Thyrotropin and Thyroid Antibodies as Predictors of Hypothyroidism: A 13-Year, Longitudinal Study of a Community-Based Cohort Using Current Immunoassay Techniques

John P. Walsh, Alexandra P. Bremner, Peter Feddema, Peter J. Leedman, Suzanne J. Brown, and Peter O'Leary

Department of Endocrinology and Diabetes (J.P.W., S.J.B.), Sir Charles Gairdner Hospital, Nedlands, and Schools of Medicine and Pharmacology (J.P.W., P.J.L.), and Population Health (A.P.B.), University of Western Australia, Crawley, Western Australia 6009, Australia; Diagnostica Stago (P.F.), Doncaster, Victoria 3108, Australia; Laboratory for Cancer Medicine (P.J.L.), University of Western Australia Centre for Medical Research, Western Australian Institute for Medical Research, Perth, Western Australia 6000, Australia; Department of Endocrinology and Diabetes (P.J.L.), Royal Perth Hospital, Perth, Western Australia 6847, Australia; Office of Population Health Genomics (P.O.), Western Australian Department of Health, Perth, Western Australia 6004, Australia; School of Women's and Infants' Health and School of Pathology and Laboratory Medicine (P.O.), University of Western Australia, Crawley, Western Australia 6009, Australia; and Centre for Population Health Research (P.O.), Curtin Health Innovation Research Institute, Curtin University of Technology, Bentley, Western Australia 6845, Australia

Context: Longitudinal studies of risk factors for hypothyroidism are required to inform debate regarding the TSH reference range. There are limited longitudinal data on the predictive value of thyroid antibodies measured by automated immunoassay (as opposed to semiquantitative methods).

Methods: We measured TSH, free T_4, thyroid peroxidase antibodies (TPOAbs), and thyroglobulin antibodies (TgAbs) using the Immulite platform on sera from 1184 participants in the 1981 and 1994 Busselton Health Surveys. Outcome measures at follow-up were hypothyroidism, defined as TSH greater than 4.0 mU/liter or on thyroxine treatment; and overt hypothyroidism, defined as TSH above 10.0 mU/liter or on thyroxine treatment. Receiver-operator characteristic analysis was used to determine optimal cutoffs for baseline TSH, TPOAbs, and TgAbs as predictors of hypothyroidism.

Results: At 13 yr follow-up, 110 subjects (84 women) had hypothyroidism, of whom 42 (38 women) had overt hypothyroidism. Optimal cutoffs for predicting hypothyroidism were baseline TSH above 2.5 mU/liter, TPOAbs above 29 kIU/liter, and TgAbs above 22 kIU/liter, compared with reference range upper limits of 4.0 mU/liter, 35 kIU/liter, and 55 kIU/liter, respectively. In women with positive thyroid antibodies (TPOAbs or TgAbs), the prevalence of hypothyroidism at follow-up (with 95% confidence intervals) was 12.0% (3.0–21.0%) when baseline TSH was 2.5 mU/liter or less, 55.2% (37.1–73.3%) for TSH between 2.5 and 4.0 mU/liter, and 85.7% (74.1–97.3%) for TSH above 4.0 mU/liter.

Conclusions: The use of TSH cutoffs of 2.5 and 4.0 mU/liter, combined with thyroid antibodies, provides a clinically useful estimate of the long-term risk of hypothyroidism. **(*J Clin Endocrinol Metab* 95: 1095–1104, 2010)**

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

doi: 10.1210/jc.2009-1977 Received September 21, 2009. Accepted December 4, 2009.
First Published Online January 22, 2010

Abbreviations: NACB, National Academy of Clinical Biochemistry; ROC, receiver-operator characteristic; TgAb, thyroglobulin antibody; TPOAb, thyroid peroxidase antibody.

Autoimmune hypothyroidism is a common disorder, with a prevalence between 1 and 10%, depending on the population studied and the definition used (1–3). The most sensitive diagnostic marker is a raised serum TSH concentration, making it essential that the reference range for TSH is soundly based. Conventionally, this is based on the 95% confidence interval of log-transformed TSH concentrations from healthy individuals, which in iodine-sufficient populations gives an upper limit of approximately 4.0–4.5 mU/liter (1, 4–8). Several authorities believe that the upper limit of the TSH reference range should be lowered to 2.5 or 3 mU/liter, which remains controversial (2, 3, 9–13). The arguments in favor of this include the increased prevalence of thyroid antibodies and risk of developing hypothyroidism in people whose TSH concentrations are in the upper part of the reference range.

Clearly longitudinal studies examining the association between baseline TSH and the development of hypothyroidism are crucial to inform this debate, but few have been published. The most influential is the Whickham Survey, in which a baseline serum TSH concentration above 2 mU/liter was associated with an increased risk of hypothyroidism at 20 yr follow-up (14). In women who were euthyroid at baseline, positive thyroid antibody status was a strong predictor of hypothyroidism, with similar predictive value to raised TSH at baseline. In the Whickham Survey, baseline measurements of TSH were carried out using a first-generation RIA with a reference range of less than 6 mU/liter (15), which may give higher measured TSH values than the third-generation immunoassays now in general use (10, 16, 17). Thyroid antibody status was based on the detection of antimicrosomal, anticytoplasmic, and antithyroglobulin antibodies by semiquantitative methods of red cell agglutination, particle agglutination, and immunofluorescence. These methods are less sensitive than the quantitative, automated immunoassays for thyroid peroxidase antibodies (TPOAbs) and thyroglobulin antibodies (TgAbs) now used, and the pathological significance of a mildly raised TPOAb or TgAb concentration measured by immunoassay is uncertain (10).

There is therefore a need for longitudinal studies examining risk factors for the development of hypothyroidism using current techniques to measure thyroid antibodies and TSH. In a recent study from China, Teng and colleagues (18–20) confirmed that TPOAb and TgAb concentrations measured by immunoassay were risk factors for the development of hypothyroidism, but the follow-up period of 5 yr was relatively short.

We previously reported a cross-sectional analysis of the prevalence of thyroid disease in participants in the 1981 Busselton Health Survey in Busselton, Western Australia (6). The majority of subjects participated in a follow-up survey in 1994. Accordingly, we examined risk factors for the presence of hypothyroidism at 13 yr follow-up.

Subjects and Methods

The Busselton Health Study (http://bsn.uwa.edu.au) includes cross-sectional health surveys of residents of Busselton, a rural town in Western Australia with a predominantly white, iodine-sufficient population (21). Detailed descriptions of the surveys have been published previously (22). The Busselton Thyroid Study is based on the 1981 and 1994 surveys, in which participants completed a health questionnaire, underwent physical examination, and gave a venous blood sample in the morning after an overnight fast. In 2001 archived sera from 2108 participants in the 1981 survey were assayed for TSH, free T_4, TPOAb, and TgAb concentrations using an Immulite 2000 chemiluminescent analyzer (Siemens Healthcare Diagnostics Products, Deerfield, IL), as previously described (6, 23). Of these 2108 subjects, 1328 also attended the 1994 survey and had a blood sample collected. In 2007 archived sera from these subjects were assayed for TSH, free T_4, and TPOAb concentrations using the same immunoassay platform. All serum samples had been securely stored at −70 C in air-tight polypropylene tubes that were filled to capacity and had not been thawed during storage. Reference ranges derived from a cross-sectional analysis of the cohort [as previously described (6)] were as follows: TSH, 0.4–4.0 mU/liter; free T_4, 0.7–1.8 ng/dl (9–23 pmol/liter); TPOAb, less than 35 kIU/liter; and TgAb less than 55 kIU/liter. Positive thyroid antibody status was defined as elevated concentration of either TPOAb or TgAb.

The primary outcome measure (as determined at the 1994 visit) was hypothyroidism, defined as serum TSH greater than 4.0 mU/liter or on treatment with T_4, and the secondary outcome measure was overt hypothyroidism, defined as serum TSH greater than 10.0 mU/liter or on T_4 treatment. The rationale for this was that an elevated serum TSH concentration is the most sensitive indicator of hypothyroidism, but the need for routine T_4 replacement for people with mildly elevated TSH concentrations up to 10 mU/liter is uncertain (3, 9, 11, 12, 24–28). We excluded subjects with the following baseline characteristics: raised serum TSH with low free T_4, treatment with T_4 or antithyroid drugs, evidence of hyperthyroidism (defined as serum TSH <0.1 mU/liter) or missing serum TSH value. We also excluded those with discordant thyroid function test results that suggested pituitary disease or antibody interference (*e.g.* increased TSH and increased free T_4) in either survey and subjects on amiodarone or lithium treatment because of the confounding effects of these drugs on thyroid function. Subjects with mildly reduced baseline TSH concentrations between 0.1 and 0.4 mU/liter were not excluded because the pathological significance of this is uncertain, and TSH often returns to the reference range on repeat testing (29). Subjects with raised TSH and free T_4 within the reference range at baseline were not excluded because this too can normalize on repeat testing (29, 30), and we wanted to examine the predictive value of raised TSH for comparison with the results of the Whickham Survey.

Baseline characteristics of subjects who participated in the 1994 follow-up study were compared with those of subjects who died before 1994 and subjects who declined to participate using

χ^2 tests or t tests. Characteristics of the study cohort at the baseline and follow-up visits were compared using paired *t* tests or McNemar's tests. We examined each of the following baseline variables as potential predictors of hypothyroidism using logistic regression models: age, sex, TSH, parity (defined as number of live births), body mass index, smoking (never smoked, former smoker, current smoker), TPOAbs, and TgAbs. Variables considered in the univariate analyses were entered into multivariate logistic regression models including TPOAbs and TgAbs as either continuous or categorical variables. Variables that were not significant at the 5% level in either the univariate analyses or the multivariate models were removed stepwise, with variables that were significant in the univariate analyses retained for adjustment purposes. Functional forms of covariates (*e.g.* TSH^2, in case of a quadratic relationship) and interactions were explored, and TSH^2 was included because it significantly improved goodness of fit.

Receiver-operator characteristic (ROC) curves were used to determine the cutoffs for each of baseline TSH, TPOAbs, and TgAbs that optimized both sensitivity and specificity as predictors of hypothyroidism and overt hypothyroidism. Univariate and multivariate logistic regression models were used to examine the association between TPOAb concentration and outcomes in TPOAb-positive subjects using arbitrarily selected, but potentially clinically relevant, TPOAb concentration cutoffs of 3 times the upper reference limit (105 kU/liter) and 10 times the reference limit (350 kU/liter). Similar methodology was used to explore the association between TgAb concentration and outcomes using groups based on TgAb cutoffs of 22 and 55 kIU/liter identified from the ROC analysis and our previous cross-sectional analysis (6) respectively.

Prevalence estimates (with 95% confidence intervals) for hypothyroidism and overt hypothyroidism at follow-up were determined for categories of baseline TSH and thyroid antibody status and age-adjusted odds ratios calculated using logistic regression models. Using the optimal TSH cutoff identified from ROC analyses, split logistic regression models were used to examine the odds of hypothyroidism being present at follow-up in subjects with baseline TSH below and above the 2.5 mU/liter threshold, with further adjustment for sex, age, and antibody status.

Analyses were conducted using PASW Statistics 17.0.2 (SPSS Inc., Chicago, IL) and R version 2.9.1 (R Foundation for Statistical Computing, http://www.R-project.org). Significance was set at 0.05. The study was approved by the Busselton Population Medical Research Foundation and the Royal Perth Hospital Ethics Committee.

Results

Demographics and subject disposition

Of the 2108 subjects in the 1981 cross-sectional sample, 1804 were alive in 1994, and of these 1461 (81%) attended the 1994 survey. A flow chart detailing subject disposition is shown in Fig. 1, and baseline demographic data are provided in Table 1. Subjects who died before 1994 tended to be older, were more likely to be male and more likely to have smoked than survivors, but did not differ significantly from survivors in prevalence of self-reported thyroid disease or goiter, thyroid antibody status, or baseline TSH. Subjects who were alive but did not participate in the 1994 survey were significantly older than those who did take part but did not differ significantly from them in prevalence of self-reported thyroid disease, thyroid antibody status, or baseline TSH. After excluding subjects with raised TSH combined with low free T_4 or on T_4 treatment at baseline, those with hyperthyroidism, and those with missing data or who breached other exclusion criteria, the final study sample consisted of 1184 subjects. The mean time between study visits was 13.0 yr (range 12.3–14.0).

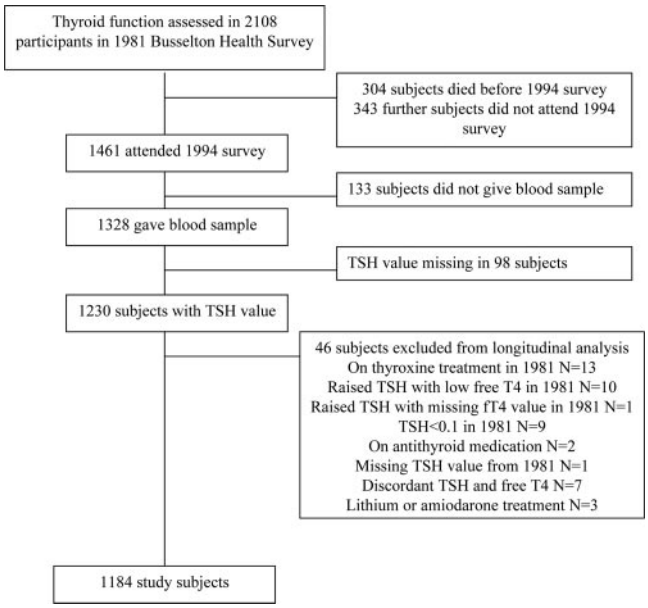

FIG. 1. Subject disposition scheme showing how the study cohort of 1184 subjects was derived. fT4, free T_4.

Outcome measures and predictors of hypothyroidism

The thyroid status of the 1184 study subjects at the baseline and follow-up visits is summarized in Table 2. The prevalence of positive TPOAbs increased from 11.1% in 1981 to 15.1% in 1994 ($P < 0.001$, McNemar's test). A change in TPOAb status occurred in 6.5% of subjects: from negative to positive in 5.2% and from positive to negative in 1.3%. At baseline, 1110 subjects (93.7%) had serum TSH concentrations between 0.1 and 4.0 mU/liter and none were on T_4 treatment. At follow-up, 29 subjects (2.4%) had commenced T_4 treatment and a further 81 (6.8%) had elevated serum TSH concentrations, of whom three had low free T_4 concentrations. Therefore, 110 subjects (9.3%) including 84 women had hypothyroidism at follow-up (defined as TSH >4 mU/liter or on T_4 treatment). Of these, 42 subjects (3.5% of the cohort) including 38 women had overt hypothyroidism (defined as TSH >10 mU/liter or on T_4 treatment).

In univariate analyses, the following baseline variables were significantly associated with hypothyroidism at fol-

TABLE 1. Baseline characteristics of members of the original 1981 cohort (n = 2108, categorized by vital status in 1994 and participation or nonparticipation in the 1994 survey) and the 1184 study subjects

	Attended 1994 survey (n = 1461)	Did not attend 1994 survey (n = 343)	*P* value[a]	Deceased in 1994 (n = 304)	*P* value[b]	Study subjects (n = 1184)
Age, mean (SD) (yr)	46.2 (14.9)	48.9 (18.5)	0.005	68.7 (10.6)	<0.001	45.8 (14.5)
Female, n (%)	747 (51)	177 (52)	0.9	121 (40)	<0.001	611 (52)
History of thyroid disease or goiter, n (%)	50 (3.4)	12 (3.5)	0.9	13 (4.3)	0.5	25 (2.1)
Smoking status, n (%)						
Current	275 (19)	76 (22)	0.13	65 (21)	<0.001	219 (19)
Former	423 (29)	107 (32)		130 (43)		330 (28)
Never	752 (52)	156 (46)		108 (36)		625 (53)
BMI, mean (SD) (kg/m^2)	25.2 (3.8)	26.2 (4.1)	<0.001	26.3 (3.9)	<0.001	25.2 (3.8)
TSH, median (interquartile range) (mU/liter)	1.42 (0.97–2.03)	1.45 (1.03–2.18)	0.5	1.54 (1.06–2.26)	1.0	1.43 (0.98–2.02)
TPOAb positive, n (%)	180 (12.3)	45 (13.1)	0.7	34 (11.2)	0.6	132 (11.1)
TgAb positive, n (%)	101 (6.9)	18 (5.2)	0.3	16 (5.3)	0.3	76 (6.4)
Thyroid antibody positive (TPOAb or TgAb), n (%)	213 (14.6)	50 (14.6)	0.9	35 (1.2)	0.2	158 (13.3)
Women only: parity, median (range)	2 (0–9)	2 (0–10)	0.4	3 (0–7)	0.9	2 (0–9)

[a] *P* values for comparison between the 1461 subjects who attended the 1994 survey and the 343 subjects who were alive in 1994 but did not attend.

[b] *P* values for comparison between the 1461 subjects who attended the 1994 survey and the 304 subjects who were deceased in 1994.

low-up: age, gender, TSH, TPOAb concentration, TgAb concentration, TPOAb-positive status, and TgAb-positive status, whereas smoking status and parity at baseline were not significant predictors (Table 3). We also analyzed outcomes with regard to parity and smoking status at follow-up, age at menopause, and change in weight between baseline and follow-up visits: none was a significant predictor of hypothyroidism. Of the 76 subjects with positive TgAb, 26 were TPOAb negative; of these 4 (15.4%) had hypothyroidism at follow-up, which was not a significantly increased risk compared with TPOAb-negative, TgAb-negative subjects (odds ratio 1.80, 95% confidence interval 0.52–4.82).

TABLE 2. Characteristics of the 1184 study subjects at baseline and follow-up

	Baseline (n = 1184)	Follow-up (n = 1184)
Age, mean (SD) (yr)	45.8 (14.5)	58.8 (14.4)
TSH, median (interquartile range) (mU/liter)	1.43 (0.98, 2.02)	1.73 (1.18, 2.51)
TPOAb positive, n (%)	132 (11.1)	179 (15.1)
On T_4 treatment	0 (0%)	29 (2.4%)
Not on T_4 treatment		
Serum TSH (mU/liter)		
Less than 0.4	13 (1.1%)	17 (1.4%)
0.4 to 4.0	1110 (93.7%)	1057 (89.3%)
4.01–10.0	45 (3.8%)	68 (5.7%)
Greater than 10.0	16 (1.4%)	13 (1.1%)

In multivariate logistic regression models including TPOAbs and TgAbs as either continuous or categorical variables, female gender and baseline TSH were the strongest predictors of hypothyroidism, whereas age was not significant (Table 3). The predictive value of thyroid antibodies was greatly attenuated in the multivariate model, with only TgAbs (as a continuous variable) remaining significant.

Optimal cutoffs for TSH, TPOAbs, and TgAbs

Using ROC curve analysis, the baseline TSH cutoff associated with the optimal combination of sensitivity and specificity for predicting outcomes was 2.4 mU/liter for hypothyroidism (sensitivity 76%, specificity 90%) and 2.6 mU/liter for overt hypothyroidism (sensitivity 79%, specificity 90%) (Supplemental Fig. 1 published as supplemental data on The Endocrine Society's Journals Online web site at http://jcem.endojournals. org). Results were similar for males and females analyzed separately, with cutoffs for hypothyroidism of 2.6 mU/liter for women (sensitivity 76%, specificity 92%) and 2.3 mU/liter for men (sensitivity 77%, specificity 88%). On this basis, we selected 2.5 mU/liter as the threshold for further examining outcomes by categories of baseline TSH. The positive predictive value of a serum TSH concentration above 2.5 mU/liter at baseline for the presence of hypothyroidism at follow-up was 47%, whereas the negative predictive value was 97% (Table 4). By contrast, for baseline TSH above 4 mU/liter (the upper limit of the refer-

TABLE 3. Results of logistic regression analysis of potential risk factors at baseline for the presence of hypothyroidism at follow-up

	Odds ratio	95% CI	*P*
Univariate analysis			
Age (yr)	1.02	1.01, 1.04	0.002
Female sex	3.35	2.16, 5.38	<0.001
TSH, mU/liter	3.50	2.83, 4.41	<0.001
BMI (kg/m^2)	1.02	0.97, 1.07	0.49
Smoking status			
Former smoker	0.86	0.55, 1.36	0.52
Current smoker	0.59	0.32, 1.07	0.08
Parity	0.96	0.83, 1.10	0.15
TPOAb (kIU/liter)	1.004	1.003, 1.005	<0.001
TgAb (kIU/liter)	1.005	1.003, 1.008	<0.001
Antibody status			
TPOAb positive	12.9	8.35, 20.2	<0.001
TgAb positive	6.80	4.01, 11.4	<0.001
Multivariate analysis			
Age (yr)	1.01	0.99, 1.03	0.40
Female sex	2.50	1.39, 4.45	0.002
TSH (mU/liter)	3.59	2.74, 4.71	<0.001
TSH^2	0.98	0.97, 0.99	<0.001
TPOAb (kIU/liter)	1.001	1.000, 1.002	0.12
TgAb (kIU/liter)	1.003	1.000, 1.005	0.03
Multivariate analysis			
Age (yr)	1.01	0.99, 1.03	0.50
Female sex	2.53	1.41, 4.53	0.002
TSH (mU/liter)	3.41	2.58, 4.51	<0.001
TSH^2	0.98	0.96, 0.99	0.02
TPOAb positive	1.92	0.94, 3.90	0.07
TgAb positive	1.50	0.65, 3.44	0.34

For continuous variables, the odds ratio shown is per unit increase in explanatory variable. Smoking status refers to comparison with never-smokers. CI, Confidence interval; BMI, body mass index.

ence range), the positive predictive value for hypothyroidism was 84% and the negative predictive value 95%.

For TPOAb, the optimal cutoffs identified by ROC analysis were 29 kIU/liter for hypothyroidism (sensitivity 52%, specificity 92%) and 32 kIU/liter for overt hypothyroidism (sensitivity 69%, specificity 90%) (Supplemental Fig. 1). These cutoffs were close to the upper limit of the reference range of 35 kIU/liter derived from cross-sectional analysis of participants in the 1981 study (6), which was associated with 53% sensitivity and 93% specificity for hypothyroidism and 67% sensitivity and specificity 91% for overt hypothyroidism. Applying the cutoff of 29 kIU/liter to the cohort of 1184 subjects resulted in reclassification of 12 subjects and only minor changes in sensitivity and specificity, whereas applying the cutoff of 32 kIU/liter did not reclassify any participant. Accordingly, we did not reanalyze outcomes using the lower cutoffs. Using logistic regression models, we examined the association between categories of TPOAb concentration and outcomes in TPOAb-positive subjects (Table 5). The risk of hypothyroidism and overt hypothyroidism increased markedly across groups with higher TPOAb concentrations, but the association was greatly attenuated by adjustment for age, sex, and TSH.

For TgAbs, ROC curve analysis (Supplemental Fig. 1) identified an optimal cutoff of 22 kIU/liter for both hypothyroidism (49% sensitivity, 84% specificity) and overt hypothyroidism (63% sensitivity, 83% specificity). This cutoff differed substantially from the reference range limit of 55 kIU/liter derived from the cross-sectional analysis, which was associated with 25% sensitivity and 94% specificity for hypothyroidism and 24% sensitivity and 94% specificity for overt hypothyroidism. To explore this further, we examined outcomes in groups based on TgAb cutoffs of 22 and 55 kIU/liter (Table 5). In unadjusted analyses, the risk of hypothyroidism and overt hypothyroidism was significantly increased in subjects with TgAb concentration of 22–55 kIU/liter and those with TgAb greater than 55 kIU/liter, but after adjustment for age, sex, and TSH, neither category was associated with significantly increased risk compared with lower values. The small number of subjects with TgAbs above 55 kIU/liter precluded further analysis of outcomes according to TgAb concentration.

Risk of hypothyroidism according to gender, baseline TSH, and antibody status

We used TSH cutoffs of 2.5 and 4.0 mU/liter (derived from the ROC analysis and the reference range, respectively) together with gender and antibody status to examine the absolute and relative risks of hypothyroidism in subgroups of subjects (Tables 6 and 7). For men, the risk of hypothyroidism was very low in subjects with baseline TSH of 2.5 mU/liter or less and increased pro-

TABLE 4. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of baseline serum TSH greater than 2.5 mU/liter or greater than 4 mU/liter for the presence of hypothyroidism and overt hypothyroidism at follow-up

	Baseline serum TSH concentration							
	Greater than 2.5 mU/liter				Greater than 4 mU/liter			
	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Hypothyroidism	73	91	47	97	45	99	84	95
Overt hypothyroidism	79	88	19	99	64	94	31	99

TABLE 5. Study outcomes analyzed by baseline TPOAb concentration and TgAb concentration

	TPOAb concentration (kIU/liter)			
	<35 (n = 1052)	**35–105 (n = 43)**	**106–350 (n = 38)**	**>350 (n = 51)**
Hypothyroidism, n (%)	55 (5.2)	6 (14.0)	16 (42.1)	33 (64.7)
Odds ratio (95% CI)				
Unadjusted	1.0	2.9 (1.2–7.3)	13.2 (6.6–26.5)	33.2 (17.6–62.7)
Adjusted for age, sex, TSH, TSH^2	1.0	1.1 (0.4–3.3)	2.1 (0.8–5.5)	4.3 (1.7–10.9)
Overt hypothyroidism, n (%)	14 (1.3)	2 (4.7)	7 (18.4)	19 (37.3)
Odds ratio (95% CI)				
Unadjusted	1.0	3.6 (0.8–16.4)	16.7 (6.3–44.4)	44.0 (22.3–95.5)
Adjusted for age, sex, TSH, TSH^2	1.0	2.9 (0.6–13.9)	4.3 (1.2–15.8)	7.5 (2.4–23.5)
	TgAb concentration (kIU/liter)			
	<22 (n = 963)	**22–55 (n = 145)**	**>55 (n = 76)**	
Hypothyroidism, n (%)	57 (5.9)	26 (17.9)	27 (35.5)	
Odds ratio (95% CI)				
Unadjusted	1.0	3.6 (2.2–5.9)	9.3 (5.4–16.0)	
Adjusted for age, sex, TSH, TSH^2	1.0	1.1 (0.5–2.3)	2.1 (1.0–4.6)	
Overt hypothyroidism	16 (1.7)	16 (11.0)	10 (13.2)	
Odds ratio (95% CI)				
Unadjusted	1.0	7.2 (3.5–14.7)	9.2 (4.0–21.2)	
Adjusted for age, sex, TSH, TSH^2	1.0	2.7 (1.0–7.1)	1.9 (0.6–6.2)	

Data are given as number and percentage unless otherwise shown. Reference ranges: TPOAb, less than 35 kIU/liter; TgAb less than 55 kIU/liter. CI, Confidence interval.

gressively across higher categories of TSH. Of 36 men with positive thyroid antibodies and TSH between 0.1 and 4.0 mU/liter, only 2 (5.6%) developed hypothyroidism, of whom 1 (2.8%) had overt hypothyroidism. The small number of men with positive antibodies and outcome measures precluded more detailed analysis.

For women, the risk of hypothyroidism was lowest in antibody-negative subjects with baseline TSH of 2.5 mU/liter or less and increased progressively across categories of TSH and antibody status. In women with positive thyroid antibodies at baseline (defined as TPOAb or TgAb concentration above the reference range), the prevalence of hypothyroidism at follow-up (with 95% confidence intervals) was 12.0% (3.0–21.0%) when baseline TSH was 2.5 mU/liter or less, 55.2% (37.1–73.3%) for TSH between 2.5 and 4.0 mU/liter, and 85.7% (74.1–97.3%) for baseline TSH above 4.0 mU/liter, suggesting that these TSH cutoffs provided useful risk stratification.

Using split regression models, the odds ratio and probability for hypothyroidism at 13 yr follow-up was determined in all subjects with baseline TSH below or above the 2.5 mU/liter cutoff. The results are shown graphically in Fig. 2.

Discussion

This study provides longitudinal data on risk factors for hypothyroidism over a 13-yr period, using current meth-

TABLE 6. Study outcomes analyzed by categories of baseline TSH for males

	TSH (mU/liter)			
	0.1–2.5 (n = 514)	**2.5–4.0 (n = 43)**	**>4.0 (n = 16)**	***P* for trend**
Hypothyroidism	10	4	12	
	1.9%	9.3%	75.0%	<0.001
Odds ratio[a]	1.0	4.9	136	
95% CI		1.5, 16.4	36.9, 501	
Overt hypothyroidism	1	1	2	
	0.20%	2.3%	12.5%	0.001
Odds ratio[a]	1.0	16.1	375	
95% CI		0.9, 279	14.7, 9574	

Data are shown as number, percentage, and for females, 95% confidence interval (CI) for the percentage (where this could be calculated). *P* values are shown for the trend across groups. Positive thyroid antibody status was defined as TPOAb or TgAb concentration above the laboratory reference range.

[a] Adjusted for age.

TABLE 7. Study outcomes analyzed by categories of baseline TSH and for females further analyzed by thyroid antibody status

	0.1–2.5 negative antibodies (n = 448)	0.1–2.5 positive antibodies (n = 50)	2.5–4.0 negative antibodies (n = 39)	2.5–4.0 positive antibodies (n = 29)	>4.0 negative antibodies (n = 10)	>4.0 positive antibodies (n = 35)	*P* for trend
Hypothyroidism	14	6	11	16	7	30	
	3.1%	12.0%	28.2%	55.2%	70.0%	85.7%	<0.001
	(1.5–4.7)	(3.0–21.0)	(14.1–42.3)	(37.1–73.3)	(41.6–98.4)	(74.1–97.3)	
Odds ratio[a]	1.0	4.2	12.1	38.1	71.6	185	
95% CI		1.5, 11.5	5.0, 29.3	15.4, 94.2	16.5, 311	61.7, 553	
Overt hypothyroidism	7	1	1	4	4	21	
	1.6%	2.0%	2.6%	13.8%	40.0%	60.0%	<0.001
	(0.4–2.7)					(43.8–76.2)	
Odds ratio[a]	1.0	1.4	1.8	10.4	49.7	108	
95% CI		0.2, 11.3	0.2, 14.7	2.8, 38.1	10.8, 229	37.2, 312	

Data are shown as number and percentage, and for females, 95% confidence interval (CI) for the percentage (where this could be calculated). *P* values are shown for the trend across groups. Positive thyroid antibody status was defined as TPOAb or TgAb concentration above the laboratory reference range.

[a] Adjusted for age.

ods for measurement of TSH and thyroid antibodies. The results will help inform debate regarding the reference range for TSH and provide a tool for clinicians to estimate the long-term risk of hypothyroidism in patients based on gender, TSH, and thyroid antibody status.

As expected from previous longitudinal studies (14, 18, 31), age, female gender, thyroid antibodies, and baseline TSH were associated with hypothyroidism in univariate analyses. Smoking was not associated with a significant reduction in the risk of hypothyroidism, in contrast to the results of cross-sectional studies (32–34), but the number of smokers was small and statistical power accordingly limited. Parity was not a risk factor for hypothyroidism, consistent with cross-sectional analyses of this and another cohort (35, 36) but in contrast to another study in which parity was associated with autoimmune thyroiditis (37). In the multivariate analysis, female gender and TSH were the strongest independent predictors of hypothyroidism, whereas age was no longer significant, and thyroid antibodies were of borderline significance.

For baseline TSH, ROC analysis identified a threshold of 2.5 mU/liter as associated with optimal sensitivity and specificity in predicting hypothyroidism. This is broadly consistent with data from Whickham (14) and China (18–20), in which the risk of hypothyroidism was higher if baseline TSH was above 2 mU/liter. Based in part on the Whickham Survey, it has been argued that the upper limit of the TSH reference range should be lowered to 2.5 or 3 mU/liter (9–11, 24). Our study demonstrates that individuals with serum TSH between 2.5 and 4 mU/liter are indeed at increased risk of hypothyroidism, but the majority of such subjects did not develop hypothyroidism by 13 yr follow-up. For this reason, we do not support lowering the upper limit of the TSH reference range to 2.5 mU/liter, but it is certainly reasonable to regard TSH concentrations of 2.5–4 mU/liter as a category of intermediate risk, especially in women with positive thyroid antibodies. Follow-up thyroid function testing for such individuals is appropriate, as already recommended (2, 3).

In our previous cross-sectional study (6), we derived reference ranges for TPOAbs and TgAbs based on 95% confidence intervals from 2026 subjects with no history of thyroid disease. For comparison, we also used the approach recommended by National Academy of Clinical Biochemistry (NACB) guidelines of deriving these reference ranges from males aged under 30 yr with serum TSH between 0.5 and 2.0 mU/liter (10). In the present study, the optimal TPOAb cutoffs identified by ROC analysis (29 kIU/liter for hypothyroidism and 32 kIU/liter for overt hypothyroidism) were close to the reference range upper limits derived from the whole cohort (35 kIU/liter) and that

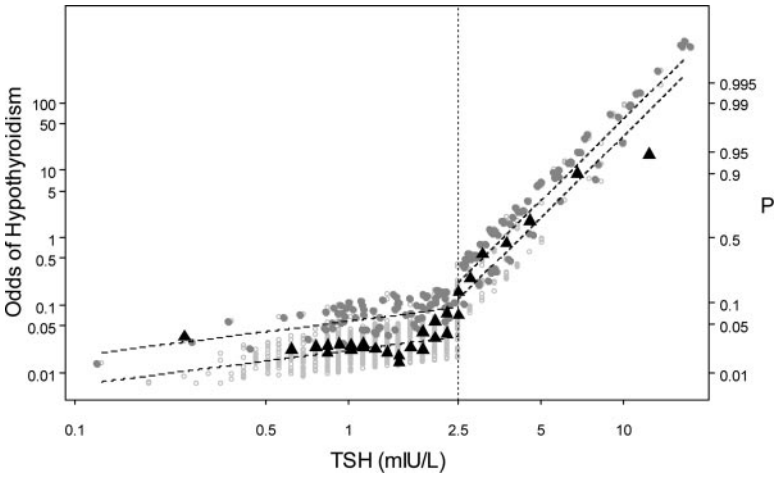

FIG. 2. Predicted odds of hypothyroidism from a logistic regression model split at a baseline TSH of 2.5 mU/liter (*vertical dotted line*). For the purposes of illustration, TSH values were grouped in 50 bins of equal size on the natural logarithmic scale: ●, Antibody positive; ○ antibody negative; ▲ actual odds ratios from smoothed raw data. *Dashed lines* represent the predicted odds of hypothyroidism for antibody positive (*upper*) and antibody negative (*lower*) subjects, averaged over other covariates. The right-hand axis shows the conversion of odds ratios to the probability of hypothyroidism.

derived by the NACB-recommended approach (30 kIU/liter). For TPOAb-positive subjects, the risk of hypothyroidism was highest in subjects with the highest TPOAb concentrations, whereas mildly elevated TPOAb concentrations of up to 3 times normal had no independent predictive value after adjustment for age, sex, and TSH.

For TgAbs, the cutoff of 22 kIU/liter from the ROC analysis differed substantially from the cutoff of 55 kIU/liter derived from our cross-sectional analysis and was closer to the cutoff of 28 kIU obtained using NACB guidelines (6). TgAb concentrations between 22 and 55 kIU/liter were associated with a greater risk of hypothyroidism than lower levels, but this was not significant after adjustment for age, sex, and TSH. If the 22 kIU/liter cutoff were adopted as reference range limit, the prevalence of TgAb positivity in the cohort would increase from 6.4 to 17.3%, and the prevalence of positive thyroid antibodies (TPOAbs or TgAbs) from 13.3 to 21.1%. Unless positive TgAb status (in the absence of TPOAbs) is shown to be an independent risk factor for hypothyroidism, we do not believe that such a drastic change to the reference interval is warranted, but our data suggest that this question warrants further study.

In the Whickham Survey, positive thyroid antibody status in women who were euthyroid at baseline (defined as serum TSH <6 mU/liter) was a strong predictor of hypothyroidism at follow-up, with an associated risk of 2.1% per year (14). It is difficult to compare our results with those because of differences in assay methods, TSH reference range, and follow-up duration. We found, however, that useful risk stratification is achieved by examining outcomes according to whether baseline TSH is in the lower or upper part of the reference range. For example, in antibody-positive women with baseline TSH less than 2.5 mU/liter, the risk of hypothyroidism was approximately 1% per year and the risk of overt hypothyroidism 0.2% per year, whereas in antibody-positive women with baseline TSH between 2.5 and 4.0 mU/liter, the risks were much higher, at 4 and 1% per year, respectively. (This use of annualized estimates of risk may not be strictly valid because it assumes a constant incidence of hypothyroidism over time, but it is the only practical way of comparing results from studies with different durations of follow-up.) Our results, as summarized in Tables 6 and 7, will allow clinicians to use patients' serum TSH and thyroid antibody status to estimate the long-term risk of hypothyroidism or overt hypothyroidism, as preferred.

The strengths of our study include its large, community-based cohort and the long follow-up duration of 13 yr. Our study also has limitations. First, we were able to study only survivors in the cohort and have no information on the ultimate thyroid status of those who died or declined to participate in the 1994 survey. However, the participation rate among survivors was high (81%), and baseline thyroid and thyroid antibody status did not differ significantly between participants and nonparticipants, making this unlikely to be an important source of bias. Second, the definition of hypothyroidism included physician-prescribed T_4 replacement, and we had no access to serum TSH concentrations at the time of diagnosis for independent verification.

In conclusion, in this 13-yr longitudinal analysis of a community-based cohort, female gender and TSH were the strongest risk factors for the presence of hypothyroidism at follow-up. The use of TSH cutoffs of 2.5 and 4.0 mU/liter, combined with thyroid antibodies as measured by automated immunoassay, provides a clinically useful estimate of the long-term risk of hypothyroidism.

Acknowledgments

We thank the Busselton Population Medical Research Foundation for approving the study, Graham Maier for data extraction, John Beilby for assistance with stored sera, and Valdo Michelangeli for his major contribution in the establishment of the Busselton Thyroid Study.

Address all correspondence and requests for reprints to: Clin A/Professor John P. Walsh, Department of Endocrinology and

Diabetes, Sir Charles Gairdner Hospital, Nedlands, Western Australia 6009. E-mail: john.walsh@health.wa.gov.au.

Disclosure Summary: The authors have no disclosures.

References

1. **Hollowell JG, Staehling NW, Flanders WD, Hannon WH, Gunter EW, Spencer CA, Braverman LE** 2002 Serum TSH, T(4), and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). J Clin Endocrinol Metab 87:489–499
2. **Roberts CG, Ladenson PW** 2004. Hypothyroidism. Lancet 363: 793–803
3. **Biondi B, Cooper DS** 2008 The clinical significance of subclinical thyroid dysfunction. Endocr Rev 29:76–131
4. **Bjoro T, Holmen J, Krüger O, Midthjell K, Hunstad K, Schreiner T, Sandnes L, Brochmann H** 2000 Prevalence of thyroid disease, thyroid dysfunction and thyroid peroxidase antibodies in a large, unselected population. The Health Study of Nord-Trondelag (HUNT). Eur J Endocrinol 143:639–647
5. **Jensen E, Hyltoft Petersen P, Blaabjerg O, Hansen PS, Brix TH, Kyvik KO, Hegedüs L** 2004 Establishment of a serum thyroid stimulating hormone (TSH) reference interval in healthy adults. The importance of environmental factors, including thyroid antibodies. Clin Chem Lab Med 42:824–832
6. **O'Leary PC, Feddema PH, Michelangeli VP, Leedman PJ, Chew GT, Knuiman M, Kaye J, Walsh JP** 2006 Investigations of thyroid hormones and antibodies based on a community health survey: the Busselton thyroid study. Clin Endocrinol (Oxf) 64:97–104
7. **Kratzsch J, Fiedler GM, Leichtle A, Brüugel M, Buchbinder S, Otto L, Sabri O, Matthes G, Thiery J** 2005 New reference intervals for thyrotropin and thyroid hormones based on National Academy of Clinical Biochemistry criteria and regular ultrasonography of the thyroid. Clin Chem 51:1480–1486
8. **Hamilton TE, Davis S, Onstad L, Kopecky KJ** 2008 Thyrotropin levels in a population with no clinical, autoantibody, or ultrasonographic evidence of thyroid disease: implications for the diagnosis of subclinical hypothyroidism. J Clin Endocrinol Metab 93:1224–1230
9. **Baskin HJ, Cobin RH, Duick DS, Gharib H, Guttler RB, Kaplan MM, Segal RL** 2002 American Association of Clinical Endocrinologists medical guidelines for clinical practice for the evaluation and treatment of hyperthyroidism and hypothyroidism. Endocr Pract 8:457–469
10. **Baloch Z, Carayon P, Conte-Devolx B, Demers LM, Feldt-Rasmussen U, Henry JF, LiVosli VA, Niccoli-Sire P, John R, Ruf J, Smyth PP, Spencer CA, Stockigt JR** 2003 Guidelines Committee, National Academy of Clinical Biochemistry Laboratory medicine practice guidelines. Laboratory support for the diagnosis and monitoring of thyroid disease. Thyroid 13:3–126
11. **Wartofsky L, Dickey RA** 2005 The evidence for a narrower thyrotropin reference range is compelling. J Clin Endocrinol Metab 90: 5483–5488
12. **Surks MI, Goswami G, Daniels GH** 2005 The thyrotropin reference range should remain unchanged. J Clin Endocrinol Metab 90:5489–5496
13. **Brabant G, Beck-Peccoz P, Jarzab B, Laurberg P, Orgiazzi J, Szabolcs I, Weetman AP, Wiersinga WM** 2006 Is there a need to redefine the upper normal limit of TSH? Eur J Endocrinol 154:633–637
14. **Vanderpump MP, Tunbridge WM, French JM, Appleton D, Bates D, Clark F, Grimley Evans J, Hasan DM, Rodgers H, Tunbridge F, Young ET** 1995 The incidence of thyroid disease in the community: a twenty-year follow-up of the Whickham Survey. Clin Endocrinol (Oxf) 43:55–68
15. **TunbridgeWM, Evered DC, Hall R, Appleton D, Brewis M, Clark F, Evans JG, Young E, Bird T, Smith PA** 1977 The spectrum of thyroid disease in a community: the Whickham survey. Clin Endocrinol (Oxf) 7:481–493
16. **Pekary AE, Hershman JM** 1984 A new monoclonal-antibody two-site solid-phase immunoradiometric assay for human thyrotropin evaluated. Clin Chem 30:1213–1215
17. **Nicoloff JT, Spencer CA** 1990 The use and misuse of the sensitive thyrotropin assays. J Clin Endocrinol Metab 71:553–558
18. **Teng W, Shan Z, Teng X, Guan H, Li Y, Teng D, Jin Y, Yu X, Fan C, Chong W, Yang F, Dai H, Yu Y, Li J, Chen Y, Zhao D, Shi X, Hu F, Mao J, Gu X, Yang R, Tong Y, Wang W, Gao T, Li C** 2006 Effect of iodine intake on thyroid diseases in China. N Engl J Med 354: 2783–2793
19. **Guan H, Shan Z, Teng X, Li Y, Teng D, Jin Y, Yu X, Fan C, Chong W, Yang F, Dai H, Yu Y, Li J, Chen Y, Zhao D, Shi X, Hu F, Mao J, Gu X, Yang R, Chen W, Tong Y, Wang W, Gao T, Li C, Teng W** 2008 Influence of iodine on the reference interval of TSH and the optimal interval of TSH: results of a follow-up study in areas with different iodine intakes. Clin Endocrinol (Oxf) 69:136–141
20. **Li Y, Teng D, Shan Z, Teng X, Guan H, Yu X, Fan C, Chong W, Yang F, Dai H, Gu X, Yu Y, Mao J, Zhao D, Li J, Chen Y, Yang R, Li C, Teng W** 2008 Antithyroperoxidase and antithyroglobulin antibodies in a five-year follow-up survey of populations with different iodine intakes. J Clin Endocrinol Metab 93:1751–1757
21. **Li M, Eastman CJ, Waite KV, Ma G, Zacharin MR, Topliss DJ, Harding PE, Walsh JP, Ward LC, Mortimer RH, Mackenzie EJ, Byth K, Doyle Z** 2006 Are Australian children iodine deficient? Results of the Australian National Iodine Nutrition Study. Med J Aust 184:165–169
22. **Knuiman MW, Jamrozik K, Welborn TA, Bulsara MK, Divitini ML, Whittall DE** 1995 Age and secular trends in risk factors for cardiovascular disease in Busselton. Aust J Public Health 19:375–382
23. **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
24. **McDermott MT, Ridgway EC** 2001 Subclinical hypothyroidism is mild thyroid failure and should be treated. J Clin Endocrinol Metab 86:4585–4590
25. **Chu JW, Crapo LM** 2001 The treatment of subclinical hypothyroidism is seldom necessary. J Clin Endocrinol Metab 86:4591–4599
26. **Surks MI, Ortiz E, Daniels GH, Sawin CT, Col NF, Cobin RH, Franklyn JA, Hershman JM, Burman KD, Denke MA, Gorman C, Cooper RS, Weissman NJ** 2004 Subclinical thyroid disease: scientific review and guidelines for diagnosis and management. JAMA 291:228–238
27. **Helfand M** 2004 U.S. Preventive Services Task Force screening for subclinical thyroid dysfunction in nonpregnant adults: a summary of the evidence for the U.S. Preventive Services Task Force. Ann Intern Med 140:128–141
28. **Gharib H, Tuttle RM, Baskin HJ, Fish LH, Singer PA, McDermott MT** 2005 Consensus statement #1: subclinical thyroid dysfunction: a joint statement on management from the American Association of Clinical Endocrinologists, the American Thyroid Association, and The Endocrine Society. J Clin Endocrinol Metab 90:581–585; discussion 586–587
29. **Meyerovitch J, Rotman-Pikielny P, Sherf M, Battat E, Levy Y, Surks MI** 2007 Serum thyrotropin measurements in the community: five-year follow-up in a large network of primary care physicians. Arch Intern Med 167:1533–1538
30. **Díez JJ, Iglesias P, Burman KD** 2005 Spontaneous normalization of thyrotropin concentrations in patients with subclinical hypothyroidism. J Clin Endocrinol Metab 90:4124–4127
31. **Geul KW, van Sluisveld IL, Grobbee DE, Docter R, de Bruyn AM, Hooykaas H, van der Merwe JP, van Hemert AM, Krenning EP, Hennemann G** 1993 The importance of thyroid microsomal antibodies in the development of elevated serum TSH in middle-aged women: associations with serum lipids. Clin Endocrinol (Oxf) 39:275–280
32. **Belin RM, Astor BC, Powe NR, Ladenson PW** 2004 Smoke exposure is associated with a lower prevalence of serum thyroid auto-

antibodies and thyrotropin concentration elevation and a higher prevalence of mild thyrotropin concentration suppression in the third National Health and Nutrition Examination Survey (NHANES III). J Clin Endocrinol Metab 89:6077–6086

33. **Asvold BO, Bjøro T, Nilsen TI, Vatten LJ** 2007 Tobacco smoking and thyroid function: a population-based study. Arch Intern Med 167:1428–1432
34. **Pedersen IB, Laurberg P, Knudsen N, Jørgensen T, Perrild H, Ovesen L, Rasmussen LB** 2008 Smoking is negatively associated with the presence of thyroglobulin autoantibody and to a lesser degree with thyroid peroxidase autoantibody in serum: a population study. Eur J Endocrinol 158:367–373
35. **Walsh JP, Bremner AP, Bulsara MK, O'Leary P, Leedman PJ, Feddema P, Michelangeli V** 2005 Parity and the risk of autoimmune thyroid disease: a community-based study. J Clin Endocrinol Metab 90:5309–5312
36. **Bülow Pedersen I, Laurberg P, Knudsen N, Jørgensen T, Perrild H, Ovesen L, Rasmussen LB** 2006 Lack of association between thyroid autoantibodies and parity in a population study argues against microchimerism as a trigger of thyroid autoimmunity. Eur J Endocrinol 154:39–45
37. **Friedrich N, Schwarz S, Thonack J, John U, Wallaschofski H, Völzke H** 2008 Association between parity and autoimmune thyroiditis in a general female population. Autoimmunity 41:174–180