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Age-specific thyroid hormone and thyrotropin reference intervals for a pediatric and adolescent population

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Abstract

Background: Establishment of reliable reference intervals remains valuable for confirming validity and advancing standardization across methods and populations. Moreover, knowledge of the measurement uncertainty (U) and of the reference change value (RCV) has important applications in clinical chemistry.

Methods: Starting from the information available in the laboratory data base (29,901 subjects) an initial selection was carried out by eliminating all subjects with a clinical or laboratory pathological report; data from 7581 0- to 20-year-old subjects (53.87% girls) remained in the study. These subjects, divided into nine age groups, were used to define reference distribution percentiles (2.5th, 50th and 97.5th) of serum thyrotropin (TSH), triiodothyronine (T3), thyroxine (T4), and free T4 (fT4), as well as U and RCV of these assays.

Results: In early infancy, T4 and fT4 values were higher than in the older age groups. Serum T4 95th percentile reference value, useful for the diagnosis of hyperthyroidism, was 142.9 in 20-year-old boys and 230.4 nmol/L in early infants and serum T3 95th percentile was 2.6 and 3.5 nmol/L, respectively, while fT4 2.5th percentile reference value, useful for the diagnosis of hypothyroidism, was 9.6 and 13.0 pmol/L, respectively. Serum TSH 97.5th percentile showed less age variation, 4.38–4.88 mIU/L. Performance of the four assays resulted in approximately 20% Us, reflecting simple and complex imprecision, trueness, analytical and functional sensitivity. RCV of serum TSH (58.6%) was larger than for thyroid hormones (28.3%–34.7%), probably due to the high biological variation of this hormone.

Conclusions: We have established reference interval for TSH and thyroid hormones, as well as Us for assessing reliability of measurements, and RCVs to alert users on the presence of clinical significant changes.

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Introduction

Thyroid hormones influence almost all aspects of normal development during childhood and adolescence. Primary care pediatricians tend to evaluate thyroid function as a screening test in children and adolescents with various non-specific complaints. Overt abnormalities in thyroid function are common endocrine disorders affecting 5%–10% of individuals over a lifespan (1). Clinical symptoms and signs are often non-specific, and the diagnosis and monitoring of therapy depends crucially on measurements of thyroid hormones [triiodothyronine (T3), total thyroxine (T4), free T4 (fT4) and thyrotropin (TSH)] in blood (2).

Reference intervals are necessary for understanding laboratory results in individuals or in diagnosis-related groups. The different types of assays often lead to results that may vary considerably. Reference intervals are described in a few published studies but given the relatively frequent and importance of thyroid function testing, establishment of reliable reference intervals remains valuable for confirming validity and advancing standardization across methods and populations (3–6).

The application of ISO/IEC 15189 (International Organization for Standardizations's Technical Committee 212) for accreditation of clinical laboratories requires the knowledge of the measurement uncertainty (U). U is the interval of possible values among which the true value is placed with a high degree of probability; there is no agreement, whether it is more convenient to report results only as ranges or to include U, as additional data in analytical reports (7).

Reference change value (RCV) is a term that expresses the difference that has to be observed for a change of patient values, to be considered clinically important. RCV includes analytical variations, depending on assays, and biological variations. Data on the biological variations of analyte concentration have important uses in clinical chemistry, including judging the usefulness of conventional population-based reference ranges (8), assessing the true significance of changes in results obtained for serial specimens from a single patient, and determining the standards of performance, or analytical goals, required to facilitate optimal patient care. Even though optimal analytical goals are desirable, they are seldom reached in clinical practice, and assay performances similar to ours are usually reported (9).

In this study, we have established reference interval for serum thyroid hormones and TSH, as a function of age (0–20 years), in a pediatric and adolescent population, and we have calculated U and RCV for these determinations as useful tools in the interpretation of results of thyroid function studies.

Materials and methods

Subject selection and blood sampling

The data base of the endocrinology laboratory of Garrahan Pediatric Hospital of Buenos Aires includes laboratory data, pharmaceutical and clinical information. The study was approved by the Hospital de Pediatria Garrahan Internal Review Board. Between 2003 and 2007, thyroid function (serum TSH, T3, T4, fT4), anti thyroid antibodies (in some cases anti thyrotropin receptor antibodies), was studied in 29,901 children and adolescents of the two sexes, from birth to 20 years of age, attending the outpatient clinic. Blood was drawn by venipuncture into serum tubes without additives. Serum was obtained by centrifugation at 2000 *g* over 10 min, and assays were carried out within the day of sampling. In many patients, results of non-thyroid hormones and routine laboratory determinations (hemogram, cholesterol, triglycerides, urea, creatinine and tests for hepatic function) were available. To eliminate abnormal values several selection/exclusion criteria were applied. Initially, all subjects with any clinical diagnosis in hospital records, either at admission or during follow-up (51.2%), were excluded. Subjects with altered non-thyroid hormone laboratory records were also eliminated: abnormal routine laboratory determinations (11.07%) or other hormone assays (7.23%), as well as altered TSH-TRH tests or positive thyroid antibodies (3.59%). Serum thyroid hormone and serum TSH outlier results (1.556%) were detected by MedCal software, following the recommendation of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) (10). The interquartile range (IQR) between the lower and upper quartiles of distribution (Q_1 and Q_3) was estimated ($IQR=Q_3-Q_1$) for the four determinations. Outliers were identified as any data outside of: Q_1–1.5×IQR and Q_3+1.5×IQR. For serum TSH a logarithmic transformation of data was necessary (see Statistical analyses). Analyses were assessed including interaction terms, i.e., an outlier value in a single determination resulted in the elimination of all assays for that particular subject. Data from 7581 (25.354%) children (girls: 53.87%) remained in the study. Age grouping criteria was to choose shorter age intervals during the first year of life and 3-year intervals throughout the rest of prepuberty and adolescence. Age distribution and number of subjects were as follows: 1 week–2.9 months (mo): 241 (girls: 47.12%); 3–5.9 mo: 144 (girls: 48.12%); 6–11.9 mo: 274 (girls: 49.17%); 1–2.9 years (y): 849 (girls: 49.85%); 3–5.9 y: 977 (girls: 49.96%); 6–8.9 y: 1266 (girls: 54.60%); 9–11.9 y: 1493 (girls: 55.29%); 12–14.9 y: 1256 (girls: 57.60%); and 15–19.9 y: 1081 (girls: 56.87%).

Assay methods

Undiluted serum samples were assayed for T3, T4, fT4 and TSH using an automated assay system (Abbott AxSYM, Abbott Diagnostics, Abbott Park, IL, USA). T3, fT4 and TSH were assessed by microparticle enzyme immunoassay (MEIA) and T4 by fluorescence polarization immunoassay (FPIA).

Assay performance was studied according to the Clinical and Laboratory Standard Institute (CLSI) evaluation protocols (EP): EP15 A2 (user demonstration of performance for precision and accuracy), EP6 A2 (evaluation of the linearity of quantitative methods), and EP17 A2 (protocol for determination of analytical sensitivity and functional sensitivity). All verifications were acceptable, and are shown in Table 1.

Total error (TE) of each assay was estimated as TE = bias + 1.65 times coefficient of variation (CV)%. All values were below Allowable Total Error (Table 2). Bias was calculated from an external quality control system (CEMIC, Progba, Buenos Aires, Argentina).

Quality assessment

The clinical endocrinology laboratory where the measurements were performed participates in a National Quality Award program (Argentina). Parameters were controlled and results were documented and evaluated using a peer group internal quality control program (Unity, Bio-Rad Laboratories, Irvine, CA, USA), and an external quality control system (CEMIC). During evaluation of reference intervals, the internal control was assessed by Westgard rules (11). Bias was estimated comparing with a mean of the external quality control program, and it was taken as our best estimate of trueness.

Measurement uncertainty and reference change value

Percentage U was estimated by the "Top Down" method as proposed by the Analytical Methods Committee (7, 12), where:

%U = measurement uncertainty (or expanded uncertainty) = standard combined uncertainty (Uc) times k

k = 2 for 95% confidence interval, and $Uc= (U^2 \text{ precision} + U^2 \text{ trueness})^{½}$

U precision = %CV; U trueness = $[(\text{peer group \%CV}/(\text{n laboratories})^{½})^2 + (\text{bias}^2)]^{½}$.

RCV was calculated according to Harris & Brown (8, 13):

$RCV = 2^{½}\, Z\cdot[(CV_a)^2 + (CV_i)^2]^{½}$ where Z: 1.96 for 95% confidence interval.

Table 1 Assay performance.

	TSH	T3	T4	fT4
Simple precision	3.43–5.05%	3.46–8.22%	2.46–5.14%	2.63–5.19%
[intraassay (within run) CV]	(0.25–6.00) mIU/L	(1.1–3.1) nmol/L	(77.2–154) nmol/L	(9.0–38.6) pmol/L
Complex precision [interassay (run to run) CV]	8.25%	9.01%	8.9%	9.63%
Bias	5.73%	3.75%	2.85%	3.76%
Linearity	Up to 100 mIU/L	Up to 7 nmol/L	Up to 308.9 nmol/L	Up to 51.5 pmol/L
Analytical sensitivity	0.02 mIU/L	0.1 nmol/L	8.23 nmol/L	1.3 pmol/L
Functional sensitivity	0.06 mIU/L	0.2 nmol/L	14.4 nmol/L	3.4 pmol/L

To convert thyroid hormone values to metric units use the following factors. T3 nmol/L ÷ 1.538: T3 ng/mL; T4 nmol/L ÷ 12.87: T4 µg/dL and fT4 pmol/L ÷ 12.87: fT4 ng/mL.

Table 2 Total error.

	TSH	T3	T4	fT4
CV, %, times 1.65	8.32	13.56	8.48	8.56
Bias, %	5.73	3.75	2.85	3.76
Total error, %	14.05	17.31	11.33	12.32
Allowable total error, %	24.60	27.03	20.00	23.70

$RCV = 2.77 \cdot [(CV_a)^2 + (CV_i)^2]^{½}$

CV_a is the analytical coefficient of variation, and CV_i the biological variability coefficient, obtained from Westgard web page (www.westgard.com).

Statistical analyses

For patient inclusion, we eliminated the outlier's results using MedCal software, if one result was an outlier, the patient was eliminated from the database. Since, serum TSH has non-normal distribution; logarithm transformation was used for normalization before outlier testing.

Percentiles and regression coefficients were calculated with the Statistic 9 program. In accordance with the IFCC (14), the range between the 2.5th and the 97.5th percentile was taken as the reference interval. The 50th percentile was taken as the center of distribution.

Results

Fiftieth percentile and reference interval

We analyzed a total of 7581 children, who were sub-grouped according to age (Table 3, Figure 1). Since no significant difference was found between the means and ranges of thyroid hormones and TSH concentrations in age-matched male and female subjects, data of the two sexes were combined for the calculation of percentiles.

In the neonatal period, the 2.5th, 50th and 97.5th percentile concentrations of serum T4, T3, fT4 and TSH showed some fluctuations. T4 and fT4 values were higher than in the older age groups. As previously reported (15), TSH ($p<0.001$), T3 ($p<0.0001$), T4 ($p<0.0001$) and fT4 ($p<0.001$) decreased significantly with chronological age. At the level of higher and lower limits, which are of particular clinical interest, some values showed marked variation with age. The 97.5th percentile reference value of serum T4, useful for the diagnosis of hyperthyroidism, is 142.9 nmol/L (11.1 µg/dL) in 20-year-old boys and 230.4 nmol/L (17.9 µg/dL) in early infants, while the 2.5th percentile reference value of fT4, useful for the diagnosis of hypothyroidism, is 9.6 pmol/L (0.75 ng/dL) and 13.0 pmol/L (1.01 ng/dL), respectively.

As shown in Table 4, performance of the four assays resulted in Us of approximately 20%, indicating that in terms of simple and complex imprecision, trueness, analytical and functional sensitivity these assays are suitable for measuring T4, T3, fT4 and TSH in children's serum.

RCV of serum TSH (58.6%) was larger than for thyroid hormones (28.3%–34.7%), probably due to the high biological variation of this hormone (Table 3). As a conclusion from these data, the highest serum TSH value within the reference interval, which might be considered "non-elevated" after accounting for the analytical and biological variability (RCV), would be, for the different age groups, as follows: 1 w–3 mo: 2.57; >3 mo–6 mo: 2.48; >6 mo–12 mo: 2.53; >1 y–3 y: 2.55; >3 y–6 y: 2.57; >6 y–9 y: 2.78; >9 y–12 y: 2.79; >12 y–15 y: 2.72 and >15 y–20 y: 2.86 mIU/L.

Discussion

Data on results of serum thyroid hormones and TSH in children and adolescents are limited (16, 17). In the last years, studies based in computerized databases have demonstrated to be useful in the assessment of populations (18–20). The present study used data of 29,901 children and adolescents of the two sexes, from birth to 20 years of age, in whom thyroid function was studied. Patients with clinical and/or laboratory altered records were excluded, and patients with one outlier result of any serum thyroid hormone or TSH were eliminated from the database; data of 7581 children and adolescents remained in the study, and they were divided in age groups.

Table 3 Percentiles (2.5th, 50.0th, 97.5th) of serum TSH, T3, T4 and fT4 concentration per age group.

Age, n	TSH, µIU/L mIU/L			T3, nmol/L			T4, nmol/L			fT4, pmol/L		
	2.5th	50th	97.5th	2.5th	50th	97.5th	2.5th	50th	97.5th	2.5th	50th	97.5th
1 w–3 m (241)	0.92	2.30	4.38	1.4	2.4	3.5	91.4	150.6	230.4	13.0	17.2	26.8
>3 m–6 m (144)	0.79	2.24	4.23	1.6	2.7	3.7	96.5	146.7	216.2	11.6	16.3	23.3
>6 m–12 m (274)	0.84	2.37	4.31	1.6	2.6	3.5	81.1	131.3	203.4	11.1	16.2	24.4
>1 y–3 y (849)	0.97	2.46	4.35	1.5	2.6	3.5	86.2	132.6	186.6	11.4	16.3	24.8
>3 y–6 y (977)	0.97	2.49	4.38	1.6	2.5	3.4	88.8	127.4	173.8	12.0	16.6	25.0
>6 y–9 y (1266)	0.82	2.36	4.74	1.5	2.4	3.3	86.2	124.8	164.7	11.7	16.2	24.6
>9 y–12 y (1493)	0.88	2.53	4.76	1.3	2.3	3.2	82.4	118.4	158.3	10.9	16.0	25.2
>12 y–15 y (1256)	0.88	2.28	4.65	1.2	2.1	3.1	81.1	106.8	150.6	10.4	14.9	24.7
>15 y–20 y (1081)	0.71	1.86	4.88	1.1	1.8	2.6	78.5	106.8	142.9	9.6	14.8	25.0

m, months; w, week; y, years. To convert thyroid hormone values to metric units use the following factors: T3 nmol/L ÷ 1.538: T3 ng/mL; T4 nmol/L ÷ 12.87: T4 µg/dL and fT4 pmol/L ÷ 12.87: fT4 ng/mL.

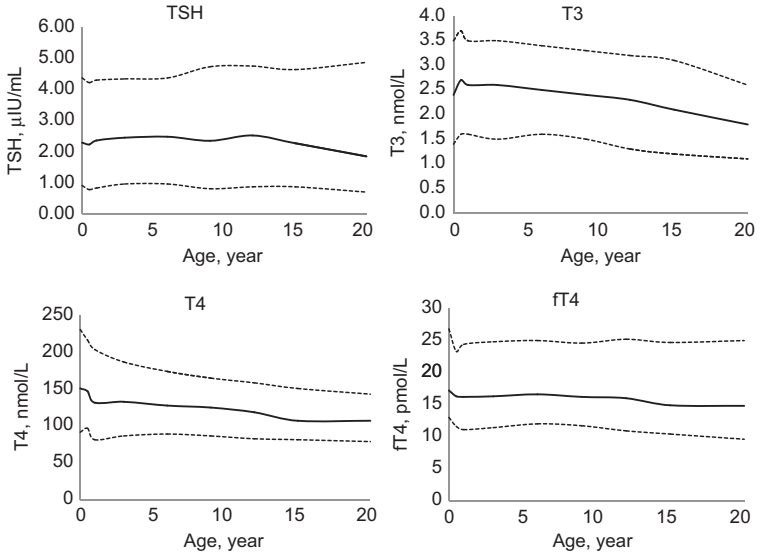

Figure 1 Reference values as a function of age, (----) 2.5th and 97.5th, (——) 50.0th percentiles.

As published by Ermlinger et al. (3) for the German population, serum TSH, T4, fT4, and T3 in the first years of life are higher than in the rest of prepuberty and during adolescence. Our values of TSH and T4 were somewhat higher at all ages tested, while those of fT4 and T3 were similar. These differences might be explained by population differences, stressing the need for using references values for each population. The method we used for sample selection might explain a higher age range of our results, but the high number of subjects contributes to the robustness of our study. The dissociation between serum T4 and fT4 profiles with advancing child age (steeper T4 decrease) might be secondary to differences in serum thyroxine binding globulin (TBG), which increases with age up to 5 years old. It is possible that this induces an increment in TBG-bound T4 keeping fT4 relatively stable during the rest of prepuberty. Decreases in both serum TBG (3) and T4 are observed during puberty, while fT4 remains moderately changed.

Table 4 Evaluation of serum TSH, T3, T4 and fT4, U and RCV.

	TSH	T3	T4	fT4
U (precision)=%CV_a	8.25	9.01	8.97	9.63
Peer group %CV	9.50	13.70	9.90	10.90
n	36	14	16	33
% Bias	–5.51	0.82	1.42	3.24
% U (bias)	5.73	3.75	2.85	3.76
Combined U	10.05	9.76	9.41	10.34
% U	20.09	19.52	18.83	20.67
% CV_i	19.30	8.70	4.90	7.60
% RCV	58.60	34.69	28.31	33.98

Combined U, U(precision) combined with U(trueness); CV_a, analytical coefficient of variation; CV_i, biological coefficient of variation; n, number of laboratories participating in the Peer Group Program; peer group %CV, peer group coefficient of variations; %RCV, reference change value; %U, measurement uncertainty (or expanded); U, uncertainty.

The serum concentration of thyroid hormones and TSH are important for the assessment of thyroid function and for differential diagnoses in non-thyroid complaints. There is strong evidence supporting the convenience that patient values and reference intervals should belong to the same population under study (19). From the age of 1 year onwards, mean concentrations of thyroid hormones did not show significant differences among age groups, until puberty, thereafter showing a gradual decrease towards adult concentrations, both at the level of the highest (97.5th percentile) and lowest limits (2.5th percentile), which are of clinical interest; some values showed marked variation with age, and these limits are useful for the diagnosis of hyperthyroidism or hypothyroidism, respectively.

The distribution of TSH serum values is not Gaussian (21), the 50th percentile varied between 1.86 and 2.53 mIU/mL and the tail end of the upper limit (97.5th percentile) between 4.23 and 4.88 mIU/mL. This peculiar distribution of serum TSH has generated a long debate to define normal values. Different authors have proposed higher limits for diagnosing thyroid disease (18, 22, 23). Our data does not support this proposal.

During the study, all the assays passed successfully evaluation by a peer group internal quality control program and by an external quality control system.

U has two components, uncertainty relative precision and uncertainty relative trueness. Uncertainty relative precision is the reproducibility within the laboratory, and is usually expressed as the mean of the percentage coefficient of variation determined during at least 6 months. Our assays were successfully validated by the internal quality control program for more than 6 months; even though in this period, different operators, reagent lots and calibrations were used. Uncertainty relative

trueness is linked to reproducibility between laboratories. We used the bias obtained from the Peer Group Internal Quality Control program. Bias was added as a component of the peer group coefficient of variation of the participating laboratories.

Defining uncertainty is a requirement of the ISO/IEC 15189, since this is essential information of every analytical platform. Uncertainty reflects assay performance, the higher the uncertainty the poorer the assay. It also incorporates the concept that a reported value is not an absolute truth but a range of values. This is particularly important in the extremes of the reference distribution. There is not an agreement if it is necessary to inform results as a range or just to include the uncertainty as additional information in analytical reports. We consider our U was acceptable for the four assays, because it was obtained from assays with acceptable performance according to CLSI norms.

RCV is a term that expresses the difference that has to be observed, such as a change of patient values, to consider it clinically important. It is not only depending on analytical causes. To define it, we used the equation described by Harris and Brown (13) which has two components, mean of analytical CV, obtained from an internal quality control, and coefficient of biological variability obtained from tables (Westgard home page: www.westgard.com). This is the first time that results of thyroid hormones and TSH are presented with this type of controls, for this analytical platform. This is useful in the analytical laboratory, not only to check on possible reasons for analytical differences and to control for pre- or post-analytical errors, but also as an alert indicator to confirm that a change is outside biological variations (24). Even though our results are only applicable to our analytical platform, variations are similar to that reported by other laboratories and they can be used as a general guide. In our assays, serum thyroid hormone % RCV was 34.69%, 28.31% and 33.98% for T3, T4, and fT4, respectively. The relatively high value of 58.60% for TSH % RCV is explained by the high biological variation of serum TSH (19.30%), and highlights the need to evaluate other thyroid hormones, and other parameters, when a TSH value is in the limits of the reference interval.

In conclusion, we have established reference interval for TSH, T3, T4, and fT4 in our population that will be useful for an appropriate interpretation of individual serum concentrations in children and adolescent patients with thyroid disorders and/or belonging to diagnosis-related groups. To consider U for thyroid hormones and TSH is useful in assessing reliability of measurements of analytical procedures in the laboratory, and to know RCV is useful to alert laboratory professionals and physicians that a clinical significant change has taken place in a particular patient test result.

Conflict of interest statement

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References

1. Vanderpump MP, Tunbridge WM. The epidemiology of thyroid disease. In: Bravermam LE, U Tiger RD, editors. Werner & Ingba's The thyroid: a fundamental and clinical text, 8th ed. Philadelphia: Lippincott-Raven Publishers, 2000:467–73.
2. Ladenson PW, Singer PA, Ain LB, Bagchi N, Bigos ST, Levy EG, et al. American Thyroid Association Guidelines for detection of thyroid dysfunction. Arch Inter Med 2000;160:1573–5.
3. Elmlinger MW, Kühnel W, Lambrecht H-G, Ranke MB. Reference intervals from birth to adulthood for serum thyroxine (T4), triiodothyronine (T3), free T3, thyroxine binding globulin (TBG) and thyrotropin (TSH). Clin Chem Lab Med 2001;39: 973–9.
4. Sapin R, Schlienger JL. Thyroxine (T4) and tri-iodothyronine (T3) determinations: techniques and value in the assessment of thyroid function. Ann Biol Clin (Paris) 2003;61:411–20.
5. Chan MK, Seiden-Long I, Aytekin M, Quinn F, Ravalico T, Ambruster D, et al. Canadian Laboratory Initiative on Pediatric Reference Interval Database (CALIPER): pediatric reference intervals for an integrated clinical chemistry and immunoassay analyzer, Abbott ARCHITECT ci8200. Clin Biochem 2009;42:885–91.
6. Soldin SJ, Cheng LL, Lam LY, Werner A, Le AD, Soldin OP. Comparison of FT4 with log TSH on the Abbott Architect ci8200: pediatric reference intervals for free thyroxine and thyroid-stimulating hormone. Clin Chim Acta 2010;411:250–2.
7. Maroto A, Boqué R, Riu J, Rius FX. Effect of non-significant proportional bias in final measurement uncertainty. Analyst 2003;128:373–8.
8. Eugene H, Toshio Y. On the calculation of a "Reference Change" for comparing two consecutive measurements. Clin Chem 1983;29:25–30.
9. 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 interpretation of results. Clin Chem 1988;32: 962–6.
10. Solberg HE. The IFCC recommendation on estimation of reference intervals. The RefVal program. Clin Chem Lab Med 2004;42:710–4.
11. Westgard JO. Design of internal quality control for reference value studies. Clin Chem Lab Med 2004;42:863–7.
12. Magnusson B, Naykki T, Hovind H, Krysell M. Handbook for calculation of measurement uncertainty in environmental laboratories, 2nd ed. Nordtest report. Espoo, Finland: Nordtest, 2003.
13. Queralto J, Boyd J, Harris E. On the calculation of reference change value, with examples from a long-term study. Clin Chem 1993;39:1398–403.
14. International Federation of Clinical Chemistry Scientific Committee, Clinical Section Expert Panel on Theory of Reference Values and International Committee for Standardization in Haematology. Part 5. Statistical treatment on theory of reference value: determinations of reference limits. J Clin Chem Clin Biochem 1987;25:645–56.
15. Zurakowski D, Di Canzio J, Majzoub J.A. Pediatric reference interval for serum thyroxine, triiodothyronine, thyrotropin, and frre thyroxine. Clin Chem 1999;45:1087–91.
16. Wiedemam G, Joentz-Mentzel L, Panse R. Establishment of reference intervals for thyrotropin, triiodothyronine, thyroxine and free thyroxine in neonates, infants, children and adolescents. Eur J Clin Chem Clin Biochem 1993;31:277–88.

17. Soldin SJ, Morales A, Albalos F, Lenherr, S, Rifai N. Pediatric reference intervals on the Abbott IMx for FSH, LH, TSH, T4, T3, free T3, T-uptake, IgE and ferritin. Clin Biochem 1995;28:603–6.
18. Lazar L, Ben-David Frumkin R, Battat E, Lebental Y, Philip M, Meyerovitch J. Natural history of thyroid function tests over 5 years in a large pediatric cohort. J Clin Endocrinol Metab 2009;94:1678–82.
19. Hollowell JG, Staehling NW, Flanders WD, Hannon H, 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;87:489–99.
20. Aoki Y, Belin RM, Clickner R, Jeffries R, Phillips L, Mahaffey KR. Serum TSH and total T4 in the United States Population and their association with participant characteristics: National Health and Nutrition Examination Survey (NHANES 1999–2002). Thyroid 2007;17:1211–23.
21. Surks MI, Goswami G, Daniels GH. Controversy in clinical endocrinology. The thyrotropin reference range should remain unchanged. J Clin Endocrinol Metab 2005;90: 5489–96.
22. Fatourechi V. Upper limit of normal serum thyroid-stimulating hormone: a moving and now an aging target? J Clin Endocrinol Metab 2007;92:4560–62.
23. Andersen S, Pedersen KM, Bruun NH, Laurberg P. Narrow individual variations in serum T4 and T3 in normal subjects: a clue to understanding of subclinical thyroid disease. J Clin Endocrinol Metab 2002;87:1068–72.
24. Andersen S, Bruun NH, Pedersen KM, Laurberg P. Biologic variation is important for interpretation of thyroid function tests. Thyroid 2003;11:1069–78.