

Age-Related Changes in Thyroid Function: A Longitudinal Study of a Community-Based Cohort

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Context: In cross-sectional studies, serum TSH concentrations increase with age. This has not been examined longitudinally, and it is uncertain whether the TSH increase reflects healthy aging or occult thyroid failure.

Methods: We measured serum TSH, free T_4 , thyroid peroxidase, and thyroglobulin antibodies in 1100 participants in the 1981 and 1994 Busselton Health Surveys and derived a reference group of 908 individuals without thyroid disease or thyroid antibodies. We examined changes in thyroid function longitudinally and, in 781 participants, explored associations with the *CAPZB* polymorphism rs10917469.

Results: At 13 yr follow-up, mean serum TSH increased from 1.49 to 1.81 mU/liter, a change in mean TSH (Δ TSH) of 0.32 mU/liter [95% confidence interval (CI) 0.27, 0.38, $P < 0.001$], whereas mean free T_4 concentration was unchanged (16.6 vs. 16.6 pmol/liter, $P = 0.7$). The TSH increase was most marked in the elderly, such that gender-adjusted Δ TSH increased by 0.08 mU/liter (95% CI 0.04, 0.11) for each decade of baseline age. People with higher baseline TSH values had proportionally smaller increases in TSH, with each additional 1.0 mU/liter of baseline TSH associated with a 0.13 mU/liter decrease (age and gender adjusted) in Δ TSH (95% CI 0.09, 0.16). The Δ TSH did not differ significantly by *CAPZB* genotype.

Conclusions: Aging is associated with increased serum TSH concentrations, with no change in free T_4 concentrations. The largest TSH increase is in people with the lowest TSH at baseline. This suggests that the TSH increase arises from age-related alteration in the TSH set point or reduced TSH bioactivity rather than occult thyroid disease. (*J Clin Endocrinol Metab* 97: 1554–1562, 2012)

Homeostatic mechanisms ensure that, in the absence of disease, circulating concentrations of TSH and T_4 are tightly regulated. In healthy individuals undergoing serial thyroid function testing, variation observed in each person over time (intraindividual variation) is less than the

differences between individuals (interindividual variation) (1). This suggests that individuals have different set points (individual means) for pituitary-thyroid axis function. Twin studies suggest that TSH set points are under a strong genetic influence, with heritability estimates of ap-

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Abbreviations: BMI, Body mass index; Δ BMI, change in BMI between study visits; CI, confidence interval; Δ free T_4 , change between 1981 and 1994 in free T_4 ; IQR, interquartile range; NHANES III, National Health and Nutrition Examination Survey III; SNP, single-nucleotide polymorphism; TPOAb, thyroid peroxidase antibody; TgAb, thyroglobulin antibody; Δ TSH, change between 1981 and 1994 in TSH.

proximately 65% (2, 3). Recent studies have shown that common variants in or close to the *PDE8B*, *TSHR*, and *CAPZB* genes are associated with differences in serum TSH concentrations (4–6), but most of the heritability of TSH remains unexplained (7).

Aging is associated with changes in pituitary-thyroid axis function as well as an increased prevalence of autoimmune and nodular thyroid disease (8). Previous studies suggested that, in the absence of thyroid disease, aging was associated with reduced TSH secretion (8, 9). However, more recent data from the National Health and Nutrition Examinations Survey III (NHANES III) show that, in conditions of iodine sufficiency, serum TSH concentrations increase with age in people with no clinical or biochemical evidence of thyroid disease (10). Thus, in a reference group of 14,376 NHANES III participants free of overt hypothyroidism or hyperthyroidism, with no history of thyroid disease and with negative tests for thyroid peroxidase and thyroglobulin antibodies, there was a progressive shift in the serum TSH distribution curve toward higher TSH values with increasing age (11). Similar results have now been reported from other communities (12, 13). Based on these data, some authorities recommend the adoption of age-specific reference ranges for TSH (14, 15), although this remains a matter of debate (16, 17). A limitation of these studies is their cross-sectional design, and there are no large, longitudinal studies examining changes in serum TSH and free T_4 concentrations in individuals without thyroid disease.

If TSH concentrations do increase with age, this could arise from physiological or pathological processes. Many endocrine systems exhibit changes with aging in the absence of overt disease, such as the age-related decline in secretion of somatotropin, IGF-I, dehydroepiandrosterone sulfate and, in men, testosterone (9). If similar changes occur in thyroid function, an age-related fall in circulating T_4 concentrations (in the absence of thyroid disease) could result in increased TSH secretion. Alternatively, an age-related increase in TSH could occur without a reduction in circulating T_4 if, for example, there is a reduction with aging in TSH bioactivity or the responsiveness of the thyroid to TSH. A third possibility is that the TSH increase in older people results from occult thyroid disease. Serum thyroid antibody testing does not detect all cases of lymphocytic thyroiditis (18), and so the reference group in NHANES III may still have included some participants with autoimmune thyroid disease. In that case, the age-related increase in TSH might reflect a higher prevalence of occult thyroid disease in older people rather than being a feature of healthy aging (19).

To address these uncertainties, we examined age-related changes in thyroid function in participants in the

Busselton Health Survey who were studied on two occasions, 13 yr apart. We hypothesized that if TSH increases with age in response to occult thyroid disease, then this increase should be most evident in participants with a high-normal TSH at baseline because the upper part of the TSH reference range is thought to harbor individuals with early thyroid failure (20), and the TSH increase should be accompanied by a fall in free T_4 concentrations. In addition, because only one study has explored whether the association between common genetic variants and TSH remains constant with aging (21), we examined whether a polymorphism located upstream of the *CAPZB* gene (5) was associated with differences in the age-related increase in TSH.

Materials 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 (22, 23). 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. The 1981 questionnaire included the question, “Have you ever had thyroid disease or goiter?” whereas in 1994 no specific question on thyroid disease was included. In 2001, archived sera from 2108 participants in the 1981 survey were assayed for TSH, free T_4 , thyroid peroxidase antibody (TPOAb), and thyroglobulin antibody (TgAb) concentrations using an Immulite 2000 chemiluminescent analyzer (Siemens Healthcare Diagnostics Products, Deerfield, IL) as previously described (24, 25). Of these 2108 subjects, 1328 also attended the 1994 survey and had a blood sample collected. In 2007, sera from these participants were assayed for TSH, free T_4 , and TPOAb concentrations using the same immunoassay platform, as previously described (25). Reference ranges derived from a cross-sectional analysis of the cohort (24) were as follows: TSH, 0.4–4.0 mU/liter; free T_4 , 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 TPOAb or TgAb. The fact that sera from the 1981 and 1994 surveys were assayed years apart introduced a potential bias. To address this, in 2010 we reassayed TSH and free T_4 in paired sera (from 1981 and 1994) from 65 participants, selected on the basis of serum availability and to achieve a broad range of TSH concentrations, and compared the values using the method of Bland and Altman (26). For log TSH, the 95% confidence interval (CI) for the mean difference between original and repeat measures was –0.03, 0.05, with 97% of differences lying within the limits of agreement, indicating good agreement, with no evidence of systematic bias affecting the original assay runs. The free T_4 assay had been reformulated by the manufacturer in 2009 to give lower values. As expected, repeat values were lower, with the 95% CI for the mean difference being 0.15, 0.97, and 98% of differences lying within the limits of agreement. Because of the change in method, bias affecting the original free T_4 assay runs could not be completely excluded.

We performed cross-sectional analyses of data from participants in the 1981 survey and longitudinal analyses for those who participated in both the 1981 and 1994 surveys. To derive reference groups free of thyroid disease or other confounders, we applied the following exclusion criteria: self-reported thyroid disease or goiter; overt hypothyroidism (defined as TSH >20 mU/liter or TSH >4 mU/liter accompanied by free T₄ <9 pmol/liter); overt hyperthyroidism (defined as serum TSH <0.4 mU/liter with free T₄ >23 pmol/liter); use of T₄ or antithyroid drugs; use of amiodarone, lithium carbonate, carbamazepine or phenytoin (because of the effects of these medications on thyroid function or thyroid function tests); positive thyroid antibodies (TPOAb or TgAb in 1981, TPOAb in 1994); and discordant results suggestive of pituitary dysfunction or assay interference (e.g. increased TSH with increased free T₄).

Data are reported as mean and SD or median and interquartile range (IQR) if skewed. Baseline characteristics of subjects who attended the 1994 survey were compared with those of subjects who were alive but did not attend and subjects who were deceased, using independent *t* tests or χ^2 tests. Characteristics of the longitudinal reference group at baseline and follow-up were compared using paired *t* tests or McNemar tests.

The cross-sectional analysis comprised calculation of Pearson correlations for age, log-transformed TSH, and free T₄. Linear regression models were used to determine whether these associations differed for males and females with age in decades. The reference ranges for TSH were calculated as mean \pm 2 SD of log-transformed serum TSH concentrations for each age stratum.

To perform the longitudinal analysis, the changes between 1981 and 1994 in TSH (Δ TSH) and free T₄ (Δ free T₄) were calculated for each participant. Relationships between age at baseline and each of Δ TSH and Δ free T₄, as well as between Δ TSH and Δ free T₄, were modeled using linear regression, adjusted for gender and then further adjusted for change in body mass index between study visits (Δ BMI) and smoking status. Smoking status was analyzed in six categories: three with unchanged smoking status between 1981 and 1994 (never/never, former/former, current/current) and three with changed status between surveys [current/former (stopped smoking between surveys), never/former (started and then stopped smoking between surveys), and never or former at baseline and current at follow-up (started or resumed smoking between surveys)]. Regression models were also used to model relationships between baseline TSH (as a continuous variable and in quintiles) and each of Δ TSH and Δ free T₄ and to adjust for age and gender and further adjust for smoking and Δ BMI. Error bar graphs were used to show variations in Δ TSH and Δ free T₄ over decades of age and quintiles of baseline TSH, and a probability density plot was produced to illustrate the distribution of TSH at baseline and follow-up.

Tobacco smoking is associated with reduced serum TSH concentrations and smoking cessation with a subsequent, gradual increase in TSH (27). To eliminate any confounding effect of change in smoking status between surveys, relationships were modeled for a subgroup of participants who reported that they had never smoked at both baseline and follow-up visits.

Genotyping for the single-nucleotide polymorphism (SNP) rs10917469, located upstream of the *CAPZB* gene, was determined by TaqMan allelic discrimination 5' nuclease assay (Applied Biosystems, Foster City, CA) as previously described (5). Relationships between *CAPZB* genotype and TSH at baseline

and follow-up visits, as well as Δ TSH, were modeled using regression analyses adjusted for age, gender, and body mass index (BMI). Smoking status was not a significant covariate in these analyses and was not included in the final models. TSH concentrations were log transformed before analysis.

Analyses were performed using PASW Statistics 18, release version 18.0.0 (IBM SPSS Inc., Chicago, IL; 2009, www.spss.com) and R version 2.13.1 (R Foundation for Statistical Computing, 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

Cross-sectional analysis

After excluding participants with evidence of thyroid disease, positive thyroid antibodies, missing data and those who breached other exclusion criteria, we derived a reference group of 1751 subjects for cross-sectional analysis. Figure 1 shows participant disposition and Table 1 shows data for this group. In the 1751 participants, there was a significant positive correlation between age and TSH ($r = 0.092$, $P < 0.001$), and significant negative correlations between age and free T₄ ($r = -0.146$, $P < 0.001$)

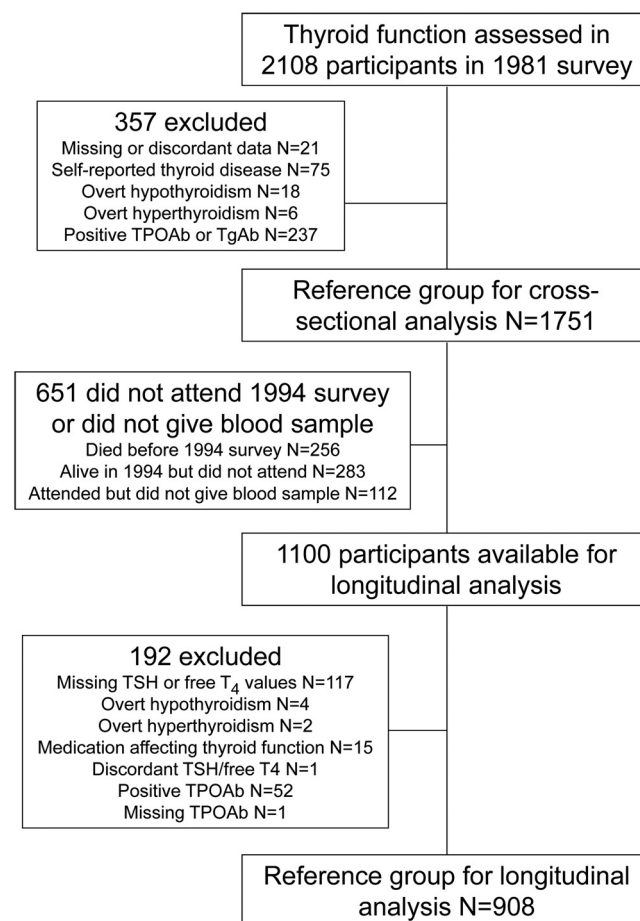


FIG. 1. Participant disposition.

TABLE 1. Baseline characteristics of the cross-sectional reference group of 1751 participants in the 1981 survey, further categorized by their vital status and participation or nonparticipation in the 1994 survey

	Cross-sectional reference group from 1981 survey (n = 1751)	Attended 1994 survey (n = 1212)	Alive in 1994 but did not attend 1994 survey (n = 283)	<i>P</i> ^a	Deceased in 1994 (n = 256)	<i>P</i> ^b
Age, mean (sd) (yr)	49.2 (17.2)	45.5 (15.1)	47.6 (18.2)	0.072	68.5 (11.0)	<0.001
Female, n (%)	792 (45.2)	564 (46.5)	133 (47.0)	0.888	95 (37.1)	0.006
Smoking status, n (%) ^c				0.200		<0.001
Current	363 (20.9)	241 (20.0)	66 (23.6)		56 (22.0)	0.494
Former	553 (31.8)	352 (29.3)	88 (31.4)		113 (44.3)	<0.001
Never	822 (47.3)	610 (50.7)	126 (45.0)		86 (33.7)	<0.001
BMI, mean (sd) (kg/m ²)	25.5 (3.75)	25.2 (3.68)	26.2 (3.96)	0.001	26.3 (3.80)	<0.001
TSH, median (IQR) (mU/liter)	1.64 (1.13–2.29)	1.36 (0.94–1.86)	1.40 (1.03–1.97)	0.262	1.49 (1.07–2.10)	0.001
Free T ₄ , mean (sd) (pmol/liter)	16.4 (2.83)	16.6 (2.83)	16.4 (2.72)	0.375	15.8 (2.85)	<0.001

^a *P* value from *t* test or χ^2 test comparing the 1212 subjects who attended the 1994 survey with the 283 subjects who were alive in 1994 but did not attend.

^b *P* values for comparisons of the 1212 subjects who attended the 1994 survey with the 256 subjects who were deceased in 1994.

^c Excluding 13 participants with unknown smoking status.

and TSH and free T₄ ($r = -0.142$, $P < 0.001$). Neither the relationship between age and TSH nor that between age and free T₄ differed significantly between men and women. Age-related reference ranges for TSH calculated from the reference group of 1751 participants are shown in Table 2.

Longitudinal analysis

Of the 1751 participants in the 1981 cross-sectional sample, 1495 were alive in 1994 and of these 1212 (81.1%) attended the 1994 survey (Table 1). Compared with those who attended the follow-up survey, participants who died before 1994 were significantly older, more likely to be male and more likely to have smoked; in addition at baseline serum TSH was significantly higher, and

free T₄ significantly lower in the deceased group, although the magnitude of these differences was small. Subjects who were alive but did not participate in the 1994 survey did not differ significantly from those who did take part, except with regard to baseline BMI, which was higher in nonparticipants.

After excluding participants with evidence of thyroid disease or positive thyroid antibodies at follow-up or because other exclusion criteria were breached, the reference group for the longitudinal analysis comprised 908 participants (Fig. 1). The mean time between study visits was 13.0 yr (range 12.3–14.0 yr). Participant characteristics at baseline and follow-up are provided in Table 3. There were significant changes in smoking status between surveys (in particular, a reduction in the number of current smokers) and a significant increase in mean BMI. The relationships between age and TSH, between age and free T₄, and between TSH and free T₄ at baseline in these 908 participants were similar to those observed in the cross-sectional reference group as a whole.

At 13-yr follow-up, the mean serum TSH concentration in the cohort had increased from 1.49 to 1.81 mU/liter, with a mean Δ TSH of 0.32 mU/liter (95% CI 0.27, 0.38, $P < 0.001$), whereas the mean free T₄ concentration was unchanged (16.6 vs. 16.6 pmol/liter, $P = 0.7$) (Table 3). The mean change in TSH did not differ significantly between genders, being 0.34 mU/liter in men and 0.31 mU/liter in women ($P = 0.62$), and neither smoking status nor change in BMI between visits was a significant predictor of Δ TSH ($P = 0.08$ and $P = 0.72$, respectively). A probability

TABLE 2. Age-related reference ranges for TSH derived from the cross-sectional reference group (n = 1751)

Age (yr)	n	TSH reference range (mU/liter)		
		Lower limit	Mean	Upper limit
<30	304	0.51	1.34	3.54
30–40	299	0.48	1.25	3.21
40–50	269	0.44	1.32	3.92
50–60	321	0.42	1.31	4.09
60–70	334	0.38	1.34	4.70
>70	224	0.52	1.66	5.28
All	1751	0.44	1.35	4.10

Reference ranges were calculated as mean \pm 2 sd of log-transformed serum TSH concentrations for each age stratum.

TABLE 3. Clinical characteristics of the longitudinal reference group (n = 908) at baseline and follow-up visits

	Baseline (1981) (n = 908)	Follow-up (1994) (n = 908)	P ^a
Age, mean (SD) (yr)	45.5 (14.5)	58.5 (14.4)	
Female, n (%)	423 (46.6)	423 (46.6)	
Smoking status, n (%) ^b			<0.001
Current	174 (19.9)	82 (9.4)	
Former	252 (28.8)	356 (40.6)	
Never	449 (51.3)	438 (50.0)	
BMI, mean (SD) (kg/m ²)	25.1 (3.63)	26.4 (3.92)	<0.001
TSH mU/liter, mean (SD)	1.49 (0.79)	1.81 (0.96)	<0.001
Median (IQR)	1.36 (0.95–1.85)	1.63 (1.12–2.22)	
Change in TSH, mean (95% CI)		0.32 (0.27, 0.38)	
Free T ₄ pmol/liter, mean (SD)	16.6 (2.85)	16.6 (2.82)	0.667
Change in free T ₄ , mean (95% CI)		0.04 (–0.16, 0.24)	

^a P value from paired *t* test or McNemar test comparing baseline with follow-up values.

^b Percentage of those with self-reported smoking status at both baseline and follow-up.

density plot of TSH distribution at baseline and follow-up demonstrated a rightward shift in TSH concentrations, with some flattening of the curve, as shown in Fig. 2. In 143 participants (16%), Δ TSH was 1.0 mU/liter or more, and in 25 (3%) it was 2.0 mU/liter or greater.

Figure 3A shows Δ TSH over the 13-yr follow-up period plotted for participants by age group. There was a significant relationship between age and Δ TSH, with the greatest increase in TSH occurring in the oldest participants. After adjustment for gender, Δ TSH increased on average by 0.08 mU/liter (95% CI 0.04, 0.11) with each additional decade of age at baseline. Results were similar after further adjustment for smoking and Δ BMI (mean Δ TSH 0.09 mU/liter; 95% CI 0.05, 0.13). There was no evidence of an accompanying fall in free T₄ in any age group (Fig. 3B); after adjustment for sex, Δ free T₄ increased on average by 0.2 pmol/liter (95% CI 0.1, 0.4) with each additional de-

cade of baseline age. There was no significant relationship between Δ TSH and Δ free T₄ in simple regression models or after adjustment for age and gender.

In Fig. 3C, Δ TSH for participants is plotted according to quintiles of TSH at baseline. Participants with higher baseline TSH values had proportionally smaller increases in TSH during follow-up. After adjustment for age and gender, each additional 1.0 mU/liter of baseline TSH was associated with a 0.12 mU/liter decrease in mean Δ TSH (95% CI 0.09, 0.16). The results were similar after further adjustment for smoking and Δ BMI (mean Δ TSH –0.13 mU/liter, 95% CI –0.16, –0.09). In participants in the highest quintile of baseline TSH (serum TSH >2.2 mU/liter), serum TSH did not differ significantly between baseline and follow-up visits. Serum free T₄ did not change significantly between study visits when participants were analyzed by quintiles of baseline TSH (Fig. 3D).

To eliminate the potential confounding effects of smoking cessation on serum TSH concentrations, we performed a subgroup analysis of 438 participants who reported that they had never smoked at both baseline and follow-up visits. In this subgroup, gender-adjusted Δ TSH increased on average by 0.09 mU/liter (95% CI 0.04, 0.14) with each additional decade of age at baseline, similar to the result for the cohort as a whole. When analyzed by baseline TSH with adjustment for age and gender, each additional millunit per liter of baseline TSH was associated with a 0.17 mU/liter decrease in mean Δ TSH (95% CI 0.07, 0.27), also similar to the result for the entire cohort.

Relationship between TSH and CAPZB genotype

Genotype data for the SNP rs10917469, located upstream of the CAPZB gene, were available for 823 of the 908 participants in the longitudinal group; in the remain-

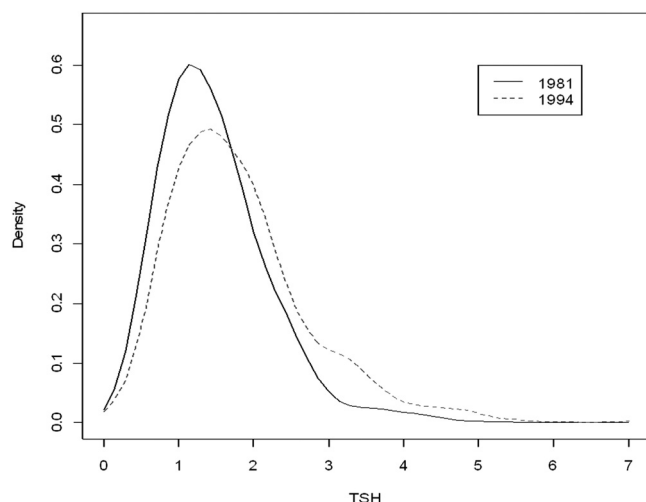


FIG. 2. Probability density plot of TSH distribution at baseline and follow-up.

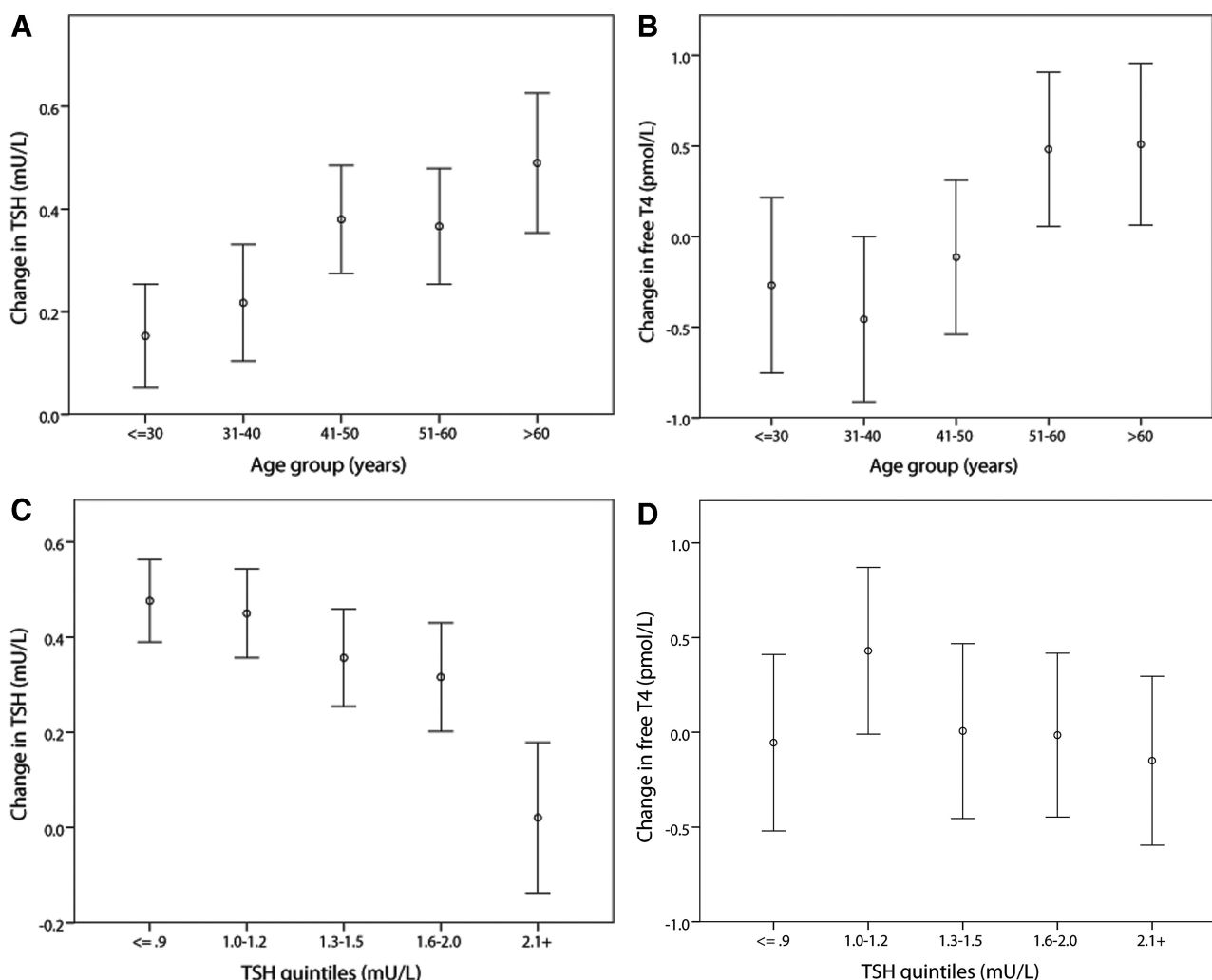


FIG. 3. Error bar plots showing means and 95% CI for each of the changes in TSH and free T₄ between baseline and follow-up over age in decades (A and B, respectively) and quintiles of baseline TSH (C and D). The numbers of participants in each age group (from youngest to oldest) are 165, 181, 183, 207, and 172.

ing subjects, DNA was not available or genotyping failed. Forty-two participants were excluded because of missing covariate data from the 1981 or 1994 visits, leaving 781 participants for analysis, in whom the minor (G) allele frequency was 0.14. On cross-sectional analysis, after adjustment for age, gender, and BMI, serum TSH concentrations differed significantly according to *CAPZB* genotype at both baseline and follow-up visits, with the highest values associated with AA genotype, and the lowest with

GG (Table 4). There was, however, no significant difference in Δ TSH between genotypes.

Discussion

In this study of a large cohort of individuals with no evidence of thyroid disease, mean serum TSH concentrations increased over a 13-yr period, with significantly greater

TABLE 4. Serum TSH concentrations analyzed by genotype for the *CAPZB* polymorphism rs10917469 for 781 members of the longitudinal reference group at baseline and follow-up visits

	Genotype			β (SE)	P
	AA (n = 578)	AG (n = 184)	GG (n = 19)		
TSH at baseline (mU/liter)	1.53 (0.84)	1.42 (0.72)	1.23 (0.49)	−0.078 (0.036)	0.031
TSH at follow-up (mU/liter)	1.90 (1.00)	1.69 (0.87)	1.47 (0.48)	−0.096 (0.039)	0.015
Δ TSH (mU/liter)	0.36 (0.83)	0.27 (0.71)	0.24 (0.42)	−0.076 (0.056)	0.176

TSH values are shown as mean (SD); β (SE) and P values are from regression analyses adjusting for age, sex, and BMI.

increases in older participants than younger participants. This confirms results from cross-sectional studies showing an increase in TSH with age in reference populations free of thyroid disease. The shift in TSH distribution curves we observed with 13 yr of aging in the present study is very similar to that seen in a cross-sectional analysis of NHANES III participants (11), and the age-related reference ranges we obtained were also similar to those recently published from NHANES III data (14). The magnitude of the mean increase in TSH in the cohort was relatively modest, but in 16% of participants, the TSH increase was 1.0 mU/liter or more [thought to indicate a significant difference at the 1% level with repeated testing in the same individual (1)], and in 3% it was 2.0 mU/liter or more, which is potentially clinically relevant.

The study provides two strong lines of evidence that the age-related increase in TSH we observed is not a result of occult thyroid disease, which has been advanced as an explanation for the cross-sectional results from NHANES III (19). First, the largest TSH increase was seen in participants with the lowest TSH concentrations at baseline. Although this may be consistent with regression to the mean, it is the opposite of what would be expected if the TSH increase arose from thyroid disease because individuals with early thyroid failure should be overrepresented in the upper part of the population distribution of TSH. Second, there was no evidence of a fall in serum free T_4 to accompany the increase in TSH either in the cohort as a whole or in the subgroups with the greatest increase in TSH, as would be expected if the TSH increase occurred because of occult thyroid failure resulting from lymphocytic thyroiditis in TPOAb-negative individuals.

The results suggest therefore that with aging, the set point for TSH secretion is altered resulting in higher serum TSH concentrations. There are several possible mechanisms for this. First, it could arise from diminished sensitivity of thyrotropes to negative feedback of thyroid hormones (*e.g.* by down-regulation of type 2 iodothyronine deiodinase activity in the pituitary), although in that case, one would expect the increased TSH secretion to result in an increase in free T_4 concentrations. This was not observed in the cohort as a whole, although there was a small increase in free T_4 in older participants. Second, there could be an age-related reduction in TSH bioactivity. Circulating TSH exists in a number of isoforms with different bioactivity, depending on the extent of glycosylation and sialylation (28), and the bioactivity of circulating TSH is reduced in both primary and central hypothyroidism (29–31). It is possible (but as yet unstudied) that TSH bioactivity declines with age so that a greater circulating concentration of immunoreactive TSH is required to maintain

the same circulating T_4 concentrations. Third, the TSH increase could arise from diminished responsiveness of the thyroid gland to TSH, such that higher TSH concentrations are required to maintain the same circulating concentrations of T_4 . Further studies are required to elucidate which of these is correct.

It is also uncertain whether the age-related increase in TSH is a result of genetic influences, environmental factors, or both. As previously shown in this and two other cohorts (5, 32), a SNP located upstream of the *CAPZB* gene was associated with differences in serum TSH between participants on cross-sectional analysis but not with changes in TSH concentration over the 13-yr study period. We previously reported a lack of association between Δ TSH in this cohort and a SNP in the *PDE8B* gene (21), suggesting that although these genes contribute to the regulation of pituitary-thyroid axis set points, they do not affect the age-related alteration in set point observed in this study. Most of the heritability of pituitary-thyroid axis set points is unexplained, and it remains to be seen whether other genetic factors are associated with the age-related increase in TSH concentrations (7).

The cross-sectional and longitudinal analyses each showed increasing TSH concentrations with age, although with some inconsistency: in the former, mean TSH appeared unchanged until age 70 yr (although the reference range upper limit increased from age 40 yr), whereas in the latter, Δ TSH was greater than zero in all age groups. We cannot fully explain this, but analysis of intraindividual change over time seems a more appropriate way to study effects of aging than comparing individuals who differ in age and other characteristics; thus, we regard the longitudinal results as more informative.

The strengths of this study include the community-based nature of the cohort, the detailed clinical and biochemical assessment of participants (allowing adjustment for potential confounders such as change in BMI and smoking), and its longitudinal design. A limitation is that although thyroid disease was excluded as far as possible, thyroid ultrasound was not performed and so we cannot be confident the cohort was strictly disease free. In reference range studies of iodine-sufficient populations, however, thyroid ultrasound results have little or no impact on normative data (33), reducing the likelihood that this is a significant confounder. Almost all the participants in our study were white, and it cannot be assumed that the results apply to other ethnic groups. TSH assay methods are less susceptible to variability than those for free T_4 , which may have affected our results; furthermore, we were unable to completely exclude assay drift affecting free T_4 .

In conclusion, we report that in a longitudinal analysis of a reference cohort, serum TSH concentrations increase

with age, and we provide evidence that this arises from an age-related alteration in TSH set point rather than from occult thyroid disease.

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