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National Health and Nutrition Examination Survey III Thyroid-Stimulating Hormone (TSH)-Thyroperoxidase Antibody Relationships Demonstrate That TSH Upper Reference Limits May Be Skewed by Occult Thyroid Dysfunction

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Context: The setting of the TSH upper reference limit impacts the diagnosis of mild hypothyroidism and is currently controversial.

Objective: Our objective was to evaluate factors influencing the TSH reference range.

Design: Nonpregnant subjects aged 12 yr and older from National Health and Nutrition Examination Survey III were used to study the relationships between TSH, thyroid peroxidase antibodies (TPOAb), and thyroglobulin antibodies in different ethnic groups.

Results: TPOAb prevalence was lowest (<3%) when TSH was between 0.1 and 1.5 mIU/liter in women and between 0.1 and 2.0 mIU/liter in men and progressively increased to above 50% when TSH exceeded 20 mIU/liter. TSH reference range parameters (2.5th, 50th, and 97.5th percentiles) were analyzed according to thyroid antibody status, race/ethnicity, and age for the 14,202 subjects made up of non-Hispanic Blacks (B), non-Hispanic whites (W), and Mexican-Americans (M) who did not report thyroid disease or taking thyroid-altering medications and whose total T_4 was within the reference range. For each age group of each ethnicity, the inclusion of antibody-positive subjects increased TSH medians and upper limits (97.5th percentiles). The TSH upper limit was lower for the entire B cohort *vs.* W or M. However, this difference was lost when age cohorts with a similar prevalence of TPOAb (B age 40–49 yr *vs.* W and M age 20–29 yr) were compared.

Conclusions: Ethnic differences in TSH were not present when populations with the same relative frequency of thyroid antibodies were compared. TSH upper reference limits may be skewed by TPOAb-negative individuals with occult autoimmune thyroid dysfunction. (***J Clin Endocrinol Metab* 92: 4236–4240, 2007**)

TSH REFERENCE LIMITS ARE calculated from the 95% confidence intervals of cohorts of individuals without evidence of thyroid dysfunction or positive thyroid peroxidase antibodies (TPOAb) and/or thyroglobulin antibodies (TgAb) (1). TSH reference ranges are typically narrower when calculated from small rigorously selected cohorts as compared with large populations (2, 3). Most studies report that the lower TSH limit (2.5th percentile) lies between 0.2 and 0.4 mIU/liter, but upper limits (97.5th percentile) vary between 2.4 and 4.2 mIU/liter as related to ethnicity or geographic location (3, 4). Some variability is methodological (5, 6), but even when using the same assay, TSH upper limits vary between 3.1 and 3.7 mIU/liter for European Caucasians (4). Similarly, TSH upper limits of 4.2 mIU/liter for non-Hispanic whites (W) and 3.6 mIU/liter for non-Hispanic Blacks (B) were reported by the National Health and Nutrition Examination Survey (NHANES) III (3). The presence of thyroid antibodies is the primary exclusion criterion used for eliminating individuals with thyroid dysfunction from TSH reference range calculations. However, studies find that 20% of subjects with mild TSH elevations and cytological evidence of lymphocytic infiltration have no thyroid antibodies detected (7). In these studies, ultrasound patterns of thyroiditis occurred even in the absence of antibodies (8). It follows that differences in the TSH upper limit likely reflect effects from including individuals with occult thyroid dysfunction who have no thyroid antibodies detected. Populations in which the dominant thyroid pathology is thyroid deficiency secondary to Hashimotos' thyroiditis display a trend for the TSH upper limit to increase with age (3, 9). An inverse relationship between TSH and age is seen in iodine-deficient populations in which the dominant thyroid pathology is nodularity and increasing thyroid autonomy with age (10).

NHANES III was a survey of individuals selected to represent the civilian, noninstitutionalized U.S. population (3). In a previous report, serum TSH, total T_4 (TT_4) and thyroid autoantibody measurements were used to establish the overall prevalence of overt and subclinical thyroid dysfunction.

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Abbreviations: B, Non-Hispanic Black; M, Mexican-American; NHANES, National Health and Nutrition Examination Survey; OH, overt hypothyroidism; OR, odds ratio; SH, subclinical hypothyroidism; TgAb, thyroglobulin antibodies; TPOAb, thyroid peroxidase antibodies; TT_4, total T_4; W, non-Hispanic white.

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Thyroid antibodies (TPOAb and/or TgAb) were detected in 12.5% of the population. When both TPOAb and TgAb were present, the odds ratios (OR) for overt hypothyroidism (OH) was 23.5 and for subclinical hypothyroidism (SH) was 11.7. These were higher than for TPOAb alone for OH (OR = 6.9) and for SH (OR = 4.0). TgAb alone was not a risk factor for OH or SH (3). In the current study, we analyzed relationships between TSH and thyroid antibodies with a focus on TSH reference limits.

Subjects and Methods

Study population

A cohort of 16,088 nonpregnant subjects from NHANES III (7821 women and 8267 men) without estrogen, androgen, thyroid, or lithium medications was used to study relationships between TSH, TPOAb, and TgAb. TSH reference range parameters (2.5th, 50th, and 97.5th percentiles) were studied in a subcohort of 14,202 individuals representing the three major ethnic groups [B, n = 2230 women and 2098 men; W, n = 2576, women and 2958 men; and Mexican-American (M), n =2047 women and 2293 men] without reported thyroid disease or taking thyroid-altering medications and having TT_4 levels within laboratory reference limits. All age groups were well represented: 12–19 yr (n = 2131, 1086 women and 1045 men); 20–29 yr (n = 2584, 1186 women and 1398 men); 30–39 yr (n = 2598, 1370 women and 1228 men); 40–49 yr (n = 2011, 974 women and 1037 men); 50–59 yr (n = 1245, 565 women and 680 men); 60–69 yr (n = 1619, 710 women and 909 men); 70–79 yr (n = 1181, 558 women and 623 men), and more than 80 yr (n = 833, 404 women and 429 men).

Laboratory methods

The immunoassay methods used to measure TT_4, TSH, TPOAb, and TgAb have been described previously (3). Manufacturers' reference ranges [TT_4, 4.5–13.2 μg/dl (58–170 nmol/liter); and TSH, 0.39–4.6 mIU/liter] were used to identify subjects with overt (low TT_4/high TSH) and subclinical (normal TT_4/highTSH) hypothyroidism. Subjects with TPOAb less than 0.5 kIU/liter and TgAb less than 1.0 kIU/liter were considered antibody negative.

Statistical analyses

Data from the NHANES III were assembled with SAS software (Research Triangle Institute, Triangle Park, NC) for analysis using Excel software.

Results

Figure 1 shows the prevalence of thyroid antibodies across TSH intervals of the cohort of 16,088. There was no associ-

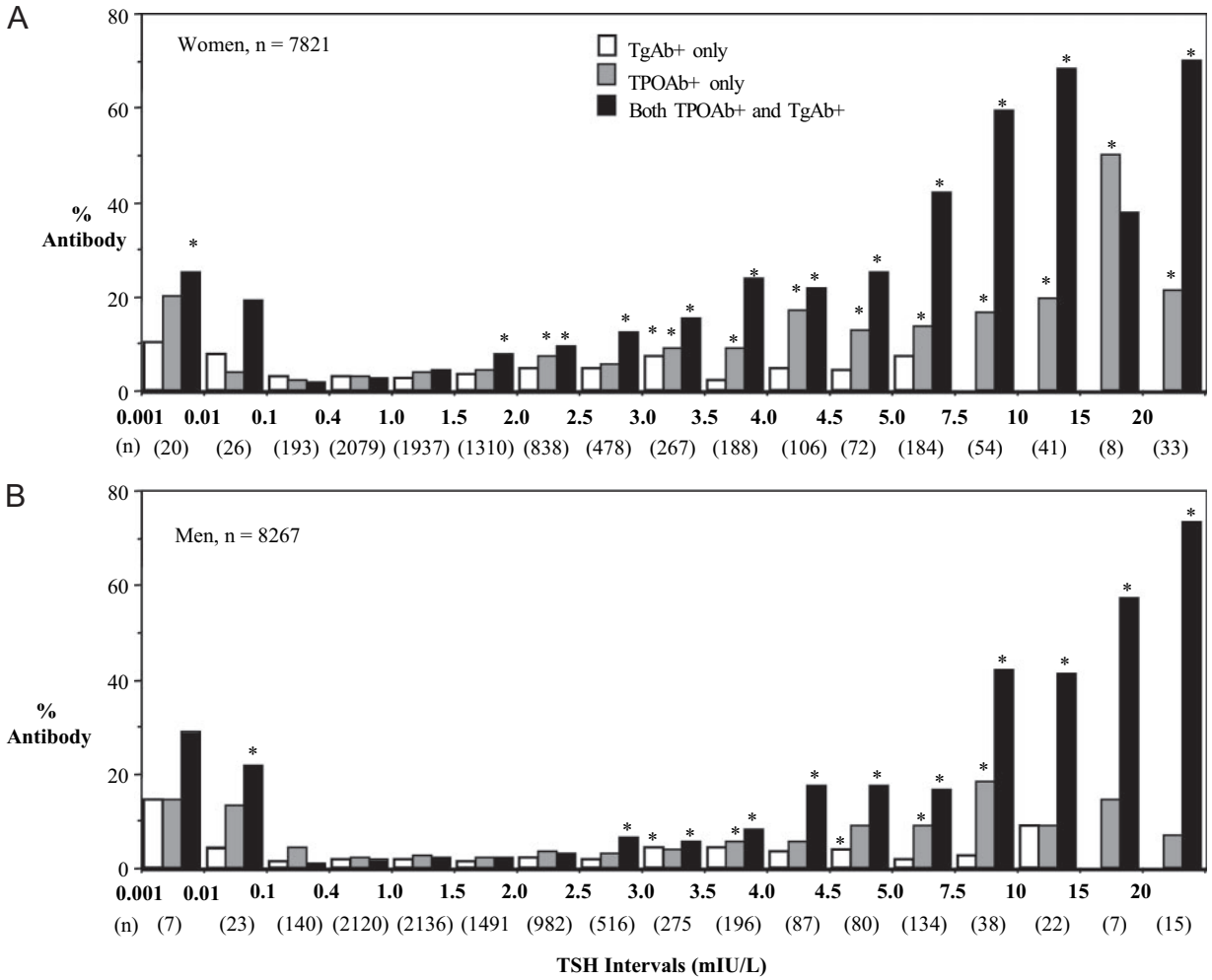

FIG. 1. Prevalence of thyroid antibodies across TSH intervals in women (A) and men (B). The *abscissa* TSH values correspond to the upper and lower limits of the intervals spanning each set of bars. *Asterisks* denote a significant difference in prevalence from the TSH range with lowest antibody prevalence, 0.1 and 1.5 mIU/liter for women and 0.1 and 2.0 mIU/liter for men.

ation between TSH and a positive TgAb alone in either women or men. The lowest TPOAb prevalence (<3%) for women was seen in a TSH range between 0.1 and 1.5 mIU/liter and for men between 0.1 and 2.0 mIU/liter. TPOAb prevalence progressively increased with TSH above 2 mIU/liter, approaching 80% when TSH was above 20 mIU/liter and both antibodies were present. However, in subjects with unequivocal TSH elevations (>10 mIU/liter), no thyroid antibodies were detected in 31% of 45 men (median age 71 yr) and 11% of 82 women (median age 74 yr).

In the subcohort of 14,202, thyroid antibody prevalence was 17.4% for W (23.2% women, 12.3% men), 5.6% for B (8.0% women, 3.1% men), and 13.1% for M (18.7% women, 8.2% men). TPOAb, either alone or associated with TgAb, was the dominant antibody detected in all ethnic groups (13.3% W, 4.7% B, and 11.0% M). Median TPOAb concentrations were higher when associated with TgAb (28 kIU/liter) than alone (6 kIU/liter).

Figure 2 shows that the prevalence of thyroid antibodies increased with age in all ethnic groups but at each age was always lower for B compared with W or M. Although the inclusion of antibody-positive subjects had no effect on the 2.5th TSH percentile, there was a trend for an increase in the 50th TSH percentiles, which reached significance only for W ($P < 0.01$). The inclusion of antibodies increased the 97.5th percentile of each age group and ethnicity ($P < 0.05$). In the absence of antibodies, there was a positive correlation between 97.5th TSH percentile and age for each ethnic group ($P < 0.01$). When age groups with similar antibody prevalence from each ethnicity were compared, 20- to 29-yr-old W and M (10.2 and 9.6% antibody prevalence) *vs.* 40- to 49-yr-old B (7.0% antibody prevalence), the TSH 97.5th percentiles calculated for antibody-negative subjects became comparable for W, M, and B (3.5, 3.5, and 3.7 mIU/liter, respectively).

Discussion

Previous studies have shown that a TSH level less than 2 mIU/liter is associated with a nadir in both thyroid antibody prevalence (<3%) and lymphocytic infiltration (evident with ultrasound) (8, 9). In this study, TPOAb prevalence progressively increased with TSH above 2 mIU/liter, approaching 80% when TSH was above 20 mIU/liter and both antibodies were present. However, 31% of men and 11% of women with TSH over 10 mIU/liter had no thyroid antibodies detected (Fig. 1). This is not an unexpected finding for older subjects (median age was 71 yr) considering that thyroid antibody prevalence decreases and atrophic thyroiditis increases with age (11). It follows that TSH reference intervals calculated from population data may be skewed by including individuals whose thyroid dysfunction cannot be detected by thyroid antibody testing alone. As shown in Fig. 2, a skewing of the TSH upper limit was evident in the older age groups of each ethnicity even when no thyroid antibodies were detected. When Hashimoto's thyroiditis is the dominant thyroid pathology affecting a population, the TSH upper limit increases with age (3, 9). This does not appear to reflect the influence of age *per se* because an inverse relationship (decreasing TSH with age) is seen when the dominant thyroid

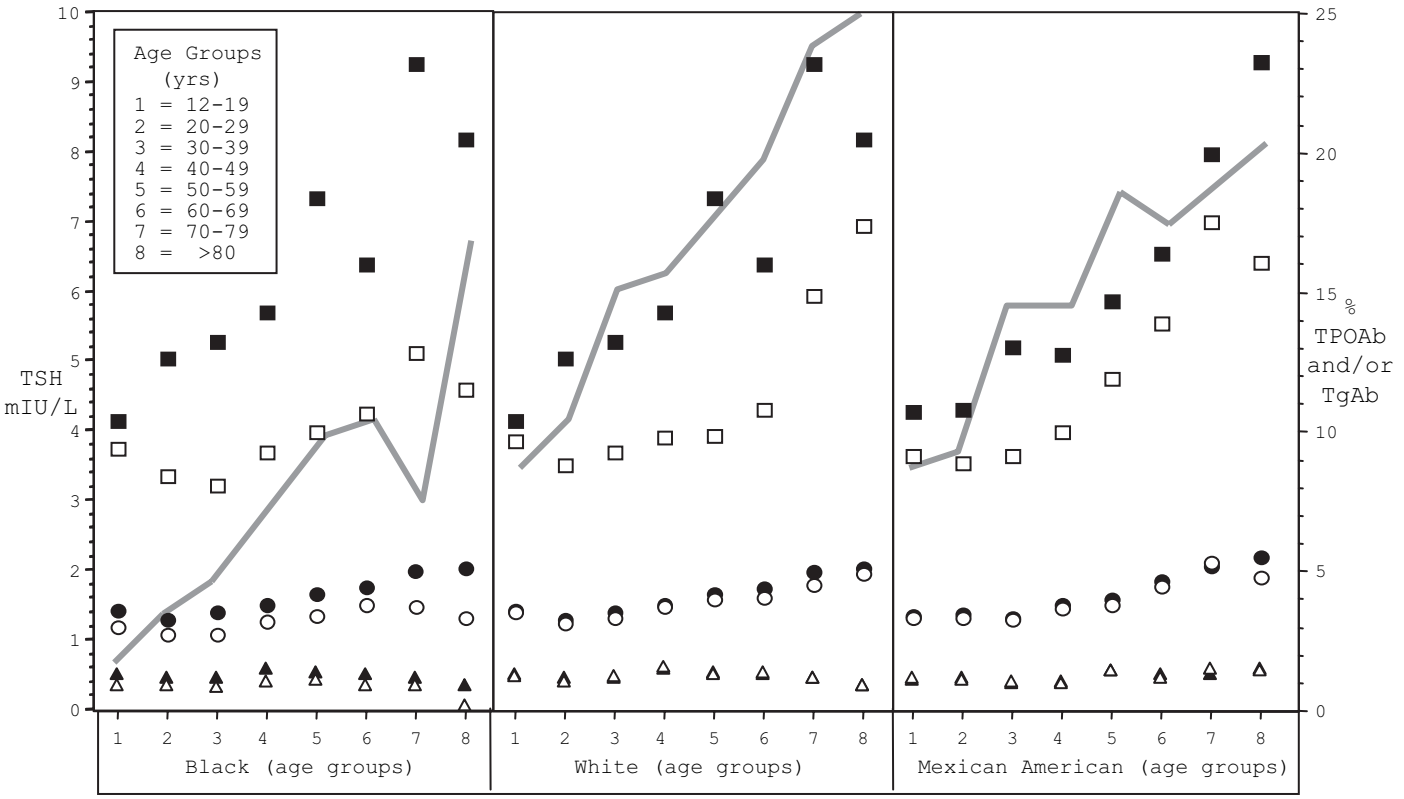

FIG. 2. The panels show TSH percentiles (5th, *triangles*; 50th, *circles*; and 97.5th, *squares*) for the eight age groups for each ethnicity: *left*, B; *middle*, W; *right*, M. *Solid symbols* represent the TSH percentiles for antibody-positive subjects, and *open symbols* represent the TSH percentiles for antibody-negative subjects. In each *panel*, the solid line links the prevalence of TPOAb and/or TgAb of each age group.

pathology is toxic multinodular goiter secondary to iodine deficiency, the prevalence of which increases with age (10). Thus, the variability in the TSH upper limit reported for different populations may reflect differences in the prevalence of occult thyroid pathology, although other factors, such as sampling time of day, the sensitivity and specificity of the TPOAb method used, and TSH method specificity may be contributing factors (4–6, 12). In NHANES III, specimens were drawn during morning, afternoon, or evening sessions, so that sampling time is unlikely to be a confounding variable (12). Although current TSH immunometric assays are all standardized against the same International Reference Preparation, significant variability may reflect differences in monoclonal antibody specificity for different circulating TSH isoforms (5). In one TSH reference range study that used both thyroid antibodies and ultrasound hypoechogenicity as exclusion criteria, a TSH upper limit of 3.7 mIU/liter was reported with one method as compared with 2.9 mIU/liter using a different method (6).

Thyroid antibody prevalence in NHANES III was lower for B compared with W or M in accord with a lower prevalence of lymphocytic thyroiditis seen for B *vs.* W at autopsy (13). Similarly, the TSH 97.5th percentile of the antibody-negative B cohort was lower than that for W or M as previously reported (3), and in each age group, antibody prevalence was lower for B compared with W or M. The inclusion of antibody-positive subjects increased the TSH 97.5th percentiles of each age group and ethnicity relative to the 97.5th percentiles calculated for antibody-negative subjects. However, when age groups with a similar thyroid antibody prevalence were compared (B age 40–49 yr *vs.* W and M age 20–29 yr), the TSH upper limits calculated for those cohorts became comparable (B 3.7 mIU/liter, W 3.5 mIU/liter, and M 3.5 mIU/liter). This suggests that occult thyroid autoimmunity skews the TSH upper limit to a degree related to the prevalence of autoimmune thyroiditis in the population being studied.

The setting of the TSH upper reference limit impacts the controversy concerning the diagnosis and efficacy of treating mild (subclinical) hypothyroidism that followed the 2002 Consensus Conference on subclinical thyroid disease (14–16). Since then, studies of various markers of thyroid hormone action on tissues have suggested that even slightly elevated TSH levels (3–10 mIU/liter) may increase the risk for atherosclerosis in susceptible individuals (17–20). The current study shows that it is not possible to establish an accurate TSH upper limit from population data such as NHANES III. Furthermore, because TSH has a low index of individuality (the ratio between the within- and between-person variability), the TSH population reference range is not expected to be a sensitive parameter for detecting thyroid dysfunction in individuals (2). For these reasons, the American Association of Clinical Endocrinologists has recommended the adoption of a TSH upper limit of 3.0 mIU/liter, a value close to the TSH upper limit determined for populations with a low prevalence of thyroid autoimmunity (*i.e.* NHANES III Blacks) (10, 21). Contracting the TSH upper limit should not necessarily increase the frequency of L-T_4 treatment in view of the Consensus Conference statement that clinical action should relate to the degree of TSH elevation together with the individual clinical context (pregnancy, lipid profile, and the presence of TPOAb) (13).

It was hoped that the NHANES III survey would provide definitive TSH reference range data (3). However, the current analysis shows that an accurate TSH reference range cannot be determined from population data, because occult thyroid dysfunction skews the TSH upper limit (3). Because of the association of thyroid antibodies with upper TSH values, we believe this dysfunction is related to autoimmune disease, but it may well be due to other unidentified physiological mechanisms. The adoption of a TSH upper limit of 3.0 mIU/liter as recommended by the American Association of Clinical Endocrinologists is consistent with the range of TSH associated with the lowest TPOAb prevalence in both men and women (Fig. 1) and is in accord with new Endocrine Society guidelines that recommend a TSH upper limit of 2.5 mIU/liter for preconception planning and pregnancy (21, 22). These recommendations, however, come with the proviso that sound clinical judgment, based on findings other than TSH concentrations, be exercised with regard to initiating treatment.

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