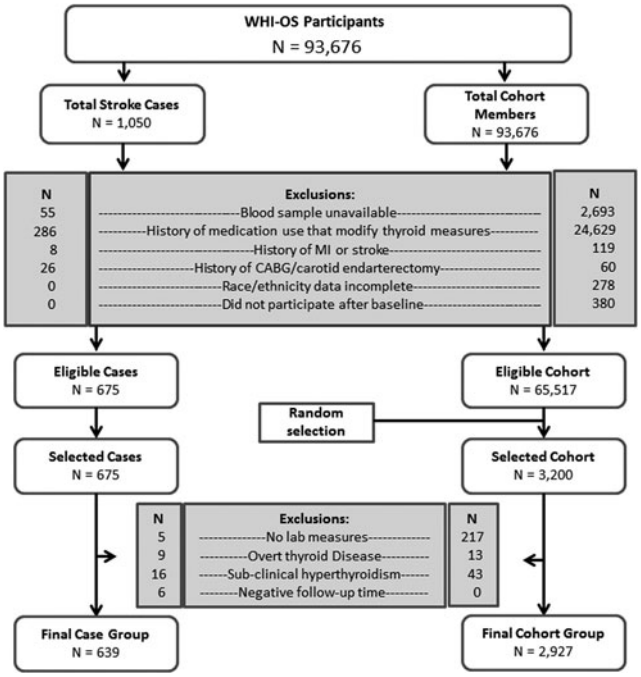

FIG. 1. Schema representing the selection process for ischemic stroke cases and subcohort from the Women's Health Initiative Observational Study (WHI-OS). Adapted with permission from LeGrys *et al.* (13).

Laboratory personnel were blind to participant characteristics including stroke or MI status. TSH was measured using a chemiluminescent immunometric assay with a detection range from 0.01 to 100 mU/L. TSH values between 0.46 and 4.68 mU/L were considered the normal reference range (euthyroid). Specimens with TSH values above or below the normal reference range were tested for free thyroxine (FT4) and thyroid peroxidase antibodies (TPOAb). FT4 was measured using a competitive chemiluminescent assay with a detection range of 0.1–12.0 ng/dL. FT4 concentrations within 0.70–1.85 ng/dL were the normal reference range. TPOAb was measured using a solid-phase, sequential chemiluminescent immunometric assay with a detection range of 10–1000 IU/mL. Specimens with TPOAb concentrations ≥35 IU/mL were considered to be TPOAb positive. All samples included in the study met quality control criteria. Samples with TSH values ≥4.69 mU/L and normal FT4 levels were classified as indicating SCH.

Statistical analyses

The sampling strategy of a case–cohort design allows resampling of cases into the subcohort portion of the population. Thus, pseudo-likelihood based methods must be used to account for the non-independent score contributions. We used weighted Cox-proportional hazards models as proposed by Barlow and colleagues (19) along with jackknife estimated robust standard errors (20) to appropriately account for the non-independence and provide reliable estimates of the hazard ratio (HR) and variance. We report unadjusted and multivariable adjusted associations between SCH and stroke. Candidate covariates were chosen *a priori* as either potential confounders or established risk factors for stroke. These included age, race/ethnicity, WHI site, years since menopause, gravidity, parity, hypertension, current diabetes, hormone therapy (HT), smoking history, alcohol consumption, physical activity, body mass index, and waist-to-hip ratio.

We developed a parsimonious model using directed acyclic graphs to include only confounders in the assessment of any SCH and its relationship to stroke. We did not impute missing data and report hazard ratios based on complete-case analysis. There were 626 cases and 2843 subcohort members available in the primary parsimonious model examining any SCH and stroke. Variables included in the parsimonious models were age, ethnicity, smoking status, HT use, gravidity, and alcohol consumption. All subsequent models were adjusted for these variables. Additional adjustment for risk factors such as diabetes status, physical activity, waist-to-hip ratio, and/or body mass index in a fully adjusted model changed effect estimates negligibly. In addition to examining the association between any SCH and stroke, we conducted separate analyses by stratum of SCH severity, restricted to exposed individuals who had mild SCH (TSH 4.69 to 6.99 mU/L), moderate/severe SCH (TSH ≥7.00 mU/L), and individuals who had moderate/severe SCH and were TPOAb-positive. All were compared to women with normal thyroid function. We used the likelihood ratio test to formally evaluate effect modification of the association between severities of SCH and stroke by age, smoking status, waist-to-hip ratio, ethnicity, alcohol consumption, gravidity, and HT use. We tested the proportional hazards (PH) assumption by visual inspection of log–log survival curves and by including an interaction term for natural log–transformed follow-up time and the SCH variables in the Cox-models. We detected no gross violations of the PH assumption.

We conducted several sensitivity analyses. First, we re-examined the association between SCH and stroke by removing individuals with potentially impending overt thyroid disease (TSH ≥20 mU/L). Second, to facilitate comparisons with other studies which report varying thresholds for SCH (8,21,22), we reanalyzed data by changing the TSH threshold for SCH, decreasing it to 4.50 mU/L and increasing it to 5.00 mU/L. Third, to examine the association between SCH and stroke without the influence of intercurrent diagnosis and treatment of thyroid disease, we restricted our analysis to women who had follow-up information on these factors in year three, and then excluded women who reported using thyroid medications. Data compilation and bivariate analyses were done in STATA 12 (College Station, TX). Weighted Cox-PH models were generated using SAS 9.3 (Cary, NC). This study was reviewed and determined to be exempt from further review by the Institutional Review Board at the University of North Carolina, Chapel Hill, and was approved by the Institutional Review Board at Vanderbilt University.

Results

The mean age of the 3566 women included in this case–cohort analysis was 67.5 years, and the majority self-identified as Caucasian/white (83.9%). Baseline thyroid measures indicated 280 women (7.8%) had SCH (TSH ≥4.69 mU/L), the majority (68.2%) of whom had mild SCH (TSH 4.69 to 6.99 mU/L), and the remaining had moderate/severe SCH (TSH ≥7.00 mU/L). Increasing age was positively associated with SCH, and whites were more likely to have SCH than other races/ethnicities. At baseline, compared with participants in the subcohort, stroke cases were more likely to be older, heavier, and more likely to have had 5 or more pregnancies. Stroke cases were also more likely to be current smokers, and have diabetes and hypertension. Stroke cases were less likely to consume alcohol and less likely to be physically active, although the latter was not statistically significant (Table 1). Baseline characteristics of the randomly sampled subcohort are very similar to the entire WHI-OS cohort (17).

Cumulative stroke incidence curves for euthyroid women, women with mild SCH, and women with moderate/severe SCH during the average 7-year follow-up period are shown in Fig. 2. Risk for stroke among women with any SCH at baseline was not appreciably different than for women with normal thyroid function at baseline (Table 2); the corresponding multivariable adjusted hazard ratio was 1.06 [95% confidence interval (CI): 0.77, 1.46]. Compared with women with normal thyroid function, HRs for women with mild SCH and moderate/severe SCH were 0.99 [CI: 0.67, 1.47] and 1.22 [CI: 0.73, 2.05], respectively. Women who had moderate/severe SCH and were also TPOAb positive had a hazard ratio of 0.83 [CI: 0.42, 1.64] (Table 3).

We found no meaningful interactions with age, smoking status, ethnicity, alcohol consumption, or gravidity (Supplementary Table S1; Supplementary Data are available online at www.liebertpub.com/thy). There was marginal evidence for effect modification by HT status on the relationship of moderate SCH and stroke risk ($p = 0.07$), with higher risk associated with moderate SCH for current (HR:

Table 1. Distribution of Baseline Characteristics and Cardiovascular Risk Factors According to Case and Subcohort Status Among 3566 Women from the Women's Health Initiative Observational Study

Characteristics	*Stroke cases (N = 639)*	*Subcohort (N = 2927)*	p-*Value**
	N (%)	*N* (%)	
Age at baseline (years)			0.006
50–64	116 (26.0)	950 (32.5)	
65–70	208 (32.5)	862 (29.4)	
71–79	265 (41.5)	1115 (38.1)	
Race or ethnic group			0.126
White	543 (85.2)	2448 (83.8)	
Black	62 (9.6)	242 (8.3)	
Hispanic	10 (1.6)	84 (2.9)	
Asian or Pacific Islander	14 (2.2)	108 (3.7)	
American Indian	4 (0.6)	15 (0.5)	
Other	4 (0.6)	24 (0.8)	
Waist-to-hip ratio			0.001
≤ 0.80	268 (42.1)	1437 (49.4)	
> 0.80–0.85	163 (25.6)	719 (24.7)	
> 0.85	205 (32.3)	755 (25.9)	
Physical activity level			0.081
None/mild	335 (53.0)	1389 (48.2)	
Moderate	179 (28.3)	871 (30.3)	
Strenuous	118 (18.7)	619 (21.5)	
Smoking			0.003
Never	337 (53.0)	1528 (53.1)	
Past	246 (38.7)	1204 (41.9)	
Current	53 (8.3)	143 (5.0)	
Alcohol			0.007
< 1 drink per week	416 (65.4)	1705 (58.7)	
1–6 drinks per week	140 (22.0)	784 (27.0)	
7+ drinks per week	80 (12.6)	417 (14.3)	
Diabetes			< 0.000
No	559 (88.7)	2777 (95.5)	
Yes	71 (11.3)	131 (4.5)	
Gravidity			
Never pregnant	54 (8.5)	293 (10.1)	
1	37 (5.8)	188 (6.5)	
2–4	353 (55.7)	1737 (59.7)	
5+	190 (30.0)	691 (23.7)	
Hormone use			0.592
Never	288 (45.1)	1342 (45.9)	
Past	101 (15.8)	496 (17.0)	
Current	250 (39.1)	1086 (37.1)	
Years from menopause			< 0.000
0–15	157 (27.7)	847 (31.8)	
16–23	176 (31.0)	956 (35.9)	
24–26	234 (41.3)	861 (32.3)	
Hypertension			< 0.000
Never	211 (33.2)	1549 (53.2)	
Past or controlled	128 (20.1)	577 (19.8)	
Current	297 (46.7)	785 (27.0)	

Numbers do not always total 3566 due to missing data for covariates.

**p*-Value: calculated using the Pearson's chi-squared test.

1.78; [CI: 0.56, 5.61]) and past (HR: 2.37; [CI: 0.52, 10.85]) HT users than for women who reported never (HR: 0.69; [CI: 0.30, 1.60]) using HT.

In our first sensitivity analysis, excluding women who had impending overt hypothyroidism (TSH ≥ 20 mU/L) yielded similar results as those for women with any SCH (HR: 1.05; [CI: 0.76, 1.45]), and those with moderate/severe SCH (HR: 1.19; [CI: 0.69, 2.05]). Similarly, in our second analysis, changing the TSH threshold from 4.69 mU/L to 4.50 mU/L or to 5.00 mU/L did not substantially change our results. Third, among women who reported information on thyroid medication use at 3 years of follow-up, exclusion of women who were using thyroid medications yielded similar results (HR: 0.91; [CI: 0.74, 1.13]) for any SCH.

Discussion

In this large case–cohort investigation of postmenopausal women from the WHI-OS, we did not find evidence to suggest an association between SCH at baseline and risk for incident ischemic stroke. Compared with women with normal thyroid function, effect estimates were only modestly elevated for women with moderate/severe SCH (TSH ≥ 7.00 mU/mL); however, the confidence interval included unity. Projecting 750 stroke cases, a 1:4 case–cohort ratio, and a type-1 error of 5%, *a priori* approximation of the minimum detectable effect estimate was 1.43 with 90% power if prevalence of SCH was 10%, and 1.81 with 80% power if prevalence of SCH was 2%. Post-hoc calculations based on population available for analysis (639 cases, 2927 comparison cases) demonstrated a 7.8% SCH prevalence; with a type-1 error rate of 5%, we had 80% power to detect an effect estimate of 1.51. Despite targeting our investigation to a population with high prevalence of SCH and relatively high incidence of stroke, the effect estimates we observed for SCH were very close to the null.

Our search of the literature identified only two prospective cohort studies (Table 3) that specifically reported on SCH and stroke risk. They have diametrically opposed and underpowered associations (15,16). Rodondi and colleagues reported larger effect estimates for participants with moderate SCH, defined as TSH 7.00 to 9.99 mU/L (HR: 2.16; [CI: 0.93, 5.00]; with 7 strokes) than for those with mild SCH, defined as TSH 4.5 to 6.9 mU/L (HR: 1.13; [CI: 0.62, 2.07]; 12 strokes), during a 4-year follow-up (15). Schultz and colleagues reported a hazard ratio of 0.74 [CI: 0.10, 5.50] for participants with SCH compared with euthyroid participants with 31 SCH cases at baseline and 28 incident stroke cases (one stroke case in the SCH group) (16). Effect estimates from both studies lack precision, as indicated by the confidence intervals. Two other studies reported the association between SCH and incident cerebrovascular disease, including stroke events. One observed a null effect-estimate (21), HR of 1.01 [CI: 0.79, 1.29], and the other observed elevated risk with an HR of 1.9 [CI: 0.5, 7.30] (22); neither performed analyses using information about severity of SCH.

The mechanisms relating overt hypothyroidism to cardiovascular and cerebrovascular conditions including coronary heart disease, MI, and stroke are well understood. Overt hypothyroidism has been associated with atherogenic lipid profiles, diastolic hypertension, impaired endothelial function, hyperhomocysteinemia, and increased C-reactive protein levels (23–26). The mechanisms relating SCH to cerebrovascular

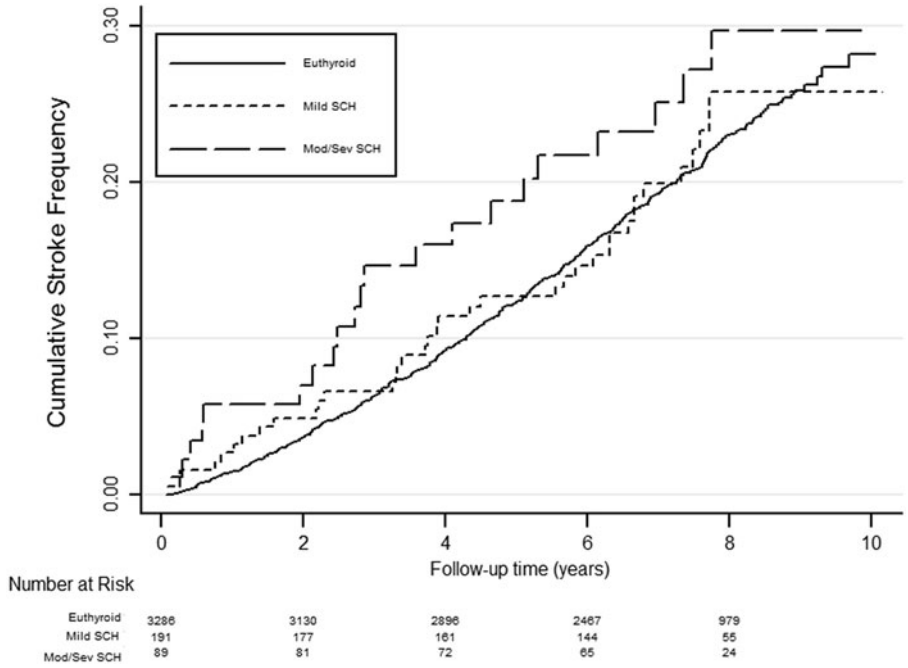

FIG. 2. Nelson-Aalen cumulative hazard estimates for ischemic stroke by severity of subclinical hypothyroidism (SCH) among women from the WHI-OS. Solid line represents euthyroid participants; short-dashed line represents participants with mild subclinical hypothyroidism; long-dashed line represents individuals with moderate/severe subclinical hypothyroidism. Mod/sev, moderate/severe; Euthyroid, thyrotropin (TSH) 0.46–4.68 mU/L; Mild SCH, TSH 4.69–6.99 mU/L; Mod/Sev SCH, TSH ≥7.00 mU/L.

TABLE 2. ISCHEMIC STROKE HAZARD RATIOS [95% CI] IN RELATION TO ANY SUBCLINICAL HYPOTHYROIDISM, ITS SEVERITY, AND PRESENCE OF THYROID PEROXIDASE ANTIBODY AMONG WOMEN FROM THE WOMEN'S HEALTH INITIATIVE OBSERVATIONAL STUDY

Thyroid condition	*N (case/cohort)*	*HR [95% CI]*
Unadjusted[a]		
Euthyroid	582/2704	1.00 [Referent]
Any SCH[b]	57/223	1.13 [0.83–1.53]
Mild[c]	36/155	1.05 [0.72–1.53]
Moderate/severe[d]	21/68	1.30 [0.79–2.15]
Moderate/severe & TPOAb positive[e]	11/51	0.90 [0.47–1.75]
Natural log-transformed TSH (continuous)[f]	639/2927	1.01 [0.99–1.04]
Unadjusted[g]		
Euthyroid	569/2626	1.00 [Referent]
Any SCH[b]	57/217	1.16 [0.85–1.57]
Mild[c]	36/151	1.07 [0.74–1.56]
Moderate/severe[d]	21/66	1.34 [0.81–2.22]
Moderate/severe and TPOAb positive[e]	11/49	0.94 [0.49–1.83]
Natural log-transformed TSH (continuous)[f]	626/2843	1.02 [0.99–1.04]
Multivariable adjusted[h]		
Euthyroid	569/2626	1.00 [Referent]
Any SCH[b]	57/217	1.06 [0.77–1.46]
Mild[c]	36/151	0.99 [0.67–1.47]
Moderate/severe[d]	21/66	1.22 [0.73–2.05]
Moderate/severe & TPOAb positive[e]	11/49	0.83 [0.42–1.64]
Natural log-transformed TSH (continuous)[f]	626/2843	1.01 [0.98–1.04]

[a]Sample size based on entire sample.
[b]Any subclinical hypothyroid vs. euthyroid.
[c]Mild subclinical hypothyroid (thyrotropin [TSH] 4.69 to 7.0 mU/L) vs. euthyroid (moderate/severe category omitted).
[d]Moderate/severe subclinical hypothyroid (TSH ≥ 7.0 mU/L) vs. euthyroid (mild category omitted).
[e]Moderate/severe and thyroid peroxidase antibody (TPOAb) positive vs. euthyroid (mild and TPOAb negative omitted).
[f]Cox regression was modeled with ln(TSH) as a continuous variable; corresponding hazard ratios are for every 20% increase in TSH.
[g]Sample size based on same observations used in the adjusted model.
[h]Adjusted for age, ethnicity, gravidity, smoking status, hormone therapy, and alcohol consumption; covariates measured at baseline only.
CI, 95% confidence interval; HR, hazard ratio; SCH, subclinical hypothyroidism.

Table 3. Characteristics of Past Studies Reporting the Relationship Between SCH and Stroke Risk

Study characteristics	*Schultz* et al. (*16*)	*Rodondi* et al. (*15*)
Average follow-up time	5 years	4 years
% Women	58.2%	50.9%
Total euthyroid	549	2392
Euthyroid stroke cases	23	131
Total SCH	31	338
SCH stroke cases	1	22
HR [95% CI]	0.77 [0.10, 5.50]	1.37 [0.62, 2.07]

Total euthyroid, total number of participants with normal thyroid at baseline; euthyroid stroke cases, total number of euthyroid participants who developed stroke during study follow-up; total SCH, total number of participants with SCH at baseline; SCH stroke cases, number of SCH participants who developed stroke during study follow-up.

conditions such as stroke are less clearly understood. Studies have shown SCH to be associated with increased carotid arterial stiffness (27), elevated serum low-density-lipoprotein cholesterol (28,29) and apolipoprotein-B (30), elevated mean platelet volume (31), and altered low-density-lipoprotein-C oxidizability (32); however, evidence has not been consistent (12). In the context of vascular diseases, it is likely that the downstream effects of overt hypothyroidism are due to reduced thyroxine levels rather than elevated TSH, which serves as an indicator of the dysfunction. If SCH is in fact associated with stroke or MI, it would likely be due to similar mechanisms as with overt hypothyroidism. Contrary to expectations, the hazard ratio for individuals with moderate/severe SCH who were also TPOAb positive was in the inverse direction in our study.

Several studies have observed improvement in cardiovascular outcomes associated with overt hypothyroidism and SCH after treatment with L-thyroxine (30,33,34), although results are inconsistent. Even though FT4 levels among patients with SCH are within ''normal range,'' there is a continuum of FT4 levels within this group, with some individuals presenting with FT4 concentrations closer to the overt hypothyroidism spectrum. This is also evident in our study; we observed a mean decrease in FT4 levels with increasing severity of SCH. Mean FT4 concentrations in individuals with mild (TSH 4.69 to 6.99 mU/L), moderate (TSH 7.00 to 9.99 mU/L), and severe SCH (TSH $\geq$10.00 mU/L) were 1.03, 0.94, and 0.86 ng/dL respectively (data not shown). Thus lower thyroxine levels in more severe forms of SCH could diminish its inhibitory effects on collagen-induced platelet aggregation and increase vascular resistance. Hypothesizing that SCH-mediated onset of coronary heart disease and stroke share similar pathways, Rodondi and colleagues conducted a meta-analysis of SCH and coronary heart disease and found elevated risk in participants with severe SCH (TSH $\geq$10.00 mU/L), but not in participants with relatively milder forms of SCH (8), a likely consequence of decreased FT4. Similarly, McQuade and colleagues reported a lack of association between mild-SCH and all-cause mortality, but reported statistically significant hazard ratios for those with moderate SCH and severe SCH, all compared with euthyroid participants (35). Additionally, studies have suggested an inverse correlation between serum FT4 levels and atherosclerosis among euthyroid patients (36,37). The antagonistic effect of estrogen use on efficacy of L-thyroxine therapy has been documented (38). Our observation of higher effect estimates for stroke in relation to moderate/severe SCH among HT users than among those who never used HT is consistent with the idea that FT4 may play a more active role in mediating the relationship between SCH and cardiovascular risk than TSH. However, evidence for this effect modification is only suggestive ($p = 0.07$) in our study and the possibility of chance as an explanation is not to be ruled out.

A plausible reason for observing no association between any SCH and stroke is potential misclassification of SCH subjects over the course of the study, which could have biased our results towards the null. We measured thyroid hormones only at baseline and were unable to track thyroid status after baseline except by claim. Somwaru and colleagues reported 46% of participants with mild SCH reverted from SCH to euthyroid status over a two-year period (39). However, in the same study as few as 10% of the participants with moderate SCH (TSH 7.0 to 9.9 mU/L) and 7% of the participants with severe SCH (TSH $\geq$10.0 mU/L) reverted back to euthyroid status during the same 2-year period. Thus, in our study, error due to misclassification in the moderate/severe group is likely to be low. Furthermore, we are unable to assess stroke risk by FT4 levels because only specimens above and below the normal TSH reference range were tested for FT4. Additionally, we did not assess stroke risk exclusively among individuals with severe SCH due to the small number of participants that met this criterion. It is also possible that an average of 7 years (interquartile range: 6.0 to 8.4 years) of follow-up was not sufficient to detect the cumulative effect of mild SCH on stroke occurrence. Finally, lacking information on lipid characteristics, we were not able to examine the mediating effects of lipids on SCH-mediated stroke occurrence. It should however be noted that even if this information were available and we assume that this model is correct, adjustment for lipid characteristics would only further work to nullify any elevated association between SCH and stroke, consequently strengthening the current conclusion of this investigation.

Despite these drawbacks, to our knowledge, this is the largest study (639 stroke cases) to date to have explicitly examined SCH and risk for stroke. The size of the study allowed us to report the marginal association between SCH and stroke, after adjusting for potential confounders. Our sample was also large enough to report effect estimates by severity of SCH. Furthermore, we conducted multiple sensitivity analyses, which did not appreciably change our results. Density-matched sampling of the comparison cohort from the entire eligible WHI-OS ensured a good approximation of the risk ratio and minimized the possibility of selection bias because cases and subcohort both originated from the same base population.

In this population of postmenopausal women with no history of cardiovascular conditions, we did not find evidence of association between SCH and risk for incident ischemic stroke. This was particularly true for mild SCH. In conjunction with biological rationale, there is still reason to speculate that individuals with more severe forms of SCH may be at increased risk for stroke. However, given the lower prevalence of moderate/severe SCH and the modest elevation of stroke risk (if any) associated with it, its impact on a population level is likely to be small and thus the utility of screening to forestall stroke outcomes extremely low or

absent. The European Commission recently funded the Thyroid Hormone Replacement for Untreated Older Adults with Subclinical Hypothyroidism Trial (40), which examines the relationship between thyroid hormone treatment and several cardiovascular and noncardiovascular outcomes. Until the results of the trial are published, efficient observational studies such as ours provide the best available evidence for clinical or policy decisions.

Acknowledgments

The WHI program is funded by the National Heart, Lung, and Blood Institute, National Institutes of Health, US Department of Health and Human Services through contracts N01WH22110, 24152, 32100-2, 32105-6, 32108-9, 32111-13, 32115, 32118-32119, 32122, 42107-26, 42129-32, and 44221. The authors thank the WHI investigators and staff for their dedication, and the study participants for making the program possible. The complete list of WHI centers and investigators can be found online at https://www.whi.org/researchers/Documents%20%20Write%20a%20Paper/WHI%20Investigator%20Long%20List.pdf This nested case–cohort study within the Women's Health Initiative Observational Study was supported by a grant funded by the NHLBI, NIH (RO1 HL076645). NHLBI had no influence on the study design, data collection, analysis and interpretation, or approval of this manuscript.

Author Disclosure Statement

The authors have nothing to disclose.

References

1. McDermott MT 2001 Subclinical hypothyroidism is mild thyroid failure and should be treated. J Clin Endocrinol Metab **86:**4585–4590.
2. Bagchi N, Brown TR, Parish RF 1990 Thyroid dysfunction in adults over age 55 years: a study in an urban US community. Arch Intern Med **150:**785–787.
3. Canaris GJ, Manowitz NR, Mayor G, Ridgway EC 2000 The Colorado thyroid disease prevalence study. Arch Intern Med **160:**526–534.
4. Vanderpump MPJ, Tunbridge WMG, French JM, Appleton D, Bates D, Clark F, Evans JG, Hasan DM, Rodgers H, Bridge F, Young ET 1995 The incidence of thyroid disorders in the community: a twenty-year follow-up of the Whickham Survey. Clin Endocrinol **43:**55–68.
5. Helfand M 2004 Screening for subclinical thyroid dysfunction in nonpregnant adults: a summary of the evidence for the US Preventive Services Task Force. Ann Intern Med **140:**128–141.
6. Stone MB, Wallace RB 2003 Medicare coverage of routine screening for thyroid dysfunction. National Academies Press, Washington, DC.
7. Tseng FY, Lin WY, Lin CC, Lee LT, Li TC, Sung PK, Huang KC 2012 Subclinical hypothyroidism is associated with increased risk for all-cause and cardiovascular mortality in adults. J Am Coll Cardiol **60:**730–737.
8. Rodondi N, den Elzen WPJ, Bauer DC, Cappola AR, Razvi S, Walsh JP, Asvold BO, Iervasi G, Imaizumi M, Collet TH, Bremner A, Maisonneuve P, Sgarbi JA, Khaw KT, Vanderpump MPJ, Newman AB, Cornuz J, Franklyn JA, Westendorp RGJ, Vittinghoff E, Gussekloo J 2010 Subclinical hypothyroidism and the risk of coronary heart disease and mortality. JAMA **304:**1365–1374.
9. Razvi S, Weaver JU, Vanderpump MP, Pearce SHS 2010 The incidence of ischemic heart disease and mortality in people with subclinical hypothyroidism: reanalysis of the Whickham Survey cohort. J Clin Endocrinol Metabol **95:**1734–1740.
10. Walsh JP, Bremner AP, Bulsara MK, O'Leary P, Leedman PJ, Feddema P, Michelangeli V 2005 Subclinical thyroid dysfunction as a risk factor for cardiovascular disease. Arch Intern Med **165:**2467–2472.
11. Hak AE, Pols HAP, Visser TJ, Drexhage HA, Hofman A, Witteman JCM 2000 Subclinical hypothyroidism is an independent risk factor for atherosclerosis and myocardial infarction in elderly women: the Rotterdam Study. Ann Intern Med **132:**270–278.
12. Pearce EN 2012 Update in lipid alterations in subclinical hypothyroidism. J Clin Endocrinol Metab **97:**326–333.
13. LeGrys VA, Funk MJ, Lorenz CE, Giri A, Jackson RD, Manson JE, Schectman R, Edwards TL, Heiss G, Hartmann KE 2013 Subclinical hypothyroidism and risk for incident myocardial infarction among postmenopausal women. J Clin Endocrinol Metab **98:**2308–2317.
14. The Women's Health Initiative Study Group 1998 Design of the Women's Health Initiative clinical trial and observational study. Control Clin Trials **109:**61–109.
15. Rodondi N, Newman AB, Vittinghoff E, de Rekeneire N, Satterfield S, Harris TB, Bauer DC 2005 Subclinical hypothyroidism and the risk of heart failure, other cardiovascular events, and death. Arch Intern Med **165:**2460–2466.
16. Schultz M, Kistorp C, Raymond I, Dimsits J, Tuxen C, Hildebrandt P, Faber J 2011 Cardiovascular events in thyroid disease: a population based, prospective study. Horm Metab Res **43:**653–659.
17. Langer RD, White E, Lewis CE, Kotchen JM, Hendrix SL, Trevisan M 2003 The Women's Health Initiative Observational Study: baseline characteristics of participants and reliability of baseline measures. Ann Epidemiol **13:** S107–S121.
18. Curb JD, Mctiernan A, Heckbert SR, Kooperberg C, Stanford J, Nevitt M, Johnson KC, Proulx-Burns L, Pastore L, Criqui M, others 2003 Outcomes Ascertainment And Adjudication Methods In the Women's Health Initiative. Ann Epidemiol **13:**122–128.
19. Barlow WE, Ichikawa L, Rosner D, Izumi S 1999 Analysis of case-cohort designs. J Clin Epidemiol **52:**1165–1172.
20. Lin DY, Wei LJ 1989 The robust inference for the Cox proportional hazards model. JASA **84:**1074–1078.
21. Cappola AR, Fried LP, Arnold AM, Danese MD, Kuller LH, Burke GL, Tracy RP, Ladenson PW 2006 Thyroid status, cardiovascular risk, and mortality in older adults. JAMA **295:**1033–1041.
22. Imaizumi M, Akahoshi M, Ichimaru S, Nakashima E, Hida A, Soda M, Usa T, Ashizawa K, Yokoyama N, Maeda R, Nagataki S, Eguchi K 2004 Risk for ischemic heart disease and all-cause mortality in subclinical hypothyroidism. J Clin Endocrinol Metab **89:**3365–3370.
23. Christ-Crain M, Meier C, Guglielmetti M, Huber PR, Riesen W, Staub JJ, Müller B 2003 Elevated C-reactive protein and homocysteine values: cardiovascular risk factors in hypothyroidism? A cross-sectional and a double-blind, placebo-controlled trial. Atherosclerosis **166:**379–386.

24. Ichiki T 2010 Thyroid hormone and atherosclerosis. Vascul Pharmacol **52:**151–156.
25. Morris MS, Bostom AG, Jacques PF, Selhub J, Rosenberg IH 2001 Hyperhomocysteinemia and hypercholesterolemia associated with hypothyroidism in the third US National Health and Nutrition Examination Survey. Atherosclerosis **155:**195–200.
26. Sundaram V, Hanna AN, Koneru L, Newman HA, Falko JM 1997 Both hypothyroidism and hyperthyroidism enhance low density lipoprotein oxidation. J Clin Endocrinol Metab **82:**3421–3424.
27. Tian L, Gao C, Liu J, Zhang X 2010 Increased carotid arterial stiffness in subclinical hypothyroidism. Eur J Intern Med **21:**560–563.
28. Iqbal A, Jorde R, Figenschau Y 2006 Serum lipid levels in relation to serum thyroid-stimulating hormone and the effect of thyroxine treatment on serum lipid levels in subjects with subclinical hypothyroidism: the Troms++ study. J Intern Med **260:**53–61.
29. Walsh JP, Bremner AP, Bulsara MK, O'Leary P, Leedman PJ, Feddema P, Michelangeli V 2005 Thyroid dysfunction and serum lipids: a community-based study. Clin Endocrinol **63:**670–675.
30. Monzani F, Di V, Caraccio N, Bertini A, Giorgi D, Giusti C, Ferrannini E 2001 Effect of levothyroxine on cardiac function and structure in subclinical hypothyroidism: a double blind, placebo-controlled study. J Clin Endocrinol Metab **86:**1110–1115.
31. Kim JH, Park JH, Kim SY, Bae HY 2013 The mean platelet volume is positively correlated with serum thyrotropin concentrations in a population of healthy subjects and subjects with unsuspected subclinical hypothyroidism. Thyroid **23:** 31–37.
32. Duntas LH, Mantzou E, Koutras DA 2002 Circulating levels of oxidized low-density lipoprotein in overt and mild hypothyroidism. Thyroid **12:**1003–1007.
33. Danese MD 2000 Effect of thyroxine therapy on serum lipoproteins in patients with mild thyroid failure: a quantitative review of the literature. J Clini Endocrinol Metab **85:**2993–3001.
34. Shakoor SKA, Aldibbiat A, Ingoe LE, Campbell SC, Sibal L, Shaw J, Home PD, Razvi S, Weaver JU 2010 Endothelial progenitor cells in subclinical hypothyroidism: the effect of thyroid hormone replacement therapy. J Clin Endocrinol Metab **95:**319–322.
35. McQuade C, Skugor M, Brennan DM, Hoar B, Stevenson C, Hoogwerf BJ 2011 Hypothyroidism and moderate subclinical hypothyroidism are associated with increased all-cause mortality independent of coronary heart disease risk factors: a PreCIS database study. Thyroid **21:**837–843.
36. Auer J, Berent R, Weber T, Lassnig E, Eber B 2003 Thyroid function is associated with presence and severity of coronary atherosclerosis. Clin Cardiol **26:**569–573.
37. Bruckert E, Giral P, Chadarevian R, Turpin G 1999 Low free-thyroxine levels are a risk factor for subclinical atherosclerosis in euthyroid hyperlipidemic patients. Journal of cardiovascular risk **6:**327–331.
38. Arafah BM 2001 Increased need for thyroxine in women with hypothyroidism during estrogen therapy. New Engl J Med **344:**1743–1749.
39. Somwaru LL, Rariy CM, Arnold AM, Cappola AR 2012 The natural history of subclinical hypothyroidism in the elderly: the cardiovascular health study. J Clin Endocrinol Metab **97:**1962–1969.
40. Quinn TJ, Gussekloo J, Kearney P, Rodondi N, Stott DJ 2012 Subclinical thyroid disorders. Lancet **380:**335–336.

Address correspondence to:
Katherine E. Hartmann, MD, PhD
Department of Obstetrics and Gynecology
Vanderbilt University School of Medicine
2525 West End Avenue, Suite 600
Nashville, TN 37203

E-mail: katherine.hartmann@vanderbilt.edu