



Reference Range of Thyroid Function Tests (Total Triiodothyronine, Total Thyroxine, and Thyroid-Stimulating Hormone) Among Iranian Adults

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Abstract

Background and aims: Thyroid hormones are the primary tests for diagnosing thyroid disorders in individuals. This study aimed to evaluate the reference ranges of serum thyroid-stimulating hormone (TSH), total thyroxine (T4), and total triiodothyronine (T3) among adults in Yazd province.

Methods: This cross-sectional study was conducted on individuals participating in the Yazd Health Study. Overall, 1,943 participants were included according to the National Academy of Clinical Biochemistry criteria. The direct method was employed to establish accurate and relevant reference ranges. Moreover, the 2.5th and 97.5th percentiles were calculated, with 95% confidence intervals for the lower and upper limits. The obtained data were analyzed using MedCalc 12.1 and SPSS 25.

Results: The mean \pm standard deviation age of the participants was 42.8 ± 11.6 years. In addition, the reference intervals derived for TSH, T4, and T3 were $0.32\text{--}4.22$ mU/L, $9.54\text{--}11.93$ pmol/L, and $92.48\text{--}194.41$ nmol/L, respectively. Furthermore, the 2.5th and 97.5th percentiles for TSH were 1.32 mU/L and 4.60 mU/L, respectively. Significant differences were observed in TSH between genders ($P=0.001$), while differences in T3 ($P=0.146$) and T4 ($P=0.087$) values between genders were not statistically significant.

Conclusion: This study established age-specific and gender-specific reference ranges for T3, T4, and TSH in a large cohort of Iranian adults. Such information can help healthcare professionals categorize patients more accurately by thyroid function levels.

Keywords: Reference values, Thyroid hormones, Adults, Iran

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Received: February 15, 2025

Revised: December 13, 2025

Accepted: December 14, 2025

ePublished: December 30, 2025



Introduction

Thyroid-stimulating hormone (TSH) is the primary test for diagnosing thyroid disorders in individuals without hypothalamic or pituitary gland complications. TSH levels in the blood indicate whether the thyroid gland is overactive (hyperthyroidism) or underactive (hypothyroidism), thereby guiding health professionals in assessing the need for further testing or treatment.¹ The reliability of the reference range for TSH significantly influences the accuracy of identifying thyroid disorders, particularly subclinical ones. In addition, subclinical hypothyroidism and hyperthyroidism exhibit elevated or decreased TSH levels, respectively, while serum free thyroxine (FT4) levels remain within the normal range.²

The debate over the optimal reference range for serum TSH levels and the clinical significance of subclinical thyroid dysfunction persists, impacting accurate

diagnosis.³ According to recommendation 2 from the National Academy of Clinical Biochemistry (NACB), establishing the normal reference range for the TSH index requires at least 120 healthy individuals with no personal or familial history of thyroid disorders.⁴ Various factors (e.g., age, gender, thyroid antibodies, smoking habits, and iodine intake) can influence TSH levels, necessitating a nuanced interpretation by healthcare providers.⁴⁻⁸

According to studies such as the National Health and Nutrition Examination Survey III in the U.S. population, the upper limits of TSH increase with age, potentially exceeding the commonly used reference range of 4.2 mU/L.⁸⁻¹⁰ This issue underscores the need for population-specific reference ranges for thyroid function tests, considering demographic variables and environmental factors that affect thyroid function (e.g., dietary iodine intake). Notably, racial disparities influence serum TSH

levels, with median levels being lower in Black individuals compared to Caucasians.^{8,11,12}

Establishing reference ranges for thyroid hormones, namely, total thyroxine (T4), total triiodothyronine (T3), and TSH, in an Iranian adult population helps define normal parameters for this group. This information enables healthcare professionals to identify potential thyroid dysfunction or abnormalities accurately.

Various factors, including age, gender, nutritional status, and body mass index, can influence the reference ranges of thyroid hormones. Studies have shown that in overweight and obese individuals, TSH levels may be slightly higher than in normal-weight individuals, while FT4 levels tend to be lower. These variations can affect the interpretation of thyroid function tests, underscoring the importance of accounting for demographic characteristics when establishing reference ranges.¹³ A recent study by Meamar et al expanded this understanding by establishing age-specific and gender-specific TSH and T4 reference ranges in Isfahan, Iran, using rigorous NACB criteria. Their cohort analysis of 1,899 participants highlighted gender-specific differences in TSH levels and age-related declines in T4 concentrations. Notably, their findings emphasized the role of thyroid autoantibodies (TPOAb and TgAb) in elevating TSH upper limits, a factor not addressed in earlier Iranian studies.¹⁴

Tailoring treatments and medications based on population-specific reference ranges ensures more effective and safer therapies, contributing to more precise healthcare. To establish appropriate reference limits for TSH and total T4 in iodine-sufficient areas, such as the Yazd Greater Area (YGA), we followed the selection criteria outlined in the NACB guidelines. It should be noted that the study by Meamar et al in Isfahan dates back to 2011, and to the best of our knowledge, no prior research on the normal range of thyroid function has been conducted in Yazd Province. Accordingly, this study aims to evaluate the reference range of serum TSH, T4, and T3 among adults aged 20–70 years in the YGA.¹⁴

Materials and Methods

Design and Participants

This cross-sectional study was performed on individuals participating in the Yazd Health Study (YaHS). The YaHS is a population-based cohort study that aims to investigate the prevalence of non-communicable diseases and their risk factors in the YGA, Iran. Adhering to WHO STEPS guidelines, the study employed a two-level clustered random sampling technique to select 9,578 residents aged 20–70 years based on YGA postcodes. The inclusion criteria for this study were adult residents of Yazd who participated in the first and second phases of the YaHS. On the other hand, the exclusion criteria included a previous history of thyroid diseases, the use of medications that affect thyroid function, and pregnancy or breastfeeding.

In the second phase of the study, of 3,500 people, 856 did not attend the examination, and 701 were excluded

for the lack of meeting the inclusion criteria, resulting in 1,943 participants.

A total of 1,943 participants were included in this study. In total, 200 clusters were randomly selected, with interviews conducted with 50 individuals per cluster, ensuring a balanced representation of 25 males and 25 females, and five individuals in each 10-year age group. YaHS, the inaugural prospective cohort study focusing on the health and diseases of the Yazd population, gathered enrollment data from 2013 to 2014, involving 10,000 residents, representing approximately 2% of the city's population. Participants have been followed through annual telephone interviews since 2015, and a repeated-measures phase has been conducted with 3,500 participants (about one-third of the initial cohort) from 2021 to 2024. The study also provided a comprehensive overview of the prevalence and risk factors of non-communicable diseases in YGA, Iran. Details of the study methodology have been published elsewhere.¹⁵

Research Instrument

Linked data from the YaHS and Yazd Central Lab records were used to assess the incidence and prevalence of hypothyroidism among adults in YGA. Data linkage between YaHS participants and Yazd Central Lab records was performed to enhance the understanding of thyroid disorders. The Yazd Central Lab, serving as the primary laboratory service provider for YGA, measured serum thyroid hormone levels using blood samples collected between 7:00 and 9:00 a.m. after a 12–14-hour fast. Participants included in the study underwent comprehensive medical history assessments and physical examinations, excluding those taking thyroid-related medications or with a history of thyroid diseases.

Statistical Analysis

The obtained data were analyzed using MedCalc (version 12.1.4.0, MedCalc Software, Belgium) and SPSS (version 25.0). The Shapiro-Wilk test was used to assess normality, and outliers were identified and removed via the Tukey method.^{7,8} Considering that TSH, T4, and T3 values were non-normally distributed, they were transformed using the Box-Cox method to achieve normality. For normally distributed parameters, both the central 95% and the 2.5th and 97.5th percentile values were calculated in accordance with International Federation of Clinical Chemistry and Laboratory Medicine recommendations. Moreover, gender differences were analyzed using the Student's t-test, and age groups were compared using analysis of variance. The level of significance in this study was set at 0.05.

Results

This study presents evaluated thyroid hormone levels (TSH, T4, and T3) in a sample of 1,943 participants. The mean \pm standard deviation age of the participants was 42.8 ± 11.6 years. Of these, 68.1% (1,324) were female, and 31.9% (619) were male. The study established reference

intervals for TSH, T4, and T3 using both parametric and nonparametric statistical methods. Specifically, TSH levels fell within the 2.5th and 97.5th percentiles, suggesting that normal serum TSH levels range from 1.32 mU/L to 4.6 mU/L (Table 1). Figures 1-3 present histograms of TSH, T3, and T4. Based on the results, TSH exhibited a clear skew, whereas T3 and T4 were approximately normally distributed.

Significant differences in mean TSH levels were observed between genders, with females exhibiting a mean TSH of 1.46 mU/L, significantly higher than that of males at 1.22 mU/L ($P=0.001$). This suggests that female participants may have higher baseline TSH levels than male participants. Conversely, no significant differences in T4 and T3 levels were found between females and males ($P>0.05$), indicating that these hormone levels are relatively consistent across genders (Table 2).

The study also examined variations in thyroid hormone levels across different age groups. Notably, TSH levels demonstrated no statistically significant change with age ($P=0.07$), whereas T3 and T4 levels exhibited substantial differences across age groups ($P=0.001$). This represents

that age may influence T3 and T4 levels, but does not significantly affect TSH levels (Table 3).

Discussion

This study provided essential reference limits for TSH, T3, and T4 levels in healthy adults from YGA. To the best of our knowledge, it is the first considerable cohort-based investigation in central Iran to establish serum TSH and FT4 reference values, offering data that support more accurate diagnosis and management of thyroid disorders. The TSH reference limits identified in this study (2.5th percentile: 0.36 mU/L and 97.5th percentile: 4.60 mU/L) can serve as benchmarks for clinical practice and research in Iran and nearby regions, aiding early detection of thyroid dysfunction. Our findings are consistent with those of a previous study from the Tehran Lipid and Glucose Study conducted in Tehran, Iran. The reference limits established for TSH were 0.32 mU/L and 5.06 mU/L for the 97.5th and 2.5th percentiles.¹⁶

In studies performed in China and Korea, the normal range for serum TSH, with 2.5–97.5% confidence intervals, was 0.48–5.50 mU/L in China and 0.62–6.68 mU/L in Korea.^{17,18} However, a separate study in Koreans reported a different TSH reference range of 0.68–3.70 mU/L.¹⁹

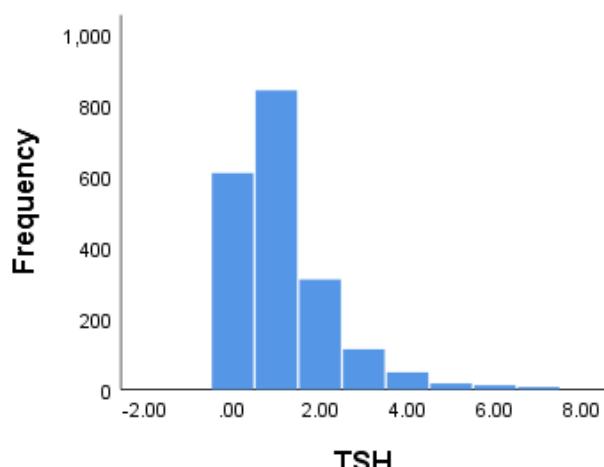


Figure 1. Frequency Distribution of TSH Values
Note. TSH: Thyroid-stimulating hormone

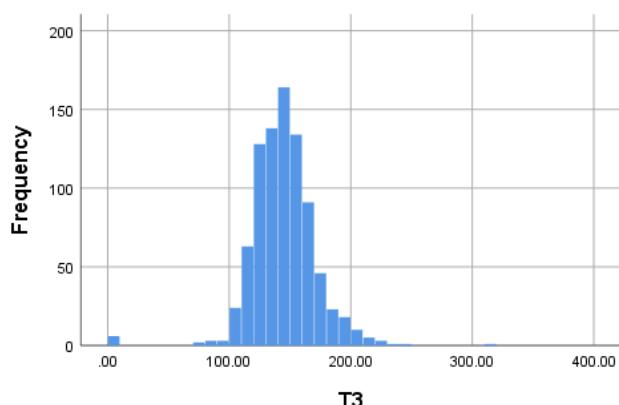


Figure 2. Frequency Distribution of T3 Values.
Note. T3: Total triiodothyronine

Table 1. Reference Values of Thyroid Gland Parameters

Parameter	N	Reference Interval					
		Parametric Method			Nonparametric Method		
		Mean	Lower Bound	Upper Bound	Median	Percentile 2.5	Percentile 97.5
TSH (mIU/L)	1943	1.38	0.32	4.27	1.32	0.36	4.60
T4 (nmol/L)	1453	9.51	6.49	11.86	9.40	6.7	12.00
T3 (nmol/L)	864	144.85	91.30	193.58	144	102	199.37

Note. TSH: Thyroid-stimulating hormone; T4: Total thyroxine; T3: Total triiodothyronine.

Table 2. Thyroid Parameters in Different Gender Groups

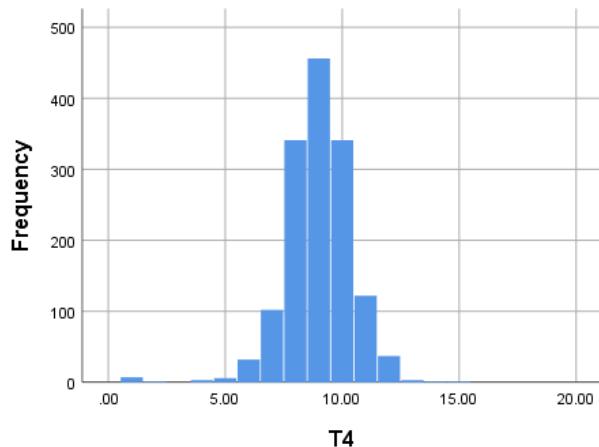
Parameter	Male			Female			P-Value
	N	Mean (95% CI)	Median (Percentile 2.5-97.5)	N	Mean (95% CI)	Median (Percentile 2.5-97.5)	
TSH (mIU/L)	619	1.22 (0.35-3.80)	1.14 (0.38-4.42)	1324	1.46 (0.31-4.42)	1.4 (0.32-4.69)	0.001
T3 (nmol/L)	72	143.09 (84.68-191.5)	142 (97.52-200)	617	145.4 (95.19-194.31)	144 (103-199)	0.146
T4 (nmol/L)	435	9.41 (16.62-11.64)	9.3 (6.70-12)	1017	9.55 (6.43-11.95)	9.5 (6.54-12.05)	0.087

Note. TSH: Thyroid-stimulating hormone; T4: Total thyroxine; T3: Total triiodothyronine; CI: Confidence interval.

Table 3. Thyroid Parameters in Different Age Groups

Parameter	Groups (Age)	N	Mean (95% CI)	Median (Percentile 2.5-97.5)	P-Value
TSH (mIU/L)	1 (20-29 years)	384	1.42 (0.35-4.24)	1.40 (0.33-4.47)	0.07
	2 (30-39 years)	390	1.48 (0.34-4.5)	1.38 (0.42-5.00)	
	3 (40-49 years)	403	1.32 (0.28-4.03)	1.25 (0.30-4.30)	
	4 (50-59 years)	389	1.38 (0.35-4.44)	1.37 (0.42-4.92)	
	5 (60-69 years)	381	1.54 (0.31-4.48)	1.24 (0.29-4.57)	
T4 (nmol/L)	1 (20-29 years)	299	9.79 (6.48-12.34)	9.7 (6.85-12.40)	0.001
	2 (30-39 years)	291	9.5 (6.42-11.73)	9.4 (6.42-12.04)	
	3 (40-49 years)	307	9.53 (6.47-11.92)	9.5 (6.7-12.33)	
	4 (50-59 years)	283	9.4 (6.62-11.57)	9.3 (6.61-11.7)	
	5 (60-69 years)	269	9.34 (6.4-11.42)	9.4 (6.3-11.45)	
T3 (nmol/L)	1 (20-29 years)	192	151.21 (88.93-205.15)	149 (99.4-211.17)	0.001
	2 (30-39 years)	179	147.2 (94.02-194.4)	145 (105.5-202)	
	3 (40-49 years)	195	143.9 (90.3-197.4)	143 (100-199)	
	4 (50-59 years)	154	139.8 (89.91-180.24)	138 (101.75-191.62)	
	5 (60-69 years)	141	140 (103.24-176.7)	143 (103-175)	

Note. TSH: Thyroid-stimulating hormone; T4: Total thyroxine; T3: Total triiodothyronine; CI: Confidence interval.

**Figure 3.** Frequency Distribution of T4 Values

Note. T4: Total thyroxine

These findings suggest that factors beyond ethnicity may influence serum TSH levels in the same population. The variations in TSH reference ranges observed in different populations, including those from China, Korea, and Iran, highlight the complexity of factors that may influence serum TSH levels. While ethnicity may contribute to observed differences in serum TSH reference ranges across populations, it is crucial to consider the interplay among environmental, genetic, lifestyle, healthcare access, and geographical factors. Nonetheless, further research is needed to better understand these influences and develop more accurate and personalized reference ranges for thyroid function tests across diverse populations.

In our population, the mean TSH level in females was higher than in males, and the difference was significant by t-test. This finding conforms to several previous reports.^{14,17,20} The results suggest that estrogen, genetic predisposition, and external influences may affect the regulation of TSH levels, implying that factors beyond thyroid function (e.g., hormonal changes, genetic

variations, and environmental factors) may contribute to the regulation of TSH levels.^{20,21} According to a study by Tunbridge et al, TSH levels significantly increased in females older than 45 years, whereas this was not found in males.⁹ However, variations in serum TSH levels can also be attributed to ethnic differences.^{10,11}

The observation of higher mean TSH levels in females than in males, along with the statistically significant difference observed by the t-test, highlights the potential influence of various factors on TSH regulation. These factors may include hormonal, genetic, and environmental influences that differ between males and females. The differences in TSH levels noted between males and females, as well as the impact of age and hormonal variations, underscore the complex interplay of factors that regulate TSH. Understanding these factors may help healthcare professionals better diagnose and manage thyroid disorders, thereby tailoring treatment strategies to the unique needs of different populations and individuals. Thus, further research is necessary to clarify the complex relationships between TSH regulation and factors influencing it.

The researchers found that TSH levels varied between different age groups; however, the differences were not statistically significant. This finding is in line with the results of other studies.^{7,8,20,21} According to three studies, including one from Iran and two from Germany, reference levels for TSH decrease with age in areas with iodine deficiency.^{16,22,23} Differences in iodine intake among regions can lead to variations in thyroid function test results. Additionally, as people age, the reference range for TSH concentrations may shift due to healthy aging, the presence of less active TSH variants, or the use of certain medications.^{24,25}

This research underlines a notable association between age and T3 and T4 hormone levels. The youngest age group

(20–29 years) exhibited the highest mean and 97.5th-percentile T4 concentrations among the age groups. However, other studies conducted in Tehran and Isfahan identified no consistent pattern in T4 concentrations,^{4,14} whereas the National Health and Nutrition Examination Survey III study reported a decline in mean total T4 with age.⁴ These findings emphasize the complexity of hormonal changes in the human body and the need for further investigation to better understand the relationship between age and hormone levels.

Previous studies demonstrated that normal thyroid hormone levels vary with physiological conditions, such as pregnancy. For instance, a survey of pregnant Iranian women established trimester-specific reference ranges for TSH, T4, and FT4. These findings underscore the necessity of using population-specific reference ranges for the accurate interpretation of thyroid function tests.²⁶

The study acknowledges that thyroid hormone levels may vary across populations and age/gender groups. These differences may be influenced by the diverse measurement methods used in practice. However, this research had two notable limitations. First, during the study period, a reliable free T4 and free T3 assay kit was unavailable, so total T4 and total T3 levels were measured instead of the more specific free hormone levels. Second, the thyroid peroxidase index, an essential marker of thyroid autoimmunity, was not assessed due to limited availability. These limitations may have affected the accuracy and comprehensiveness of the findings. Therefore, future research should address these aspects in order to produce more precise and inclusive results.

Conclusion

Overall, the study demonstrated essential aspects of thyroid function in a reasonably large sample, emphasizing differences in TSH levels between genders and revealing no significant age-related variation in TSH levels. However, the variances in T3 and T4 levels across age groups indicated that age may impact thyroid hormone dynamics. These insights are valuable for understanding normal variations in thyroid hormone levels in healthy populations and may inform clinical practice in interpreting thyroid function tests.

This lack of considerable change in TSH levels across age groups may highlight the stability of TSH production regulation over the lifespan, unlike T3 and T4, which may fluctuate in response to metabolic demands or other aging factors.

Acknowledgments

The authors would like to thank the Center for Healthcare Data Modeling and the participants for their cooperation in this research project.

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Competing Interests

The authors declare that there is no conflict of interests.

Ethical Approval

Ethical considerations included obtaining permission from the Ethics Committee of Shahid Sadoughi University of Medical Sciences (ethical code: IR.SSU.SPH.REC.1400.191) and written consent from participants.

Funding

This research received no financial support.

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