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Evaluation of Subclinical Thyroidal Dysfunction with Gender- and Age-Specific Serum TSH Reference Intervals Derived from a 2014-2023 Ligurian Population*

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ABSTRACT

Purpose: There is a need to refine the reference intervals (RI) of normal TSH values for age and gender. Aim of this study was to construct an RI of normal TSH in subjects living in Liguria, Italy. Methods: From 2014 to 2023, 1787 medical records (1336 females, 451 males) were evaluated in order to document TSH values. The RI of TSH was set at the 2.5th and 97.5th percentiles. The population was stratified into three age-groups: 18–44, 45–64 and ≥65 years. Results: Median TSH levels were significantly (P<0.0001) higher in females (1.82 mIU/l) than in males (1.43 mIU/l). This significance was maintained in all age-groups. In females, the RI were 0.46-6.31 mIU/l, 0.45-6.70 mIU/l, and 0.34-8.80 mIU/l in the 18-44 (n=542), 45-64 (n=517) and ≥65 (n=277) year age-groups. In males, the RI were 0.26-7.03 mIU/l, 0.33-6.21 mIU/l, and 0.26-8.57 mIU/l in the 18-44 (n=125), 45-64 (n=188) and ≥65 (n=138) year age-groups. When TSH values were evaluated by means of "study population RI" instead of the "pooled RI reported by manufacturers", a significantly higher percentage of females had sub-clinical hyperthyroidism (P=0.005), while a significantly lower percentage of both females and males (females P<0.0001; males P=0.03) had sub-clinical hypothyroidism. Conclusions: This study establishes new gender- and age-specific RI for TSH in our area. These TSH RI could be extensively employed in order to improve diagnosis and treatments. The reduced percentages of sub-clinical hypothyroidism in the elderly when the RI was derived from the study population, rather than from RI from manufacturers, redefines the need for treatment.

Keywords: TSH, reference intervals, age, gender, normal subjects.

INTRODUCTION

While thyroid stimulating hormone (TSH) is the key indicator of thyroid function, it exhibits high inter-individual variability (1). Personalized TSH reference intervals (RI) differ from population-based ranges and between-individual ranges (2). An individual TSH level is largely

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determined by genetic (2, 3) and several other factors, such as iodine status, body weight, smoking status, concomitant diseases, drugs, autoimmunity, time of sampling, sex and age (1, 4). It is recommended that an initially borderline TSH level be re-evaluated after at least two months. This precaution is particularly recommended in older adults with sub-clinical thyroid hypofunction (5). According to the guidelines of the National Academy of Clinical Biochemistry, a reference population is selected after the exclusion of individuals with positive thyroid antibodies, a personal history of thyroid disease, nodular or multinodular goiter and the use of thyroid medications (6). The RI of TSH is calculated as the range between the 2.5^{th} and 97.5^{th} percentiles of serum TSH levels in a large group of selected individuals (6). In clinical practice, normal RI ranges are provided by the manufacturers of TSH testing methods. However, these ranges do not consider possible gender and age differences, except for the occasional specification of trimester-specific TSH ranges in pregnancy. Moreover, it is well documented that RI differ among the different analysers used, and this can influence the prevalence of levo-thyroxine (L-T4) treatment (7), especially in sub-clinical hypothyroidism (SCH).

In the era of precision medicine, there seems to be a need to refine the normal RI of TSH in gender- and age-specific regional populations (8-15). In Italy, studies of TSH RI in large populations of normal volunteers living in the Pordenone district (Friuli; north-eastern Italy) (16) and in the Palermo area (Sicily) (17) were published several years ago. In 2022, RI were also evaluated in a large group of adults with obesity, without known thyroid disease, living in northern Italian centres in Piedmont and Lombardy (18). Recently, we published a study aimed at evaluating new age-specific TSH reference values collected from 2003 to 2023 in women in the Savona district (Liguria, north-western Italy) (19). On applying RI comprised between the 2.5^{th} and 97.5^{th} percentiles, we observed a slight increase in the number of 18-44-year-old women with sub-clinical thyrotoxicosis and a very significant reduction in the hasty diagnosis of SCH in women aged 45-65 years and >65 years (19). To reduce the variability of TSH testing and compare RI between females and males, we subsequently extended our evaluation. In the present study, local age-related RI were redefined over a shorter period of time (2014-2023 years) and differences between genders were evaluated. We also calculated the prevalence of sub-clinical thyroid dysfunction according to the RI derived from our study population and compared this with the prevalence calculated in the same population according to the median RI reported by manufacturers in the same period of time.

MATERIALS AND METHODS

Subjects

This cross-sectional retrospective single-centre study was conducted at the Endocrine Unit of the private Priamar Clinical Diagnostic Centre located in the Savona district (Liguria, Italy). The first endocrinological examination was requested mainly for thyroid, metabolic and pituitary-gonadal or adrenal health problems, or endocrinological screening. All records collected from 2014-2023 were individually reviewed in order to ensure that the subjects met our inclusion criteria. In this period of time, medical records on 3020 females and 825 males were anonymously evaluated. Age, body mass index (BMI), TSH, thyroid peroxidase antibodies (TPOAb), pharmacological treatments and thyroid ultrasonography (US) findings were collected. The effects of exclusion criteria [incomplete records, age <18 years, pregnancy, non-Caucasian ethnicity, and other reasons (impossibility or refusal of physical examination, transgender, only video consultation] are reported in figure 1. Subsequently, other records were excluded owing to interfering treatments (manly L-T4 administration), TPOAb positivity,

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hypoechoic thyroid texture on US and the presence of overt thyroid diseases. The final study sample comprised 1336 records on females and 451 records on males (Figures 1a, 1b).

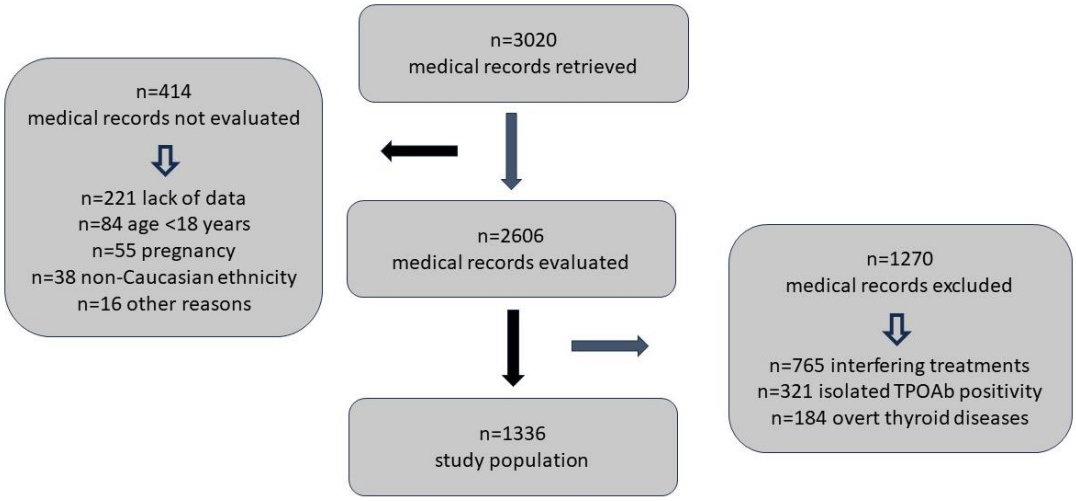

Figure 1a: Flowchart of the study (female subjects).

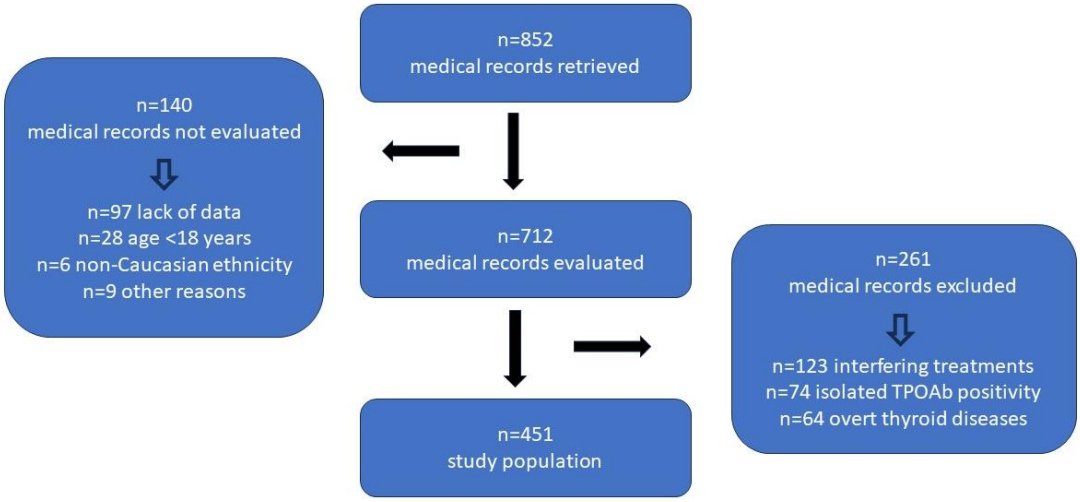

Figure 1b: Flowchart of the study (male subjects).

Subjects with isolated TPOAb positivity and negative US findings were excluded, as longitudinal evaluation was not the objective of the study and evolution to sub-clinical autoimmune thyroid disease could not be excluded. The average age of the study population at the time of the first endocrinological examination was 48.7 ± 17.5 years (±SD; range 18-89 years) in females and 53.9 ± 16.8 years (18-92 years) in males. Reasons for the endocrinological examination are reported in Supplementary material 1.

Data Collection

From the medical records, the following data were anonymously transferred to Excel files: chronological age (years), pharmacological anamnesis, smoking habits (non-smoker, previous or current smoker), body weight and height for BMI evaluation, judgement of thyroid echotexture on on-site US examination, and thyroid data (TSH, TPOAb) close to examination.

One Excel worksheet was filled in for each year from 2014 to 2023. Owing to the retrospective nature of the study, some clinical data were missing, but records were excluded from analysis only according to exclusion criteria. Missing TPOAb data did not exclude records when on-site thyroid US evaluation was available. Data were retrieved from the database from June to September 2024. Data from both study populations were arbitrarily divided into three age-groups: 18-44 years, 45-64 years and ≥65 years. In the female groups, 542 (18-44 years), 517 (45-64 years) and 277 (≥65 years) medical records were evaluable, while in the male groups, 125 (18-44 years), 188 (45-64 years) and 138 (≥65 years) medical records were evaluable.

Objectives

The primary objective was to obtain a local TSH RI from subjects in whom "healthy thyroid status" had been well defined during clinical and laboratory endocrinological examination. According to the experimental 2.5th and 97.5th percentiles of the RI of TSH, the current local percentages of sub-clinical thyroid dysfunctions were determined. A secondary objective was to compare these percentages with those from the pooled (2014-2023) TSH ranges provided by manufacturers.

Methods

BMI was calculated on the basis of weight (kg) and height (m) according to the following formula: kg/m^2. Smoking habits were investigated by applying the following score: non-smoker=0; former or current smoker=1. All US examinations were performed on-site using several machines, all equipped with linear probes working at 7.5-15 MHz. Data on normal thyroid volume are available in our district (20). Iodine status in the population of our districts has recently been deemed sufficient (21).

Assays

All diagnostic and laboratory tests were performed as part of routine clinical care. Several commercial methods were used during the study period. Negative/positive TPOAb values were assigned according to the normal range reported by the manufacturers. In the district of Savona, the neighboring districts of Imperia and Genoa, and an area of Southern Piedmont from which Savona is easier to reach, several public and accredited laboratories were available for TSH assays in the study period. Tests were performed at 12 public and 23 private centers (Supplementary material 2). All TSH calibration curves were calibrated against World Health Organization International Reference Preparation standards (WHO IR 80/558, WHO IR 81/565). The automated methods used for TSH assay were chemiluminescence microparticle immunoassay (CMIA), chemiluminescence immunoassay (CLIA), electrochemiluminescence immunoassay (ECLIA), and enzyme-linked fluorescent assay (ELFA). According to the manufacturers, the functional sensitivity of the TSH assays ranged from 0.004 to 0.07 mIU/l (median: 0.01 mIU/l).

Statistical Analysis

Statistical analysis was performed by means of GraphPad 10 software (GraphPad, San Diego, CA, USA). Data are reported as mean ± standard deviation (SD), range, median, confidence limits (Cl) of median and 2.5th - 97.5th percentiles. For statistical purposes, the functional sensitivity was set to 0.01 mIU/l, and TSH values below 0.01 mIU/l were reported as 0.01 mIU/l. Values ≤0.01 mIU/l or ≥ 10 mIU/l with out-of-range free-thyroxine levels were excluded, as these are considered to be in the clinical hyperthyroid and hypothyroid range, respectively. To compare

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continuous data, the Kruskal-Wallis non-parametric analysis of variance (ANOVA), post-ANOVA Dunn's multiple comparison test and Mann-Whitney test were used. Percentages were compared by means of Fisher's exact test. Correlations were evaluated by means of Spearman test. Significance was set at $P \leq 0.05$.

Ethical Approval

Owing to the retrospective nature of collection of clinical and hormonal data, no further formal approval from the Liguria Ethics Committee was required. A waiver of informed consent was granted because the research involved no risk to patients. Before their examination at the Priamar Clinical Diagnostic Centre, all patients had provided written informed consent to the management of data collected from their medical files and had agreed to their use for scientific purposes. Data were managed anonymously. Data collection and subsequent analysis were performed in compliance with the Helsinki Declaration.

RESULTS

Normality of TSH in the study period was determined according to the ranges provided by the manufacturers. No significant differences were noted among the lower normal TSH ranges or the upper normal TSH ranges from 2014 to 2023 (Supplementary material 2). On pooling all manufacturers' normal ranges (n=172) available from our laboratories between 2014 and 2023, the median lower normal limit of TSH was 0.35 mIU/l (95% Cl 0.35-0.35 mIU/l; range 0.20-0.55 mIU/) and the median upper normal limit of TSH was 4.68 mIU/l (95% Cl 4.30-4.90 mIU/l; range 3.07-5.66 mIU/l). The 2.5th percentile of the TSH range was 0.20 mIU/l, while the 97.5th percentile of the TSH range was 5.50 mIU/l. In the presence of normal f-T4 values, TSH values <0.20 mIU/l and >5.50 mIU/l were deemed diagnostic of sub-clinical hyperthyroidism and SCH, respectively.

Table 1: TSH and BMI values in the overall groups of female and male subjects and in the same subjects divided into the three age-groups. TSH significances female *vs* male subjects are: a) P<0.0001, b) P=0.002, c) P=0.003, d) P=0.04. BMI significance *vs* the 18-44-year age-group on post-analysis of variance Dunn's multiple comparison test is: aa) P<0.0001.

	Overall	18-44 years	45-64 years	≥65 years
Females				
Number of subjects	1336	542	517	277
Age, years (mean ± SD, range)	48.7 ± 17.5 (a) 18 - 89	30.8 ± 7.9 18-44	54.5 ± 5.6 45-64	72.8 ± 5.8 65 – 89
BMI kg/m2 (mean ± SD)	25.8 ± 5.9	24.4 ± 6.0	26.4 ± 5.8 aa	27.2 ± 5.5 aa
TSH mIU/l (median, 95% Cl of median, 2.5th-97.5th percentile)	1.82 (a) 1.72 – 1.87 0.40 - 7.46	1.78 (c) 1.69 – 1.90 0.46 – 6.31	1.83 (b) 1.70 – 1.97 0.45 – 6.70	1.79 (d) 1.56 – 1.99 0.34 – 8.80
Males				
Number of subjects	451	125	188	138
Age, years (mean ± SD, range)	53.9 ± 16.8 18 - 92	32.1 ± 8.0 18 - 44	54.7 ± 6.0 45 – 64	72.5 ± 6.1 65 - 92
BMI kg/m2 (mean ± SD)	26.9 ± 4.6	25.7 ± 4.5	27.2 ± 4.4 aa	28.0 ± 4.4 aa
TSH mIU/l (median, 95% Cl of median, 2.5th-97.5th percentile)	1.43 1.34 – 1.55 0.26 – 7.02	1.43 1.29 – 1.64 0.26 – 7.03	1.45 1.34 – 1.64 0.33 – 6.21	1.39 1.19 – 1.83 0.26 – 8.57

Table 1 shows TSH levels and age recorded in the 1336 females and 451 males from 2014 to 2023. Overall, on the first endocrinological examination, the mean age was significantly (P<0.0001) higher in males than in females. The median TSH in females (1.82 mIU/l; 95% Cl 1.72-1.87 mIU/l; 2.5th percentile 0.40 mIU/l, 97.5th percentile 7.46 mIU/l) was significantly (P<0.0001) higher than in males (1.43 mIU/l; 95% Cl 1.34-1.55 mIU/l; 2.5th percentile 0.26 mIU/l, 97.5th percentile 7.02 mIU/l) (Table 1). The study population was stratified according to age: 542 females were aged 18-44 years (mean ± SD: 30.8 ± 7.9 years), 517 were aged 45-64 years (54.5 ± 5.6 years) and 277 were aged ≥65 years (72.8 ± 5.8 years); 125 males were aged 18-44 years (32.1 ± 8.0 years), 188 were aged 45-64 years (54.7 ± 6.0 years) and 138 were aged ≥65 years (72.5 ± 6.1). In each age-group, TSH was significantly higher in females than in males (age-groups: 18-44 years P=0.003, 45-64 years P=0.002, >65 years P=0.04) (Table 1). No significant differences in TSH levels among the three age-groups emerged on ANOVA either in females or in males. The 2.5th percentile of TSH varied slightly among age-groups, both in females and in males. A progressively increasing trend in the 97.5th percentile of TSH was found across the age-groups only in females, but the highest 97.5th percentiles of TSH were found in subjects aged ≥65 years, whether females or males (Table 1).

Table 2 compares the numbers and percentages of female and male subjects with TSH values outside the normal range (<2.5th or >97.5th percentiles) according to the present study and the manufacturers' ranges.

Table 2. Numbers of female and male subjects (% in brackets) with TSH outside the normal range (<2.5th percentile or >97.5th percentile). Data are reported in the overall subjects and in the subjects divided into the 18-44-year, 45-64-year and ≥65-year age-groups, according to both the present study and the manufacturers' references. Significance value of present study *vs*. manufacturer references are: a) P<0.0001, b) P=0.007, c) P=0.005, d) P=0.03, and e) P=0.051 (near to significance).

	Age-group	Subjects TSH <2.5th percentile	Subjects TSH >97.5th percentile
	Females		
	overall	n=1336	
Manufacturers' intervals		10 (0.7%)	101 (7.6%)
Study population intervals		27 (2.0%) b	29 (2.2%) a
	18 – 44 years	n=542	
Manufacturers' intervals		4 (0.7%)	30 (5.5%)
Study population intervals		12 (2.2%)	12 (2.2%) b
	45-64 years	n=517	
Manufacturers' intervals		3 (0.6%)	31 (6.0%)
Study population intervals		12 (2.3%) d	12 (2.3%) c
	≥65 years	n=277	
Manufacturers' intervals		3 (1.1%)	40 (14.4%)
Study population intervals		5 (1.8%)	5 (1.8%) a
	Males		
	overall	n=451	
Manufacturers' intervals		3 (0.1%)	23 (5.1%)
Study population intervals		9 (0.2%)	10 (2.2%) d
	18-44 years	n=125	
Manufacturers' intervals		1 (0.8%)	5 (4.0%)

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Study population intervals		3 (2.4%)	3 (2.4%)
	45-64 years	n=188	
Manufacturers' intervals		2 (1.1%)	7 (3.7%)
Study population intervals		4 (2.1%	4 (2.1%)
	≥65 years	n=138	
Manufacturers' intervals		0 (0.0%)	11 (8.0%) e
Study population intervals		2 (1.4%)	3 (2.2%)

On applying the 2.5th – 97.5th interval derived from the study population, the overall percentage of females with TSH values <2.5th percentile was significantly (P=0.007) higher than that indicated by the manufacturers' reference values; conversely, the percentage of females with TSH values >97.5th percentile was significantly (P<0.0001) lower. The overall ratios (OR) were 0.36 (95% Cl 0.18-0.76) and 3.69 (95% Cl 2.40-5.58), respectively. In the overall male population, only the percentage of subjects with TSH values >97.5th percentile was near to being significantly (P=0.051) lower. When the data were stratified according to age-groups in females, a significantly (P=0.03; OR 0.24, 95% Cl 0.07 – 0.87) higher percentage of 45-64-year-old subjects had TSH values <2.5th percentile, while significantly lower percentages of subjects had TSH values >97.5th percentile in all age-groups (18-44 years P=0.007, OR 3.69, 95% Cl 135-4.98; 45-64 years P=0.005, OR 2.68, 95% Cl 1.41-5.17; ≥65 years P<0.0001, OR 9.18, 95% Cl 3.64-21.8) was observed (Table 2). In both females and males, BMI was significantly (P<0.0001) higher in the 45-64-year and the ≥65-year age-groups than in the 18-44-year age-group (Table 1). Overall, no correlation was noted between TSH values and chronological age, smoking status or BMI in either female or male subjects.

DISCUSSION

The diagnosis and management of thyroid dysfunction focus primarily on the measurement of TSH (1) as the most sensitive and specific marker of sub-clinical thyroid status (22, 23). RI may be device-, laboratory- and population-specific. Moreover, several other factors can influence TSH levels (1-4). Consequently, "normal" or "abnormal" TSH levels should be determined according to RI from local populations and laboratories (8 -18). In real-world practice, however, laboratories generally apply the TSH reference ranges suggested by assay manufacturers, without considering possible gender and age differences.

In this study, the overall median TSH at the first endocrinological examination was 1.43 mIU/l (2.5th percentile 0.26 mIU/l, 97.5th percentile 7.02 mIU/l) in males and 1.82 mIU/l (2.5th percentile 0.40 mIU/l, 97.5th percentile 7.46 mIU/l) in females, this latter value being slightly higher than that reported in a previous study conducted only in women (19). This difference may be attributable to some differences in the mean ages of women in the two studies (48.7±17.5 years *vs* 47.0±16.9 years), the slightly higher percentage of elderly women (≥65 years; 20.7% vs 18.7%) in the present study and, probably, a lesser variability in TSH assays in the 10-year period of data collection than in the 20-year period. Our gender-related TSH data are in accordance with the literature, which has generally, but not unanimously (24), reported higher TSH levels in females than in males (11-13). In the Italian population, similar results have been reported, with median TSH ranging from 1.39 to 1.92 mIU/l in males and from 1.46 to 2.16 mIUl in females (16-18, 25). Intra-gender differences in TSH levels in the Italian population could be due to the prevalence of adiposity (18) and, probably, differences in iodine status among groups. The reason why TSH is more elevated in females than in males is not well

known, but a genetic over-expression of subtle thyroid dysfunction in females, a different sex-steroid environment and a different central set-point TSH control could be hypothesized.

In this study, subjects were arbitrarily divided into age-groups on the basis of the conventional definitions of middle age (from about 40–45 years to about 60–65 years) and elderly (65 years and older). In the period 2014–2023, in each age-group, median TSH levels were always higher in females than in males. The declining significance of this inter-gender difference from the youngest to the oldest group may have been due to the declining number of subjects. However, a similar phenomenon has been observed by other authors (12). In our study, no significant correlation emerged between TSH and age, and no significant differences in median TSH levels among the three age-groups was found in either males or females. In the study by Yamada et al. [12], the increase in TSH from 30-39-year-old to 60-96-year-old subjects ranged from 0.24 to 0.40 mIU/l in females and from 0.19 to 0.21 mIU/l in males, according to the TSH assay methods used. The authors reported a gender difference in this increase, but the difference reached significance only in women when Siemens reagents were used (12). Lu et al. (13) also reported an increase in median TSH levels with increasing age (from 20-30 years to 50-70 years; 0.445 mIU/l in females and 0.25 mIU/l in males). By contrast, a decreasing trend in median TSH with age has been reported in all Italian studies (16, 17, 25, 26) in both sexes, the main differences being: -0.48 mIU/l from 20-24 years to 70-79 years in females in the study by Tozzoli et al. (16) and -0.38 mIU/l from 20-25 years to 70-80 years in males in the study by Lo Sasso et al. (17).

The main difference between all studies conducted in Italy, including ours, and those conducted in China and Japan concerns ethnicity. However, other factors, such as differences in iodine intake, are probably involved, too. While past or current smoking has been associated with lower TSH levels (27), the effect of smoking in our subjects, as in other research (28), can be considered marginal or absent. BMI can be considered an index of adiposity in epidemiological studies, and we observed a significant increase in BMI with ageing in both sexes. However, our data did not reveal a significant correlation between BMI and TSH. Mele et al. (18) reported a significant upward trend in TSH levels across incremental BMI classes only in females and, interestingly, in a sub-group of subjects, a positive correlation between leptin and TSH regardless of age, gender and smoking habit. Finally, the few other studies available have reported both positive (29) and negative (30) correlations.

In our previous study (19), which involved only women, a significant difference emerged when the RI of TSH was evaluated on the basis of population-derived data in comparison with the pooled reference ranges reported by manufacturers. Significantly more women aged 18-44 years were considered to have sub-clinical hyperthyroidism, while significantly fewer women in the 45-64-year and >65-year age-groups were considered to have SCH (19). In present study, these findings were confirmed both overall and in some age-groups. Specifically, more females with sub-clinical hyperthyroidism were found in the 45-65-year age-group and fewer females and males with SCH were found in the $\geq$65-year age-group. It is well known that the distribution curve of normal TSH is shifted to the right in the elderly (31), and several, but not all studies (16, 17), have shown an increasing trend in the 97.5th percentile of TSH with age. In the Padoan et al. (26) study, a slightly increasing TSH trend of the 97.5th was seen in females (from 4.96 mIU/l to 5.48 mIU/l) and males (from 4.88 mIU/l to 2.7 mIU/l) on passing from the ≤35-year group to the ≥70-year group. Other studies have reported an age-related increase in TSH, with the 97.5th percentile exceeding 7.0 mIU/l in individuals aged over 80 years (31). However, in

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Han subjects stratified into three age-groups (young: 18–44 years, middle-aged: 45–59 years, and elderly: >60 years), Luxia et al. (32) observed in females, but not in males, a similar gradual increase in TSH, which peaked in middle age and subsequently declined. Our study therefore suggests that, in the district of Savona and nearby areas, from 2014 to 2023, there may have been an over-treatment of subjects with SCH. The adoption of TSH RI derived from a "study population" could prevent treatment decisions linked only to the use of manufacturer-derived TSH RI (33). An approach that prevents over-treatment has also been reported in subjects >65 years of age in other studies (12, 24). Indeed, in a recent meta-analysis involving participants with a median age of 59 years (range 18–106 years), f-T4 above the 85th percentile and TSH below the 20th percentile, the authors reported a higher risk of all-cause mortality and cardiovascular mortality (34). From our present and previous (19) study, the suspicion of sub-clinical hyper-function seems slightly more frequent on applying the "study range" instead of the "manufacturer" range, especially in the group of women aged ≤65 years. This observation suggests that the lower limit of the reference TSH range may also need to be reassessed (34).

The present study has the limitation that the number of male subjects was quite low and the group of "normal healthy" women aged ≥ 65 years was smaller than the other two age-groups. However, our study population was larger than the minimum recommended limit in direct studies aimed at determining a normal range. Finally, the subjects who underwent endocrinological examination at our centre might not be representative of the general population of our districts, owing to the expense of attending a private centre. Moreover, no age-related TSH range was available at public healthcare centres in Liguria. In accordance with Razvi et al. (7), we sought to determine a TSH RI, above all in the elderly (35), based more on clinical outcomes than on statistical techniques. A further limitation of our study could be the incompleteness of data on thyroid hormones on the first endocrinological examination.

In conclusion, this is the first study in Liguria aimed at establishing new age-specific RI for TSH in both genders. This new age-related range could be more extensively employed in order to improve diagnoses. The main result of implementing age- and gender-related normal TSH levels between the 2.5th and 97.5th percentiles seems to be a slight increase in the number of ≤44-year-old women with sub-clinical hyperthyroidism and a very significant reduction in the hasty diagnosis of SCH in male and female subjects aged ≥65 years. Therapies for thyroid dysfunction must be started when TSH is outside age-related ranges, according to the patient's clinical condition and when this finding is confirmed some time later.

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Declarations
Ethical approval and consent to participate: All procedures were carried out in accordance with the ethical standards of the institution and with the 1975 Helsinki Declaration, as revised in 2008. Informed consent was obtained from all participants.

Availability of data and materials: The datasets used and/or analysed in the present study are available from the corresponding author on reasonable request.

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Authors' contributions: M.G. and M.S. carried out this research. M.G. and M.S. were responsible for data collection. M.G. wrote the manuscript. Both authors reviewed and approved the final version of the manuscript.

References

1. Browning MC, Ford RP, Callaghan SJ, Fraser CG. Intra- and interindividual biological variation of five analytes used in assessing thyroid function: implications for necessary standards of performance and the interpretation of results. Clin Chem. 1986;32:962-966.
2. Kuś A, Sterenborg RBTM, Haug EB, Galesloot TE, Visser WE, Smit JWA, et al. Towards personalized TSH reference ranges: a genetic and population-based approach in three Independent cohorts. Thyroid. 2024;34:969-979. doi: 10.1089/thy.2024.0045.
3. Taylor PN, Porcu E, Chew S, Campbell PJ, Traglia M, Brown SJ, et al.; UK0K Consortium. Whole-genome sequence-based analysis of thyroid function. Nat Commun. 2015 Mar6;6:5681. doi: 10.1038/ncomms6681.
4. Razvi S. Thyroid-function reference ranges in the diagnosis of thyroid dysfunction in adults. Nat Rev Endocrinol. 2024 May;20:253-254. doi: 10.1038/s41574-024-00972-2.
5. van der Spoel E, van Vliet NA, Poortvliet RKE, Du Puy RS, den Elzen WPJ, Quinn TJ, et al. Incidence and determinants of spontaneous normalization of subclinical hypothyroidism in older adults. J Clin Endocrinol Metab. 2024;109:e1167-e1174. doi: 10.1210/clinem/dgad623.
6. Baloch Z, Carayon P, Conte-Devolx B, Demers LM, Feldt-Rasmussen U, Henry JF, et al.; Guidelines Committee, National Academy of Clinical Biochemistry. Laboratory medicine practice guidelines. Laboratory support for the diagnosis and monitoring of thyroid disease. Thyroid. 2003;13:3-126. doi: 10.1089/105072503321086962.
7. Razvi S, Jabbar A, Addison C, Vernazza J, Syed A, Soran H, et al. Variation in the reference range limits of thyroid function tests and association with the prevalence of levothyroxine treatment. Eur J Endocrinol. 2023 Feb 14;188:lvad016. doi: 10.1093/ejendo/lvad016.
8. Park SY, Kim HI, Oh HK, Kim TH, Jang HW, Chung JH, et sl. Age- and gender-specific reference intervals of TSH and free T4 in an iodine-replete area: data from Korean National Health and Nutrition Examination Survey IV (2013-2015). PLoS One. 2018 Feb 1;13:e0190738. doi: 10.1371/journal.pone.0190738.
9. Vadiveloo T, Donnan PT, Murphy MJ, Leese GP. Age- and gender-specific TSH reference intervals in people with no obvious thyroid disease in Tayside, Scotland: the Thyroid Epidemiology, Audit, and Research Study (TEARS). J Clin Endocrinol Metab. 2013; 98:1147-1153. doi: 10.1210/jc.2012-3191.
10. Wang X, Li Y, Zhai X, Wang H, Zhang F, Gao X, et al. Reference Intervals for serum thyroid-stimulating hormone based on a recent nationwide cross-sectional study and meta-analysis. Front Endocrinol (Lausanne). 2021 Jun 1;12:660277. doi: 10.3389/fendo.2021.660277.
11. Xie T, Su M, Feng J, Pan X, Wang C, Tang T. The reference intervals for thyroid hormones: a four year investigation in Chinese population. Front Endocrinol (Lausanne). 2023 Jan 6;13:1046381. doi: 10.3389/fendo.2022.1046381.
12. Yamada S, Horiguchi K, Akuzawa M, Sakamaki K, Yamada E, Ozawa A, et al. The Impact of age- and sex-specific reference ranges for serum thyrotropin and free thyroxine on the diagnosis of subclinical thyroid dysfunction: a multicenter study from Japan. Thyroid. 2023;33:428-439. doi: 10.1089/thy.2022.0567.
13. Lu Y, Zhang WX, Li DH, Wei LH, Zhang YJ, Shi FN, et al. Thyroid hormone reference intervals among healthy individuals in Lanzhou, China. Endocrinol Metab (Seoul). 2023;38:347-356. doi: 10.3803/EnM.2023.1638.
14. Tran C, Topliss DJ, Schneider HG, Seeman E, Clayton-Chubb D, Neumann JT, et al. Establishing the TSH reference intervals for healthy adults aged over 70 years: the Australian ASPREE cohort study. Clin Chem Lab Med. 2024;63:611-620. doi: 10.1515/cclm-2024-0848.

URL: http://dx.doi.org/10.14738/bjhr.1204.19217.

15. Benamour M, Burlacu MC, Petrossians P, Unuane D, Van den Bruel A, Poorten VV, et al. Urgent need to adopt age-specific TSH upper reference limit for the elderly - a position statement of the Belgian thyroid club. Clin Chem Lab Med. 2024 Sep 23. doi: 10.1515/cclm-2024-1025. [Epub ahead of print]

16. Tozzoli R, D'Aurizio F, Metus P, Steffan A, Mazzon C, Bagnasco M. Reference intervals for thyrotropin in an area of Northern Italy: the Pordenone thyroid study (TRIPP). J Endocrinol Invest. 2018;41:985-994. doi: 10.1007/s40618-018-0825-0.

17. Lo Sasso B, Vidali M, Scazzone C, Agnello L, Ciaccio M. Reference interval by the indirect approach of serum thyrotropin (TSH) in a Mediterranean adult population and the association with age and gender. Clin Chem Lab Med. 2019;57:1587-1594. doi: 10.1515/cclm-2018-0957.

18. Mele C, Mai S, Cena T, Pagano L, Scacchi M, Biondi B, et al. The pattern of TSH and fT4 levels across different BMI ranges in a large cohort of euthyroid patients with obesity. Front Endocrinol (Lausanne). 2022 Oct 13;13:1029376. doi: 10.3389/fendo.2022.1029376.

19. Giusti M, Sidoti M. Women-specific reference ranges for serum TSH in Liguria: the impact of age and year of collection in a single-center cross-sectional study. Thyroid Res. 2025 Mar 11;18:8. doi: 10.11

20. Giusti M, Sidoti M. Normal thyroid volume in subjects evaluated in a primary ambulatory setting in Liguria. Minerva Endocrinol (Torino). 2023;48:19-26. doi: 10.23736/S2724-6507.20.03312-X

21. Conte L, Comina M, Monti E, Sidoti M, Vannozzi O, Di Ciolo L, et al. Urinary iodine concentration in a cohort of adult outpatients with thyroid diseases in Liguria 14 Years after the law on salt iodization. Nutrients. 2019 Dec 19;12:11. doi: 10.3390/nu12010011.

22. Gosi SKY, Kaur J, Garla VV. Subclinical hypothyroidism. 2024 Feb 15. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan–.

23. Biondi B, Cooper DS. Subclinical hyperthyroidism. N Engl J Med. 2018;378:2411-2419. doi: 10.1056/NEJMcp1709318.

24. Yildiz Z, Dağdelen LK. Reference intervals for thyroid disorders calculated by indirect method and comparison with reference change values. Biochem Med (Zagreb). 2023 Feb 15;33:010704. doi: 10.11613/BM.2023.010704.

25. Clerico A, Trenti T, Aloe R, Dittadi R, Rizzardi S, Migliardi M, et al.; Italian Section of the European Ligand Assay Society (ELAS). A multicenter study for the evaluation of the reference interval for TSH in Italy (ELAS TSH Italian Study). Clin Chem Lab Med. 2018;57:259-267. doi: 10.1515/cclm-2018-0541.

26. Padoan A, Clerico A, Zaninotto M, Trenti T, Tozzoli R, Aloe R, et al. Percentile transformation and recalibration functions allow harmonization of thyroid-stimulating hormone (TSH) immunoassay results. Clin Chem Lab Med. 2020;58:1663-1672. doi: 10.1515/cclm-2019-1167.

27. Asvold BO, Bjøro T, Nilsen TI, Vatten LJ. Tobacco smoking and thyroid function: a population-based study. Arch Intern Med. 2007;167:1428-32. doi: 10.1001/archinte.167.13.1428.

28. Sawicka-Gutaj N, Gutaj P, Sowiński J, Wender-Ożegowska E, Czarnywojtek A, Brązert J, et al. Influence of cigarette smoking on thyroid gland--an update. Endokrynol Pol. 2014;65:54-62. doi: 10.5603/EP.2014.0008.

29. Abdi H, Faam B, Gharibzadeh S, Mehran L, Tohidi M, Azizi F, et al. Determination of age and sex specific TSH and FT4 reference limits in overweight and obese individuals in an iodine-replete region: Tehran Thyroid Study (TTS). Endocr Res. 2021;46:37-43. doi: 10.1080/07435800.2020.1854778.

30. Ivo CR, Duarte V, Veríssimo D, Silva J, Passos D, Lopes L, et al. Thyrotropin and body mass index, are they related? Horm Mol Biol Clin Investig. 2022;44:1-4. doi: 10.1515/hmbci-2022-0002.

31. Taylor PN, Lansdown A, Witczak J, Khan R, Rees A, Dayan CM, Okosieme O. Age-related variation in thyroid function - a narrative review highlighting important implications for research and clinical practice. Thyroid Res. 2023 Apr 3;16(1):7. doi: 10.1186/s13044-023-00149-5. Erratum in: Thyroid Res. 2023 May 30;16(1):20. doi: 10.1186/s13044-023-00163-7.

32. Luxia L, Jingfang L, Songbo F, Xulei T, Lihua M, Weiming S, et al. Correlation between serum TSH levels within normal range and serum lipid profile. Horm Metab Res. 2021;53:32-40. doi: 10.1055/a-1191-7953.

33. Dirks NF, den Elzen WPJ, Hillebrand JJ, Jansen HI, Boekel ET, Brinkman J, et al. Should we depend on reference intervals from manufacturer package inserts? Comparing TSH and FT4 reference intervals from four manufacturers with results from modern indirect methods and the direct method. Clin Chem Lab Med. 2024;62:1352-1361. doi: 10.1515/cclm-2023-1237.

34. Xu Y, Derakhshan A, Hysaj O, Wildisen L, Ittermann T, Pingitore A, et al.; Thyroid Studies Collaboration. The optimal healthy ranges of thyroid function defined by the risk of cardiovascular disease and mortality: systematic review and individual participant data meta-analysis. Lancet Diabetes Endocrinol. 2023;11:743-754. doi: 10.1016/S2213-8587(23)00227-9.

35. Zhai X, Li Y, Teng X, Teng W, Shi X, Shan Z. Thyrotropin reference interval in older adults. Endocr Connect. 2025 Jan 2;14(2):e240435. doi: 10.1530/EC-24-0435.

Evaluation of subclinical thyroidal dysfunction with gender- and age-specific serum TSH reference intervals derived from a 2014-2023 Ligurian population.

Supplementary material 1

The 1st endocrinological examination was requested for various reasons. The table reports the reasons on the basis of medical history, symptoms referred, signs observed and available biochemical/instrumental data. Reasons were categorized according to the endocrine system whose dysfunction was hypothesized by patients and/or their general practitioner.

	Whole group	**18-44-year age-group**	**45-64-year age-group**	**≥65-year age-group**
	Females			
Numbers of subjects	1336	542	517	277
Reasons for examination				
Pituitary (%)	7.3	11.8	4.6	3.2
Thyroid (%)	42.7	20.7	59.1	55.2
Parathyroid and Bone (%)	6.4	1.3	7.2	14.8
Adrenal (%)	4.1	2.8	5.0	5.0
Gonadal (%)	17.3	39.9	2.3	1.1
Diabetes and Metabolism (%)	12.9	12.5	12.8	14.1
Other reasons (%)	9.3	10.7	9.0	6.8
	Males			
Numbers of subjects	451	125	188	138
Reasons for examination				
Pituitary (%)	4.7	2.4	5.3	5.8
Thyroid (%)	36.6	23.2	38.2	46.4
Parathyroid and bone (%)	4.4	1.6	3.6	8.0
Adrenal (%)	5.5	10.4	3.6	3.6

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Gonadal (%)	23.7	39.2	24.4	8.7
Diabetes and Metabolism (%)	16.7	11.2	15.4	23.9
Other reasons (%)	8.4	12.0	9.5	3.6

Regarding the thyroid gland, most examinations were performed because of non-specific symptoms (generally asthenia and palpitations), family history of thyroid diseases, previously treated thyroid dysfunction, incidental minimal (cystic) US findings, and misinterpretation of laboratory data. In these subjects, clinical examination excluded current thyroid dysfunction. The second most common reason for endocrine examinations was presumed or diagnosed gonadal symptoms. In females, there was an age-related decreasing trend due to the prevalence of menstrual dysfunction in young subjects, while an age-related increasing trend was seen in young and middle-aged males, owing to the prevalence of erectile dysfunctions. In our regional area, a minimal percentage of patients were first examined in a private center for metabolic reasons (mainly metabolic syndrome and diabetes). Finally, in both sexes, the prevalence of requests for endocrine examinations due to suspected pituitary, parathyroid or bone disease and adrenal dysfunction was minimal. Other reasons were generally: examination for non-thyroidal neck disease, endocrine examination included in a medical check-up, pre-gestational evaluation or examination of a couple, and examination for non-endocrine disease.

Evaluation of subclinical thyroidal dysfunction with gender- and age-specific serum TSH reference intervals derived from a 2014-2023 Ligurian population.

Supplementary materials 2

Table - The list of the public (a) and private (b) centers is reported below. ASL = Azienda Sanitaria Locale.

1	ASL 1 Imperia (a)
2	ASL 2 Savona (a)
3	ASL 3 Genova (a)
4	ASL 4 Chiavari (a)
5	Ospedale Santa Corona (Pietra Ligure) (a)
6	Ospedale Galliera (Genoa) (a)
7	Policlinico San Martino (Genoa) (a)
8	Ospedale Gaslini (Genoa) (a)
9	Laboratorio Analisi Garoglio (Bordighera) (b)
10	ACCAD Sanremo (b)
11	Casa San Michele (Albenga) (b)
12	Lab Marazolo (Tovo San Giacomo) (b)
13	Tiesseti/Bianalisi (Vado Ligure) (b)
14	IS.A.C (Savona) (b)
15	Analysis (Savona) (b)
16	Biomedical (Genoa) (b)
17	Istituto Salus (Genoa) (b)
18	Synlab/Baluardo (Genoa (b)
19	Casa della Salute (Genoa) (b)
20	Laboratorio Albaro (Genoa) (b)
21	Laboratorio SrL (Genoa) (b)
22	Laboratorio Valle Scrivia (Genoa) (b)
23	Analyst Genova (Genoa) (b)
24	Analisi Mediche Liguria (Genoa) (b)
25	Emolab (Genoa) (b)
26	Cerba Health Care (Genoa) (b)
27	Laboratorio Manara (Genoa) (b)
28	BioLab (Rapallo) (b)
29	Bio-Data (Lavagna) (b)
30	SMeL (Sestri Levante) (b)
31	ASL22 AL/Ospedale Alessandria (a)

32	ASL Novi Ligure (Alessandria) (a)
33	ASL CN 1 (Cuneo/Mondovì/Ceva) (a)
34	Lab Santa Maria Novi (AL) (b)
35	Azienda Ospedaliera Pisana (Pisa) (a)

In the period 2014-2023, the reference intervals of TSH were obtained from 12 public and 23 private centers. In this period, all yearly reference intervals of TSH were available only from 2 public territorial centers (ASL 2 Savonese and ASL 3 Genovese) and from 2 public hospitals located in Genoa (San Martino Polyclinic Hospital, Galliera Hospital). Few yearly reference intervals (for ≤5 years) were available from 18 laboratories (6 public, 12 private). In the period 2014-2023, a few centers closed, while others opened or were renamed.

Single data collected are reported for evaluation. The following tables report manufacturers' yearly lower and upper TSH intervals.

a) lower TSH

	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023
1					0.27	0.27			0.27	0.35
2	0.33	0.33	0.33	0.38	0.38	0.38	0.38	0.38	0.38	0.38
3	0.35	0.35	0.5	0.35	0.35	0.35	0.35	0.35	0.35	0.35
4		0.34	0.38	0.38	0.38	0.38		0.38	0.38	0.38
5		0.2	0.2	0.2	0.2					
6	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27
7	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27
8		0.2		0.27			0.2		0.4	0.4
9			0.25					0.27		0.27
10				0.36						
11			0.27							
12									0.35	
13	0.25	0.25	0.25	0.35	0.35	0.35	0.35	0.35	0.35	0.35
14		0.4								
15	0.5	0.35	0.35							
16			0.4		0.5		0.5	0.5	0.5	0.5
17	0.35	0.35	0.35		0.55	0.35	0.35	0.5	0.35	0.35
18	0.4		0.4	0.4	0.4	0.55	0.55	0.55	0.35	0.35
19						0.46		0.46	0.46	0.55
20	0.35	0.35	0.35		0.4	0.35	0.35	0.35	0.35	0.35
21			0.3	0.3		0.34	0.34	0.35	0.35	0.35
22				0.25		0.25	0.25	0.25	0.25	0.25
23				0.3						
24						0.38	0.38	0.38	0.38	0.38
25		0.3	0.31	0.3	0.27	0.27	0.27	0.27	0.46	
26									0.27	0.27
27	0.3									
28						0.27				
29	0.46								0.46	0.46
30								0.35		
31			0.5			0.36				
32				0.38	0.38			0.36	0.36	0.36
33	0.35			0.35		0.35			0.25	0.35
34								0.3		

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35	0.4		0.4	0.4			0.4	0.4		0.4

b) upper TSH

	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023
1					0.27	0.27			0.27	0.35
2	0.33	0.33	0.33	0.38	0.38	0.38	0.38	0.38	0.38	0.38
3	0.35	0.35	0.5	0.35	0.35	0.35	0.35	0.35	0.35	0.35
4		0.34	0.38	0.38	0.38	0.38		0.38	0.38	0.38
5		0.2	0.2	0.2	0.2					
6	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27
7	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27
8		0.2		0.27			0.2		0.4	0.4
9			0.25					0.27		0.27
10				0.36						
11			0.27							
12									0.35	
13	0.25	0.25	0.25	0.35	0.35	0.35	0.35	0.35	0.35	0.35
14		0.4								
15	0.5	0.35	0.35							
16			0.4		0.5		0.5	0.5	0.5	0.5
17	0.35	0.35	0.35		0.55	0.35	0.35	0.5	0.35	0.35
18	0.4		0.4	0.4	0.4	0.55	0.55	0.55	0.35	0.35
19						0.46		0.46	0.46	0.55
20	0.35	0.35	0.35		0.4	0.35	0.35	0.35	0.35	0.35
21			0.3	0.3		0.34	0.34	0.35	0.35	0.35
22				0.25		0.25	0.25	0.25	0.25	0.25
23				0.3						
24						0.38	0.38	0.38	0.38	0.38
25		0.3	0.31	0.3	0.27	0.27	0.27	0.27	0.46	
26									0.27	0.27
27	0.3									
28						0.27				
29	0.46								0.46	0.46
30								0.35		
31			0.5			0.36				
32				0.38	0.38			0.36	0.36	0.36
33	0.35			0.35		0.35			0.25	0.35
34								0.3		
35	0.4		0.4	0.4			0.4	0.4		0.4

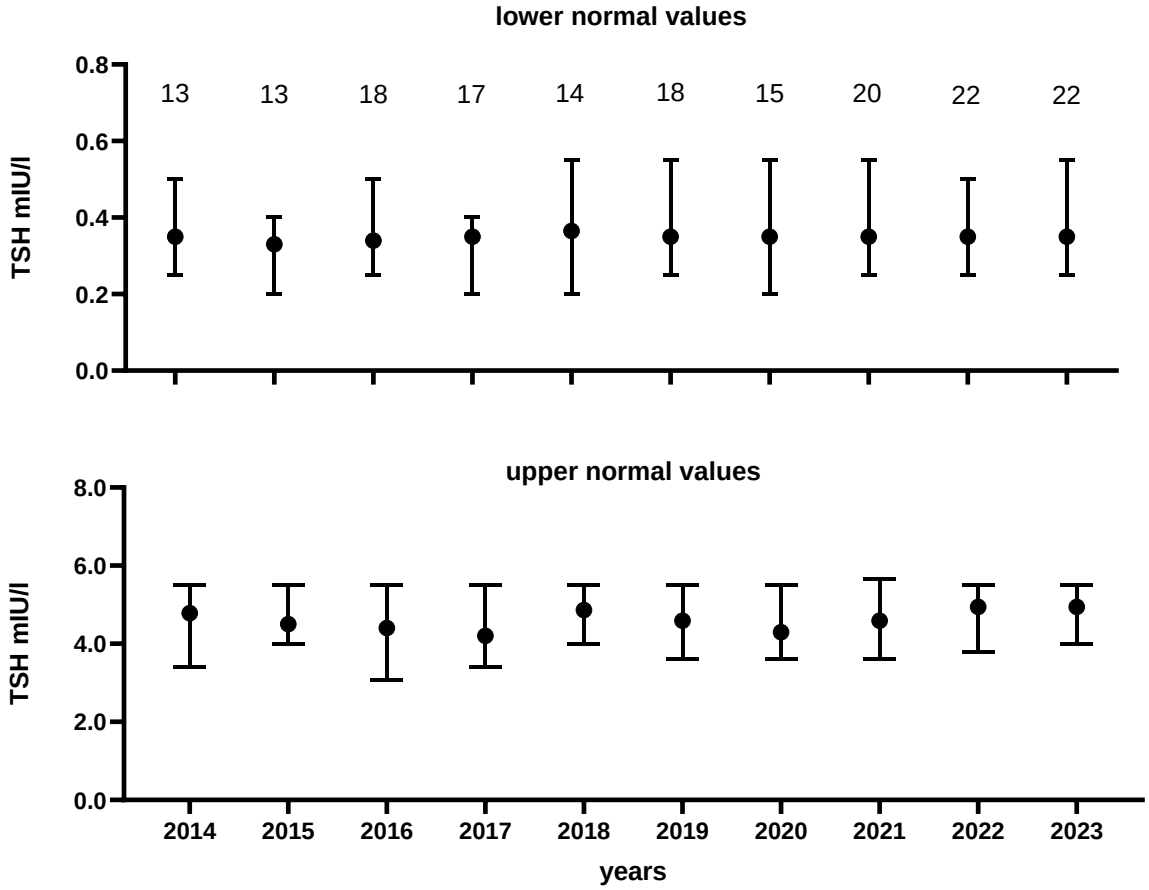
On Kruskal-Wallis ANOVA test, no differences in TSH values emerged in either lower (P=0.59) or upper (P=0.85) data collected yearly. The pooled TSH values were:

Number of values	172	172
Minimum	0.2000	3.070
25% Percentile	0.2700	4.200
Median	0.3500	4.680
75% Percentile	0.3800	5.075
Maximum	0.5500	5.660
Range	0.3500	2.590
2.5th Percentile	0.2000	3.600
97.5th Percentile	0.5500	5.500

95% CI of median		
Actual confidence level	96.08%	96.08%
Lower confidence limit	0.3500	4.300
Upper confidence limit	0.3500	4.900
Mean	0.3462	4.635
Std. Deviation	0.07742	0.5833
Std. Error of Mean	0.005903	0.04448

Lower 2.5th percentile (0.20 mIU/l) and upper 97.5th percentile (5.50 mIU/l) were employed to determine the number of subjects outside the reference interval according to the pooled TSH intervals provided by manufacturers.

In the following figure shows the median 2.5th percentile and the median 97.5th percentile of lower normal and upper normal TSH ranges, as indicated by manufacturers. The numbers at the top indicate the laboratories involved each year.



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