

# Thyroid Stimulating Hormone Reference Range: Iranian Thyroid Cohort study

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**Abstract.** *Background:* Current reference values for thyroid function tests are derived from data from different ethnicities and geographical areas. In this article, we aim to select criteria from the guidelines proposed by the National Academy of Clinical Biochemistry (NACB) and to determine the TSH and T4 reference limits in the iodine-sufficient area of Isfahan, a metropolitan city in Iran. *Materials and methods:* This study was conducted within the framework of “Isfahan Thyroid Study (ITS)”, an ongoing prospective cohort that started in 2006 (n=2523) until 2011 (n=711) and included participants above the age of twenty. We measured TSH, total T4, thyroid peroxidase antibody (TPOAb), and thyroglobulin antibody (TgAb). *Results:* Recruitment was based on the NACB criteria, 1899 participants were included in 2006(58.5% male) and 377 in 2011(62.3% male). The mean± SD age was 39.66 ±12.71 and 48.96±12.35 years in 2006 and 2011, respectively. The mean± SD for TSH was 2.0±1.19 and 2.11±1.11 mU/L and T4 was 6.67±1.47 and 8.3±2.95 µg /dl in 2006 and 2011, respectively. In 2006, the 2.5th percentile of serum TSH levels was 0.4 mU/L (males: 0.4 mU/L, females: 0.5 mU/L) and the 97.5th percentile of serum TSH was 4.96 mU/L (males: 4.72 mU/L, females: 5.3 mU/L). In 2011, the 2.5th percentile of serum TSH levels was 0.7 mU/L (males: 0.6 mU/L, females: 0.77 mU/L) and 97.5th percentiles of serum TSH was 4.9 mU/L (males: 5.7 mU/L, females: 5.57 mU/L). *Conclusion:* This study determined age and sex specific TSH and T4 reference ranges in the Isfahanian population, which could theoretically enable clinicians to classify patients more accurately. ([www.actabiomedica.it](http://www.actabiomedica.it))

**Keywords:** Reference range, TSH, Antithyroid antibodies, Iran.

## List of abbreviations:

NACB: National Academy of Clinical Biochemistry; ITS: Isfahan Thyroid Study; TPOAb: thyroid peroxidase antibody; TgAb: thyroglobulin antibody; TSH: Thyroid stimulating hormone; HPT: hypothalamo-pituitary-thyroid axis; FT4: free thyroxine; NHANES III: National Health and Nutrition Examination Survey

## Background

Thyroid stimulating hormone (TSH) is known as a sensitive indicator of hypothalamo-pituitary-thyroid axis (HPT) integrity with a reciprocal logarithmic relationship with free thyroxine (FT4) (1). Therefore, determining the TSH reference range has a critical role in the diagnosis of thyroid dysfunction (2, 3).

The upper reference limit of TSH is important for the diagnosis of subclinical hypothyroidism (4). Since adverse health outcomes occur when clinical hypothyroidism occurs (5), some studies have recommended a narrower TSH reference range (6, 7).

Guideline 22 of the National Academy of Clinical Biochemistry (NACB) states that TSH reference intervals from the 95% confidence limits of the log-transformed values of at least 120 rigorously screened normal euthyroid volunteers with no visible or palpable goiter, no family history of thyroid dysfunction, negative thyroid auto-antibodies, who are also not taking any medications that affect HPT axis, should be used to determine the TSH reference range (8).

The most commonly TSH reference intervals used are 0.5 – 5.0 mU/L or 0.4 – 4.0 mU/L based on the assay method used (9). However, these reference intervals were refuted due to the randomness of the background populations (10).

Based on the TSH distribution in a reference population of disease-free subjects from the National Health and Nutrition Examination Survey (NHANES III)(8), NACB recommended reducing the TSH reference interval to be : 0.4 – 2.5 mU/L(9). The matter of lowering the upper TSH reference limit is currently being discussed/ (11, 12).

Age, gender, thyroid autoantibodies, smoking and iodine intake and the methodological and selective criteria used for laboratory assessment could influence TSH reference range (8, 13-16). TSH and FT4 references differ among various populations. NHANES III reported that upper limits of TSH progressively increased with age to levels above 4.5 mU/L in the US population (8, 17, 18). Several studies reported the difference in serum TSH levels based on a race-specific distribution, for example median TSH level is considerably lower in Black people compared to Caucasians (8, 19, 20).

A recent Iranian study demonstrated age and sex specific TSH and T4 reference ranges in a Tehranian population (21). Therefore, we used the selection criteria proposed by NACB guidelines for determining the TSH and total T4 reference limits in the iodine-sufficient area of Isfahan, a metropolitan city of Iran.

## Methods

### *Subjects*

A population-based cohort study was performed in the iodine sufficient areas of Isfahan, a metropolitan city, of Iran, from 2006 (n=2523) to 2011 (n=711). The prospective study is detailed elsewhere (22, 23). All of the information was gathered from the «Isfahan Cohort Thyroid Study» (ITS). A total of 2523 and 711 people were selected in year 2006 and 2011 respectively and 1899 and 377 persons age  $\geq 20$  were included in 2006 and 2011, based on the NACB criteria (22, 23). All participants provided a written informed consent. The ethics committee of the Isfahan University of Medical Sciences approved the protocol for this study according to the Declaration of Helsinki.

The exclusion criteria for the study were as follows: 1) known history of thyroid disease 2) taking medicine affecting thyroid function such as levothyroxine, methimazole, carbimazole, amiodarone, lithium, iodine treatment, oral contraceptives, estrogen, glucocorticoids and anti-epileptic drugs ; 3) pregnancy or breast feeding; 4) TSH >10 or TSH<0.1mU/L ;5) positive thyroid peroxidase antibody (TPOAb) or thyroid globulin antibody (TgAb);6) history of visible or palpable goiter or nodule; 7) history of chronic disease ;8)admission to a hospital within the previous month.

### *Sampling and Laboratory measurements*

All blood samples were obtained in the morning between 07:00- 09:00 h, after 12 hours overnight fasting. Centrifugation was performed within 30 min of obtaining the blood sample and it was stored at  $-70^{\circ}\text{C}$ . TSH, T4 and TPOAb, Tg Ab were determined in all stored samples.

Serum total T4 and T3RU (T3 resin uptake) were analyzed by radioimmunoassay (Kavoshyar Co., Tehran, Iran). T4 intra- and inter-assay CV were 4.7 and 4.9%, respectively.

Normal range of T4 concentration was 4.5–12.0  $\mu\text{g/dL}$ . T3 intra- and inter-assay CV were 5.2 and 3.9%, respectively. Normal range of T3 concentration was 0.92–2.79 nmol/L.

Serum TSH concentration was assessed by immuno-

radiometric assay (KavoshyarCo., Tehran, Iran). Intra-assay and inter-assay coefficient of variation (CV) was 1.5 and 1.9%, respectively. The normal range for TSH was 0.3–3.6 mU/LmU/L. Serum TPOAb and TgAb were determined with Rapid ELISA (Genesis Diagnostic Products Corp.). The intra-assay CV for TPOAb was 7 and inter-assay was 5%, and for TgAb, it was less than 12%. TPOAb and TgAb concentrations of more than 75 and 100 IU/ mL were considered positive.

### Statistical analysis

Normality of continuous data was evaluated using Kolmogorov-Smirnov test and Q-Q plot and reported as mean  $\pm$  SD (median (interquartile range)). Categorical data were reported as frequency (percentage). Logarithmic transformation was applied for positively skewed TSH distribution. Upper limits of 97.5 and 95 and lower limits of 5 and 2.5 percentiles were calculated for providing the reference intervals for TSH and fT4 in the total sample as well as in different age, gender and thyroid antibodies groups. TSH and fT4 levels were compared between gender and thyroid antibodies groups using independent samples t-test or Mann-Whitney U test while between age group analysis variance (ANOVA) or Kruskal-Wallis tests were used. The association between TSH and fT4 levels in 2006 with counterpart values in 2011 was evaluated using Pearson and Spearman correlation coefficients. The smooth distribution curves were illustrated with locating reference limits of TSH and fT4. All statistical analyses were conducted using SPSS software version 15. (SPSS Inc. Chicago USA).

### Results

There were 2523 participants in 2006 and 711 in 2011. Mean  $\pm$  SD age was 39.03  $\pm$  12.42 years (male 40.67  $\pm$  12.26 and female 37.36  $\pm$  12.36) in 2006 and 47.29  $\pm$  11.89 years (male 48.67  $\pm$  12.02 and female 46.02  $\pm$  11.64) in 2011, respectively.

Of the participants, 1275 (50.5%) were males in 2006 and 340 (47.8%) in 2011. In 2006, a total of 134 (29.2%) subjects were TPOAb positive. In 2011, 171 (29.3%) individuals had positive TPOAb.

After applying all exclusion criteria, data of 1899 participants were included for analysis in 2006 and 377 in 2011. The mean  $\pm$  SD age was 39.66  $\pm$  12.71 years in 2006 and 48.96  $\pm$  12.35 years in 2011.

Table 1 shows the means  $\pm$  SD and reference intervals for TSH levels in the total sample and age, gender and thyroid autoantibodies groups. Of the participants, 1111 (58.5%) were male in 2006 and 235 (62.3%) in 2011.

In 2006, the 2.5th percentile of serum TSH levels was 0.4 mU/L (males: 0.4 mU/L, females: 0.5 mU/L) and 97.5th percentiles of serum TSH was 4.96 mU/L (males: 4.72 mU/L, females: 5.3 mU/L). In 2011, the 2.5th percentile of serum TSH levels was 0.7 mU/L (males: 0.6 mU/L, females: 0.77 mU/L) and 97.5th percentiles of serum TSH was 4.9 mU/L (males: 5.7 mU/L, females: 5.57 mU/L). More details are available in Table 1, Figure 1A, 1B, 1C).

The results indicate that TSH ranges are significantly gender specific. Women had higher TSH values than men (mean  $\pm$  SD; 2.19  $\pm$  1.23 *vs* 1.87  $\pm$  1.15) in 2006 ( $p < 0.001$ ) and a similar result was observed in 2011 ( $p = 0.002$ ) (Table 1 and Figure 1D).

The oldest group ( $\geq 50$ ) had the highest mean (2.04  $\pm$  1.4 mU/L), (2.21  $\pm$  1.19) with 97.5th percentile (6.67 mU/L) (5.57 mU/L) of TSH concentration in comparison to other age groups in 2006 and 2011, respectively. Comparison of TSH levels between the years 2006 and 2011 in each age group is shown in Figure 1E. In addition, Figure 1F illustrates the serum TSH levels at 2006 and 2011 in different age-sex groups.

We also described the TSH distribution in terms of TPOAb status; mean  $\pm$  SD of TSH was significantly higher in TPOAb positive participants (4.9  $\pm$  2.42) than the TPOAb negative group (3.36  $\pm$  2.18) ( $p < 0.001$ ). In 2006 the 97.5th percentile of TSH was 9.4 in the TPOAb-positive individuals, significantly higher than the TPOAb negative individuals where it was 8.28. In 2011 the 97.5th percentile for the respective groups was 14.62 and 6.3. In participants who were TPOAb-positive, there was an elevated upper limit of the TSH range both in 2006 and in 2011. Table 2 shows the means  $\pm$  SD and reference intervals for T4 levels in the total sample and age, sex and thyroid autoantibodies categories.

**Table 1.** The distribution of TSH concentrations (mU/l) in the total sample and based in age, sex and TPO, Tg antibodies groups.

	2006							2011					
	Mean± SD [Min-Max]	Percentile				P		Mean± SD [Min-Max]	Percentile				P
		5 <sup>th</sup>	95 <sup>th</sup>	2.5 <sup>th</sup>	97.5 <sup>th</sup>				5 <sup>th</sup>	95 <sup>th</sup>	2.5 <sup>th</sup>	97.5 <sup>th</sup>	
<b>Total sample</b> (n=1899)	2.0±1.19 [0.2-9.9]	0.6	3.9	0.4	4.95		<b>Total sample</b> (n=377)	2.11±1.11 [0.4-7.8]	0.7	4.3	0.7	4.9	
<b>Sex</b> <b>Female</b> (n=788)	2.19±1.23 [0.3-9.9]	0.6	4.4	0.5	5.3	p<0.001*	<b>Female</b> (n=142)	2.41±1.16 [0.4-6.6]	1.0	4.7	0.77	5.55	0.002*
<b>Male</b> (n=1111)	1.87±1.15 [0.2-9.7]	0.5	3.7	0.4	4.72		<b>Male</b> (n=235)	2.07±1.26 [0.14-9.8]	0.7	4.64	0.6	5.7	
<b>Age</b> <b>≤30</b> (n=549)	1.97±1.12 [0.3-7.5]	0.6	3.9	0.47	4.8		<b>≤30</b> (n=34)	2.13±0.94 [0.5-4.9]	0.7	3.59	0.5	4.86	
<b>31-40</b> (n=457)	1.98±1.13 [0.2-9.7]	0.5	3.9	0.4	4.5	0.83**	<b>31-40</b> (n=65)	2.0±1.02 [0.4-4.7]	0.64	4.61	0.4	6.25	0.35**
<b>41-50</b> (n=511)	2.0±1.15 [0.3-9.9]	0.7	4.0	0.5	5.12		<b>41-50</b> (n=94)	1.99±1.05 [0.5-6.6]	0.81	4.66	0.7	6.09	
<b>≥50</b> (n=382)	2.04±1.4 [0.3-9.5]	0.6	4.78	0.4	6.67		<b>≥50</b> (n=182)	2.21±1.19 [0.5-7.8]	0.73	4.87	0.66	5.57	
<b>TPO</b> <b>Positive</b> (n=134)	4.9±2.42 [0.2-9.4]	0.47	9.07	0.2	9.4	0.000*	<b>Positive</b> (n=98)	2.49±1.41 [0.14-6.8]	0.04	7.08	0.02	14.62	0.006**
<b>Negative</b> (n=325)	3.36±2.18 [0.2-9.7]	0.3	7.22	0.22	8.28		<b>Negative</b> (n=336)	2.12±1.13 [0.4-7.8]	0.7	4.7	0.53	6.3	
<b>Tg</b> <b>Positive</b> (n=135)	4.34±2.43 [0.2-8.8]	0.24	8.64	0.2	8.80	0.09*	<b>Positive</b> (n=5)	4.76±3.53 [1.1-9.8]	1.1	24.0	1.1	24.0	0.002*
<b>Negative</b> (n=324)	3.53±2.27 [0.2-9.7]	0.35	7.63	0.22	8.64		<b>Negative</b> (n=32)	2.08±1.15 [0.5-4.9]	0.05	4.3	0.02	4.9	

\*result from independent t-test or Mann-Whitney U test, \*\* result from ANOVA or Kruskal-Wallis

In 2006, the mean± SD of T4 for all negative TPOAb subjects was (6.68±1.42) which was significantly higher (p=0.01) than in positive TPOAb subjects (5.92±1.32).

In 2006, the 2.5th percentile of T4 was 3.17 and the 97.5th percentiles of T4 was 10.74 ng/dl in the total population. Those figures were 2.7 ng/dl and 10.7 for men and 5.0 and 11.6 ng/dl for women, respectively. However, in 2011, mean± SD of T4 was significantly higher in females than males (p=0.001). Both in 2006 and 2011, the oldest group (≥50) had the lowest mean (6.2±1.34) (8.16±1.58) with 97.5th percentile (7.6 mU/L) (11.43 mU/L) of T4 concentration in comparison to other age groups. A total T4 distribution curve between 2006 and 2011 is shown in Figure 2.

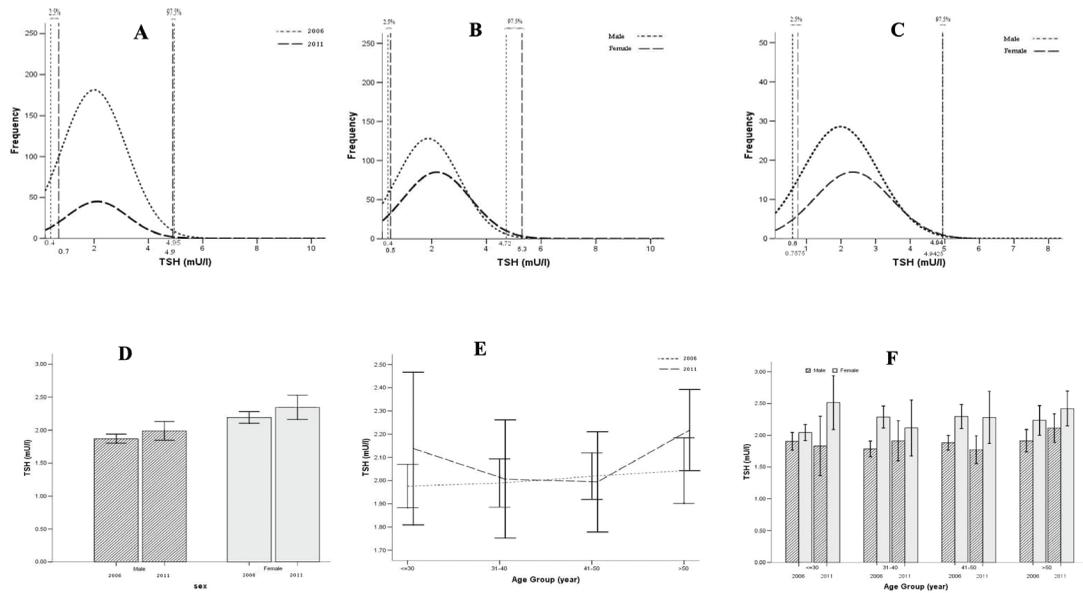
Table 3 shows different percentiles of TSH and T4 concentration in combined age- groups. In the female group in 2006, with increasing age, 97.5 percentiles of TSH have increased and a decline in T4 level

is observed.

A positive correlation between TSH levels in 2006 and 2011 in the total sample was detected as well in different age groups and both genders but significant correlations between T4 levels in 2006 and 2011 were observed in males, age≤ 30 and in the total sample (Table 4).

### Discussion

This population-based cohort study was the first to evaluate the range of serum TSH levels in the population of Isfahan, Iran .We previously reported the thyroid volume reference range (24) and recently evaluated the levels of serum TSH in the disease-free population and compared our data with the results of the NHANES in the US where the 2.5th and 97.5th percentiles of serum TSH were 0.45 and 4.12 mU/L, respectively(8).

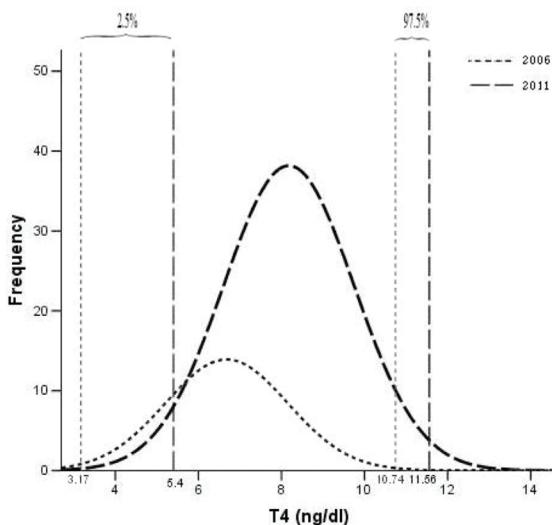


**Figure 1.** (A) TSH distribution curves in 2006 and 2011, (B) TSH distribution curves for males and females in 2006, (C) TSH distribution curves for males and females in 2011, (D) Serum TSH levels for males and females in 2006 and 2011, (E) Serum TSH levels in different age groups in 2006 and 2011, (F) Serum TSH levels in different age and sex groups in 2006 and 2011.

**Table 2.** The distribution of total T4 concentration ( $\mu\text{g}/\text{dl}$ ) in total sample and based on age, sex and TPO, Tg antibodies groups.

	2006							2011					
	Percentile							Percentile					
	Mean $\pm$ SD [Min-Max]	5 <sup>th</sup>	95 <sup>th</sup>	2.5 <sup>th</sup>	97.5 <sup>th</sup>	P		Mean $\pm$ SD [Min-Max]	5 <sup>th</sup>	95 <sup>th</sup>	2.5 <sup>th</sup>	97.5 <sup>th</sup>	P
<b>Total sample</b> (n=77)	6.67 $\pm$ 1.47 [2.7-11.6]	4.68	9.7	3.17	10.74		<b>Total sample</b> (n=377)	8.3 $\pm$ 2.95 [3.0-56.6]	5.88	11.0	5.4	11.55	
<b>Sex</b>													
<b>Female</b> (n=39)	6.67 $\pm$ 1.34 [5.0-11.6]	5.1	9.8	5	11.6	0.90*	<b>Female</b> (n=142)	8.37 $\pm$ 1.64 [4.0-13.2]	5.9	11.5	5.47	11.8	0.001*
<b>Male</b> (n=38)	6.68 $\pm$ 1.62 [2.7-10.7]	3.17	9.75	2.7	10.7		<b>Male</b> (n=235)	8.15 $\pm$ 3.2 [3.0-56.6]	5.8	10.5	5.12	11.2	
<b>Age</b>													
<b><math>\leq 30</math></b> (n=28)	6.56 $\pm$ 1.63 [3.2-11.6]	3.78	10.2	3.2	11.6		<b><math>\leq 30</math></b> (n=34)	8.36 $\pm$ 1.63 [5.0-12.1]	5.9	11.6	5.02	12.09	
<b>31-40</b> (n=15)	7.4 $\pm$ 1.59 [5.1-10.7]	5.1	10.7	5.1	10.7	0.44**	<b>31-40</b> (n=65)	8.16 $\pm$ 1.73 [3.3-12.4]	5.73	11.53	5.16	12.33	0.63**
<b>41-50</b> (n=21)	6.85 $\pm$ 1.2 [5.1-9.7]	5.12	9.58	5.1	9.7		<b>41-50</b> (n=94)	8.6 $\pm$ 5.19 [4.6-56.6]	6.1	10.97	5.41	11.89	
<b><math>\geq 50</math></b> (n=10)	6.2 $\pm$ 1.34 [2.7-7.6]	2.7	7.6	2.7	7.6		<b><math>\geq 50</math></b> (n=182)	8.16 $\pm$ 1.58 [3.0-11.9]	5.8	10.8	5.16	11.43	
<b>TPO</b>													
<b>Positive</b> (n=96)	5.92 $\pm$ 1.32 [3.7-9.1]	3.76	8.95	3.7	9.1	0.01*	<b>Positive</b> (n=98)	7.99 $\pm$ 1.48 [4.7-13.2]	5.8	12.4	4.96	13.48	0.41*
<b>Negative</b> (n=192)	6.68 $\pm$ 1.42 [2.7-11.6]	4.76	9.34	3.39	10.56		<b>Negative</b> (n=336)	8.25 $\pm$ 3.08 [3.0-56.6]	5.5	11.13	5.1	11.76	
<b>Tg</b>													
<b>Positive</b> (n=98)	6.28 $\pm$ 1.29 [3.7-9.1]	3.74	8.94	3.7	9.1	0.38*	<b>Positive</b> (n=5)	7.2 $\pm$ 0.99 [6.0-8.7]	4.9	8.7	4.9	8.7	0.08*
<b>Negative</b> (n=190)	6.57 $\pm$ 1.46 [2.7-11.6]	4.58	9.26	3.46	10.52		<b>Negative</b> (n=32)	8.43 $\pm$ 1.45 [6.0-11.5]	6.2	11.5	6.0	11.9	

\*result from independent t-test or Mann-Whitney U test, \*\* result from ANOVA or Kruskal-Wallis



**Figure 2.** Total T4 distribution curves in 2006 and 2011

However, in our study, those corresponding values were 0.4 and 4.96 mU/L for 2006 and 0.7 and 4.9 mU/L for 2011. This finding is also compatible with those of a recent single-institutional study in Tehran, Iran, that indicated the 2.5th and 97.5th percentiles of serum TSH were 0.32 mU/L and 5.06 mU/L (21).

In studies in China and Korea, the reference range of serum TSH with 2.5%-97.5% confidence intervals

were 0.48-5.50 mU/L (25) and 0.62 to 6.68 mU/L (26), respectively. However, in a study in Koreans, the TSH reference level was reported to be 0.68 to 3.70 mU/L (4). These findings proposed that other factors than race might have affected levels of serum TSH in the same population.

In our population in 2006, serum TSH appeared to be non-significantly higher in people aged above 50 without apparent risk factors for thyroid disease than in younger people. This does not agree with some extensively reviewed published reports (27). The relationship between TSH and age are not consistent (8, 28-30). Data from Germany presented that TSH reference levels decreased with age in iodine deficient areas (31, 32). On the other hand, it has been suggested that increasing median and 97.5 centile for TSH occur with aging (8, 33, 34). Diverse results may be described by the difference in iodine supply in different regions. Another explanation for a shift in reference range to higher TSH concentrations in older people is the consequence of healthy aging (35) or the presence of TSH isoforms with low bioactivity or use of different medications (36).

Surks et.al suggested that an age-specific 97.5 centile is better used instead of the fixed 4.5 mU/Liter (33). According to the accepted high prevalence

**Table 3.** The percentiles of TSH (mU/l) and total T4 (µg/dL) concentrations in combined sex-age groups.

Age Group	2006								2011									
	Male				Female				Male				Female					
	5	95	2.5	97.5	5	95	2.5	97.5	5	95	2.5	97.5	5	95	2.5	97.5		
≤30	TSH	0.5	4.1	0.4	5.02	0.6	3.9	0.5	4.8	TSH	0.52	4.67	0.5	4.9	1.1	3.6	1.1	3.6
	T4	3.2	8.5	3.2	8.5	5.0	11.6	5.0	11.6	T4	5.13	10.13	5.0	11.2	5.9	12.1	5.9	12.1
31-40	TSH	0.5	3.3	0.4	3.91	0.5	4.4	0.4	5.33	TSH	0.66	5.38	0.27	6.77	0.4	4.7	0.4	4.7
	T4	5.3	10.7	5.3	10.7	5.1	9.8	5.1	9.8	T4	5.7	11.56	3.93	12.31	5.55	11.94	5.1	13.2
41-50	TSH	0.6	3.8	0.4	4.68	0.9	4.85	0.62	5.43	TSH	0.75	3.51	0.57	5.53	0.92	6.04	0.73	6.45
	T4	5.3	9.7	5.3	9.7	5.1	8.4	5.1	8.4	T4	6.05	10.58	5.22	21.95	5.94	11.28	5.0	12.54
≥50	TSH	0.54	3.96	0.4	6.65	0.54	4.86	0.4	6.9	TSH	0.7	5.12	0.6	5.73	1.1	4.9	0.9	5.0
	T4	2.7	7.6	2.7	7.6	5.1	7.4	5.1	7.4	T4	5.6	10.36	5.0	10.86	5.8	11.5	5.4	11.8

**Table 4.** Correlation between TSH and total T4 levels in 2006 with their counterpart values in 2011.

Total	TSH							T4						
	Female	Male	≤30	31-40	41-50	≥50	Total	Female	Male	≤30	31-40	41-50	≥50	
0.60*	0.52*	0.63*	0.65*	0.52*	0.58*	0.65*	0.66*	0.39	0.86*	0.85*	0.47	-1.0*	0.42	

\*Significant at p<0.01

of subclinical hypothyroidism in older people  $\geq 50$ , it could be suggested that the upper limit of TSH that was used in NH-III: 4.5 mU/Liter (97.5 centile) (8) may not be applicable for older people. Our study has also produced results that show a much higher level of 6.67 and 5.57 mU/Liter both in 2006 and 2011 (8).

Jensen et al. showed no difference in serum TSH concentrations with respect to age, gender, or medication for individuals without thyroid antibodies and other risk factors (37). In our study, we did not observe any difference in serum TSH concentrations with respect to age, unlike to gender.

Our results indicated that gender significantly affects the TSH range. This finding is consistent with many previous reports (8, 25). These results may indicate that TSH levels are regulated by estrogen, genetic susceptibility and environmental factors (11, 38). Another study, the Wickham survey, showed that TSH levels increased markedly in women aged  $>45$  yrs. but this was not the case in men (39).

The median serum TSH level in the reference population was also higher in our study (2.0 mU/L in 2006 and 2.11 mU/L in 2011) than that in the US NHANES III data (1.39 mU/L) (8). This difference in serum TSH levels can be explained by ethnic differences (19, 40). Indeed, whether the normal limit of TSH in Middle East is similar to that in the Caucasian population has not been answered.

As regards to T4 concentrations, in our study, T4 level in the oldest group ( $\geq 50$ ) had the lowest mean and 97.5th percentile of T4 concentration in comparison to other age groups. In the Tehran study (21), no specific pattern in FT4 concentration of their population was reported but data from both the NHANES III study and our study show that the mean total T4 significantly declines with age (8).

For determining the TSH and fT4 reference ranges, we excluded individuals with TPOAb and Tg positivity but when we entered this population in our analysis, significant differences in medians and 97.5th centiles of TSH between antibody positive and negative cases were observed. This finding disagrees with studies reporting that it is not necessary to exclude thyroid antibody positive cases for determination of TSH and T4 reference limits (2, 20, 41). It is however in agreement with the guidelines published by Baloch

et al. based on designation of TSH reference limits in the disease free population (9).

In our study, TPOAb and TgAb had a role in raising the upper limit of the TSH range but without consistent effect in the lower limit of the TSH range. However, Jensen's *et al.* observed that, TPOAb was involved in increasing the upper limit of TSH, whereas TgAb decreased the lower limit of the TSH range slightly (37). Many studies have shown that autoimmune thyroid disease is one of the main pathological factors affecting TSH level (25, 42), but the role of TgAb is unclear (25), somehow, we didn't observe the consistent effect of TgAb on TSH level either in 2006 and 2011.

In addition, in 2006 we observed that the anti-TPO positive population had lower levels of T4 than negative subjects did. Previously, euthyroid subjects with positive thyroid antibodies had a lower value of T3/T4 ratio and T3 compared to subjects with negative antibodies (43).

Recent studies suggest that distinct age groups and subpopulations have unique TSH distributions and reference limits that are significantly different from limits established by the traditional approach. One explanation may be different immunoassays used in the different studies. One of our limitations is that due to financial restraints we had to measure T4 with radioimmunoassay instead of more efficient methods such as chemiluminescence. Various conditions affect the relationship between total and free thyroid hormones. Changes in the thyroid binding proteins (TBG) affect the total TH concentrations but not free TH, this happens for example when sex steroids are used. However, transient changes in free T4 and free T3 can happen (44). Another factor that can affect the evaluation is the presence of agents such as heparin in the serum that can detach T4 and T3 from their binding sites. Accordingly, total T4 is therefore rarely used and is usually utilized in conjunction with a TBG measurement or an estimate of binding proteins (45).

T3RU is used to correct the total T4; it is used as an indirect measure of serum thyroid hormone binding capacity (46).

Another limitation of our study is that at the time, we had no access to the reliable free T4 kit. Therefore, we measured total T4 and T3RU. We measured T3RU

for some patients then calculated free T4. The calculated free T4 amount was consistent with the total T4 measurement. Based on this finding, we used total T4 as a replacement free T4. The final limitation of the study is that our study is based on the Iranian population, hence may not be generalized to other populations.

## Conclusion

Our findings discuss the use of age and sex specific TSH and T4 reference ranges to diagnose, treat and monitor patients optimally.

The results of this study provide our Iranian clinicians with population-specific reference limits, which would eventually result in improved patient care. Further studies with larger number of reference subjects are necessary for broader conclusions.

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## References

- Melmed S, Polonsky K, Larsen P, Kronenberg H. Williams textbook of endocrinology. 12th. Philadelphia, PA: Saunders Elsevier. 2011.
- Demers LM, Spencer CA. Laboratory medicine practice guidelines: laboratory support for the diagnosis and monitoring of thyroid disease. *Clin Endocrinol.* 2003;58(2):138-40.
- Klee GG, Hay ID. Biochemical testing of thyroid function. *Endocrinol Metab Clin North Am.* 1997;26(4):763-75.
- Chan AO, Iu YP, Shek CC. The reference interval of thyroid-stimulating hormone in Hong Kong Chinese. *J Clin Pathol.* 2011 May;64(5):433-6. doi(2011 Mar 21):10.1136/jcp.2010.087627.
- Yoshihara A, Noh JY, Ohye H, Sato S, Sekiya K, Kosuga Y, et al. Reference limits for serum thyrotropin in a Japanese population. *Endocr J.* (2011 May 7):2011;58(7):585-8.
- Vanderpump M, Tunbridge W, French J, Appleton D, Bates D, Clark F, et al. The incidence of thyroid disorders in the community: a twenty year follow up of the Wickham Survey. *Clin Endocrinol (Oxf).* 1995;43(1):55-68.
- Guan H, Shan Z, Teng X, Li Y, Teng D, Jin Y, et al. 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).* 2008;69(1):136-41.
- Hollowell JG, Staehling NW, Flanders WD, Hannon WH, Gunter EW, Spencer CA, et al. Serum TSH, T4, and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). *J Clin Endocrinol Metab.* 2002;87(2):489-99.
- Baloch Z, Carayon P, Conte-Devolx B, Committee G. National Academy of Clinical Biochemistry. Laboratory medicine practice guidelines Laboratory support for the diagnosis and monitoring of thyroid disease. *Thyroid.* 2003;13(1):3-126.
- Schalin-Jantti C, Tanner P, Valimaki MJ, Hamalainen E. Serum TSH reference interval in healthy Finnish adults using the Abbott Architect 2000i Analyzer. *Scand J Clin Lab Invest.* 2011 Jul;71(4):344-9. doi(2011 Mar 23):10.3109/00365513.2011.568630.
- Wartofsky L, Dickey RA. The evidence for a narrower thyrotropin reference range is compelling. *J Clin Endocrinol Metab.* 2005;90(9):5483-8.
- Brabant G, Beck-Peccoz P, Jarzab B, Laurberg P, Orgiazzi J, Szabolcs I, et al. Is there a need to redefine the upper normal limit of TSH? *Eur J Endocrinol.* 2006;154(5):633-7.
- Aghini-Lombardi F, Antonangeli L, Martino E, Vitti P, Maccherini D, Leoli F, et al. The spectrum of thyroid

- disorders in an iodine-deficient community: the Pescopagano survey. *J Clin Endocrinol Metab.* 1999;84(2):561-6.
14. Laurberg P, Pedersen KM, Hreidarsson A, Sigfusson N, Iversen E, Knudsen PR. Iodine intake and the pattern of thyroid disorders: a comparative epidemiological study of thyroid abnormalities in the elderly in Iceland and in Jutland, Denmark. *J Clin Endocrinol Metab.* 1998;83(3):765-9.
  15. Surks MI, Goswami G, Daniels GH. The thyrotropin reference range should remain unchanged. *J Clin Endocrinol Metab.* 2005;90(9):5489-96.
  16. Surks MI, Hollowell JG. Age-specific distribution of serum thyrotropin and antithyroid antibodies in the US population: implications for the prevalence of subclinical hypothyroidism. *J Clin Endocrinol Metab.* 2007;92(12):4575-82.
  17. Atzmon G, Barzilai N, Hollowell JG, Surks MI, Gabriely I. Extreme longevity is associated with increased serum thyrotropin. *J Clin Endocrinol Metab.* 2009;94(4):1251-4.
  18. Bremner AP, Feddema P, Leedman PJ, Brown SJ, Beilby JP, Lim EM, et al. Age-related changes in thyroid function: a longitudinal study of a community-based cohort. *J Clin Endocrinol.* 2012;97(5):1554-62.
  19. Boucai L, Surks MI. Reference limits of serum TSH and free T4 are significantly influenced by race and age in an urban outpatient medical practice. *Clin Endocrinol (Oxf).* 2009;70(5):788-93.
  20. Boucai L, Hollowell JG, Surks MI. An approach for development of age-, gender-, and ethnicity-specific thyrotropin reference limits. *Thyroid.* 2011;21(1):5-11.
  21. Amouzegar A, Delshad H, Mehran L, Tohidi M, Khafaji F, Azizi F. Reference limit of thyrotropin (TSH) and free thyroxine (FT4) in thyroperoxidase positive and negative subjects: a population based study. *J Endocrinol Invest.* 2013 Dec;36(11):950-4. doi(2013 Jul 15):10.3275/9033.
  22. Aminorroaya A, Meamar R, Amini M, Feizi A, Tabatabae A, Imani EF. Incidence of thyroid dysfunction in an Iranian adult population: the predictor role of thyroid autoantibodies: results from a prospective population-based cohort study. *Eur J Med Res.* 2017;22(1):21.
  23. Aminorroaya A, Meamar R, Amini M, Feizi A, Nasri M, Tabatabaei A, et al. The TSH levels and risk of hypothyroidism: Results from a population based prospective cohort study in an Iranian adult's population. *Eur J Intern Med.* 2017;41:55-61.
  24. Adibi A, Sirous M, Aminorroaya A, Roohi E, Mostafavi M, Fallah Z, et al. Normal values of thyroid gland in Isfahan, an iodine replete area. *JRMS.* 2008;13(2):55-60.
  25. Li C, Guan H, Teng X, Lai Y, Chen Y, Yu J, et al. An epidemiological study of the serum thyrotropin reference range and factors that influence serum thyrotropin levels in iodine sufficient areas of China. *Endocr J.* (2011 Sep 30):2011;58(11):995-1002.
  26. Kim WG, Kim WB, Woo G, Kim H, Cho Y, Kim TY, et al. Thyroid Stimulating Hormone Reference Range and Prevalence of Thyroid Dysfunction in the Korean Population: Korea National Health and Nutrition Examination Survey 2013 to 2015. *Endocrinol Metab (Seoul).* 2017;32(1):106-14.
  27. Mariotti S, Franceschi C, Cossarizza A, Pinchera A. The aging thyroid. *Endocr Rev.* 1995;16(6):686-715.
  28. Valeix P, Dos Santos C, Castetbon K, Bertrais S, Cousty C, Hercberg S, editors. Thyroid hormone levels and thyroid dysfunction of French adults participating in the SU. VI. MAX study. *Ann Endocrinol (Paris);* 2004.
  29. Hubl W, Schmieder J, Gladrow E, Demant T. Reference intervals for thyroid hormones on the architect analyser. *Clin Chem Lab Med.* 2002;40(2):165-6.
  30. Wartofsky L, Dickey RA. The evidence for a narrower thyrotropin reference range is compelling. *J Clin Endocrinol Metab.* 2005;90(9):5483-8.
  31. Volzke H, Alte D, Kohlmann T, Ludemann J, Nauck M, John U, et al. Reference intervals of serum thyroid function tests in a previously iodine-deficient area. *Thyroid.* 2005;15(3):279-85.
  32. Völzke H, Schmidt C, John U, Wallaschofski H, Dörr M, Nauck M. Reference levels for serum thyroid function tests of diagnostic and prognostic significance. *Horm Metab Res.* 2010;42(11):809-14.
  33. Surks MI, Hollowell JG. Age-specific distribution of serum thyrotropin and antithyroid antibodies in the US population: implications for the prevalence of subclinical hypothyroidism. *J Clin Endocrinol Metab.* (2007 Oct 2):2007 Dec;92(12):4575-82.
  34. Fontes R, Coeli CR, Aguiar F, Vaisman M. Reference interval of thyroid stimulating hormone and free thyroxine in a reference population over 60 years old and in very old subjects (over 80 years): comparison to young subjects. *Thyroid Res.* 2013;6(1):13.
  35. Oliveira JH, Persani L, Beck-Peccoz P, Abucham J. Investigating the paradox of hypothyroidism and increased serum thyrotropin (TSH) levels in Sheehan's syndrome: characterization of TSH carbohydrate content and bioactivity. *J Clin Endocrinol Metab.* 2001;86(4):1694-9.
  36. Estrada JM, Soldin D, Buckley TM, Burman KD, Soldin OP. Thyrotropin isoforms: implications for thyrotropin analysis and clinical practice. *Thyroid.* 2014 Mar;24(3):411-23. doi(2013 Dec 13):10.1089/thy.2013.0119.
  37. Jensen E, Petersen PH, Blaabjerg O, Hansen PS, Brix TH, Kyvik KO, et al. 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.* 2004;42(7):824-32.
  38. Kratzsch J, Fiedler GM, Leichtle A, Brugel M, Buchbinder S, Otto L, et al. New reference intervals for thyrotropin and thyroid hormones based on National Academy of Clinical Biochemistry criteria and regular ultrasonography of the thyroid. *Clin Chem.* 2005;51(8):1480-6.
  39. Tunbridge W, Evered D, Hall R, Appleton D, Brewis M, Clark F, et al. The spectrum of thyroid disease in a community: the Whickham survey. *Clin Endocrinol (Oxf).* 1977;7(6):481-93.
  40. Spencer CA, Hollowell JG, Kazarosyan M, Braverman LE.

- 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. *J Clin Endocrinol Metab.* 2007;92(11):4236-40.
41. Boucai L, Surks MI. Reference limits of serum TSH and free T4 are significantly influenced by race and age in an urban outpatient medical practice. *Clin Endocrinol (Oxf)*. 2009 May;70(5):788-93. doi(2008 Aug 25):10.1111/j.365-2265.008.03390.x.
42. Hamilton TE, Davis S, Onstad L, Kopecky KJ. 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.* 2008;93(4):1224-30.
43. Mirjanic-Azaric B, and IS, N. S. The Impact of Serum Triiodothyronine to Thyroxine (T3/T4) Ratio in Euthyroid Subjects. *Annals Thyroid Res.* 2016;2(2):66-8.
44. Koulouri O, Moran C, Halsall D, Chatterjee K, Gurnell M. Pitfalls in the measurement and interpretation of thyroid function tests. *Best Pract Res Clin Endocrinol Metab.* 2013;27(6):745-62.
45. Spencer CA. Assay of thyroid hormones and related substances. Endotext [Internet]: MDText.com, Inc.; 2017.
46. Dunlap DB. Thyroid function tests. *Clinical Methods: The History, Physical, and Laboratory Examinations* 3rd edition: Butterworths; 1990.

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