Endocrine Journal 2013, **60** (6), 799-804

ORIGINAL

Determination of pediatric reference levels of FT3, FT4 and TSH measured with ECLusys kits

Kenji Iwaku[1)], Jaeduk Yoshimura Noh[1)], Akinobu Minagawa[2)], Yuka Kosuga[1)], Miho Suzuki[1)], Kenichi Sekiya[1)], Masako Matsumoto[1)], Hidemi Ohye[1)], Yo Kunii[1)], Ai Yoshihara[1)], Natsuko Watanabe[1)], Koji Mukasa[1)], Koichi Ito[1)] and Kunihiko Ito[1)]*

[1)] *Ito Hospital, Tokyo 150-8308, Japan*
[2)] *Internal Medicine, Saitama Medical University, Iruma-gun, Saitama 350-0495, Japan*

Abstract. Reference ranges for serum thyroid hormones free triiodothyronine (FT3), free thyroxine (FT4) and thyroid stimulating hormone (TSH) in children were set using the assay kits currently used in clinical settings. A total of 342 children (111 males and 231 females) who were negative for antithyroid antibodies (TgAb, TPOAb) and were found to have no abnormalities on ultrasonographic examination of the thyroid gland were divided into 6 age groups: 4-6 years (45 children), 7-8 years (40), 9-10 years (53), 11-12 years (65), 13-14 years (83), and 15 years (56) for the study. FT3, FT4 and TSH levels were determined by electrochemiluminescence immunoassay (ECLIA) (ECLusys FT3, FT4 and TSH).The reference range for FT3 (pg/mL) was 2.91-4.70 for the age group of 4-6 years, 3.10-5.10 for the age group of 7-8 years, 3.10-4.87 for the age group of 9-10 years, 2.78-4.90 for the age group of 11-12 years, 2.77-4.59 for the age group of 13-14 years, and 2.50-4.64 for the age group of 15 years . The reference range for FT4 (ng/dL) was 1.12-1.67, 1.07-1.61, 0.96-1.60, 1.02-1.52, 0.96-1.52, 0.95-1.53. The reference range for TSH (μU/mL) was 0.62-4.90, 0.53-5.16, 0.67-4.52, 0.62-3.36, 0.54-2.78, 0.32-3.00. Serum FT3, FT4 and TSH levels in children differ from those in adults. It is, therefore, of importance to perform evaluation of thyroid function in children using reference values appropriate for the chronological ages, because misdiagnosis of hypothyroidism or inappropriate secretion of TSH (SITSH) and oversight of mild subclinical hypothyroidism could occur if the diagnosis is made using reference values for adults.

Key words: Pediatric reference range, FT3, FT4, TSH

IT IS ESSENTIAL to establish reference ranges for pediatric age groups to diagnose abnormalities of thyroid function in children. In Japan, reference ranges for thyroid hormones free triiodothyronine (FT3) and free thyroxine (FT4) as determined by radioimmunoassay (RIA) and reference ranges for thyroid stimulating hormone (TSH) as determined by immunoradiometric assay (IRMA) have been reported [1, 2], even though they have been derived from assays of rather small sample sizes. Subsequently, a large-scale study was conducted in 1996 by a Pediatric Reference Range Study Group and reference ranges for pediatric age groups were reported [3]. More than one and half decades have elapsed since then, and reference ranges based on assays with currently used kits are being sought. However, it is difficult, for various reasons, to set reference ranges for the thyroid hormones. This report describes the setting of reference ranges for FT3, FT4 and TSH by us using samples collected from subjects diagnosed as having currently normal thyroids.

Submitted Oct. 30, 2012; Accepted Feb. 7, 2013 as EJ12-0390
Released online in J-STAGE as advance publication Apr. 6, 2013
Correspondence to: Kenji Iwaku, M.D., Ph.D., Ito Hospital, 4-3-6 Jingumae, Shibuya-ku, Tokyo 150-8308, Japan.
E-mail: k-iwaku@ito-hospital.jp
*Dr. Kunihiko Ito was deceased last autumn.

Subjects and Methods

Subjects

Of the 3210 children and adolescents aged up to 15 years old who were initially examined at the Ito Hospital between January 2003 and June 2012, 342 (111 males and 231 females) fulfilling the following criteria were enrolled in this study: absence of goiter palpable by a physician with rich clinical experience in the diagnosis/management of thyroid disorders; no thyroid nodules, including micronodules, and normal thyroid patterns, free of abnormalities such as thyroid enlargement, atrophy or abnormal echogenicity, as defined by the

Japan Society of Ultrasonics in Medicine, on ultrasound examination of the thyroid gland [4]; serologically negative for antithyroid antibodies (TgAb, TPOAb). Subjects with congenital anomalies of the thyroid gland or with a current/past history of disorders of the thyroid gland were excluded. The chief complaints at the initial visit consisted of: concern about thyroid disease in 143 subjects because of the presence of a thyroid disorder in a family member(s) in 70 subjects, and because of information gained from mass media or acquaintances despite the absence of a significant family history in 73 subjects; neck swelling pointed out by someone else, or detected at the time of a physical checkup or examination at another institution in 123 subjects; symptoms indicative of thyroid dysfunction in 51 subjects; referral from a previous clinic where the subject was found to show thyroid function values deviating from the reference ranges for adults in 13 subjects; discomfort in the neck in 4 subjects; symptom(s) of apparently other source than the thyroid in 8 subjects. Of the 342 subjects, 100 gave a family history of thyroid disorders in the parents, including chronic thyroiditis (24 cases), Basedow's disease, including remitted disease (66 cases), nodular goiter (9 cases, including 2 malignant cases), and diffuse simple goiter (1 case), while the parents of the remaining 242 subjects had no thyroid disorders. As for the age distribution, 9 subjects were 4 years old, 16 were 5 years old, 20 were 6 years old, 18 were 7 years old, and 22 were 8 years old. As subjects aged 4 to 8 years were rather few, they were further divided into two groups: the 4-6 years age group and the 7-8 years age group. Thus, the study population was divided into 6 age groups: 4-6 years (45 children), 7-8 years (40), 9-10 years (53), 11-12 years (65), 13-14 years (83), and 15 years (56). As for FT3, determination of which has been carried out with the presently used assay system since May 2004, the study population consisted of children in the 4-6 years (44 children), 7-8 years (40), 9-10 years (52), 11-12 years (64), 13-14 years (79), and 15 years (55) age group. Scattergrams of the subjects in the 6 age groups by the FT4 and TSH values were drawn up, where outliers were excluded by the rejection test. Eventually, this study was performed on a total of 324 children (104 males and 220 females) divided into 6 age groups, as follows: for FT3, 4-6 years (43 children), 7-8 years (39), 9-10 years (51), 11-12 years (61), 13-14 years (72), and 15 years (50); for FT4 and TSH, 4-6 years (43 children), 7-8 years (39), 9-10 years (52), 11-12 years (63), 13-14 years (76), and 15 years (51).

Methods

FT3, FT4 and TSH levels were determined by electrochemiluminescence immunoassay (ECLIA) using ECLusys FT3, FT4 and TSH (Roche Diagnostics GmbH, Mannheim, Germany). For measurements of TgAb and TPOAb, radioimmunoassay (RIA) was employed (TgAb Cosmic II and TPOAb Cosmic II; Cosmic Co., Tokyo, Japan) from January 2003 to May 2006. Reference values for these two parameters were set as follows: TgAb, ≤2.6 U/mL, TPOAb, ≤6.7 U/mL. Subsequently, they were determined by ECLIA using Roche ECLusys Anti-Tg and Anti-TPO (Roche Diagnostics GmbH, Mannheim, Germany), based on which the following reference values were set: TgAb, ≤40 IU/mL, TPOAb, ≤28 IU/mL. The reference values of the thyroid hormones for adults are: FT3, 2.2-4.3 pg/mL; FT4, 0.8-1.6 ng/dL; TSH, 0.2-4.5 μU/mL.

Statistical analysis

Statistical evaluation of the data was performed using the JMP, version 8.02 (SAS Institute Inc., Cary, NC). Reference values were set at 2.5-97.5%.

This study was conducted with the informed consent of the subjects and their families and with meticulous care taken to protect the confidentiality of individual subjects, in conformity with the declaration of Helsinki.

Results

With regard to the subjects background characteristics, there were no statistically significant differences in the distribution of the serum FT3, FT4 or TSH values in subjects with and without a family history of chronic thyroiditis in the parents (Table 1) (Wilcoxon/Kruskal-Wallis test).

The reference values of the thyroid parameters in the 6 age groups are presented in Table 2. All of FT3, FT4 and TSH exhibited a tendency to progressively decline with advancing age. For FT3, the upper limit of the reference range decreased with advancing age, with a peak upper limit in the 7-8 years age group and nadir lower limit in the 7-8 years and 9-10 years age group. For FT4 also, the upper limit of the reference range decreased with advancing age; the lower limit in the 7-8 years age group was lower than that in the 9-10 years age group, however, this parameter decreased with advancing age thereafter. The upper limit of the reference range for TSH decreased with advancing age, with the peak value in the 7-8 years age group, while

Table 1 Distribution of family history by parameters

family history	chronic thyroiditis		normal		*p*-value
	ranges	n	ranges	n	
FT3 (pg/mL)	2.50-4.60	24	2.60-4.83	228	0.6809
FT4 (ng/dL)	0.95-1.57	25	0.96-1.61	234	0.7034
TSH (μU/mL)	0.56-3.29	25	0.55-4.46	234	0.4499

Wilcoxon/Krukal-Wallis test

Assessment of the distribution of parameters in patients with and without a family history of chronic thyroiditis in the parents failed to reveal any statistically significant differences (Wilcoxon/Krukal-Wallis test).

Table 2 Reference range by age group

Age Group (years)	FT3 (pg/mL)	n	FT4 (ng/dL)	n	TSH (μIU/mL)	n
Adult	2.20-4.30		0.80-1.60		0.20-4.50	
4 - 6	2.91-4.70	43	1.12-1.67	43	0.62-4.90	43
7 - 8	3.10-5.10	39	1.07-1.61	39	0.53-5.16	39
9 - 10	3.10-4.87	51	0.96-1.60	52	0.67-4.52	52
11 - 12	2.78-4.90	61	1.02-1.52	63	0.62-3.36	63
13 - 14	2.77-4.59	72	0.96-1.52	76	0.54-2.78	76
15	2.50-4.64	50	0.95-1.53	51	0.32-3.00	51

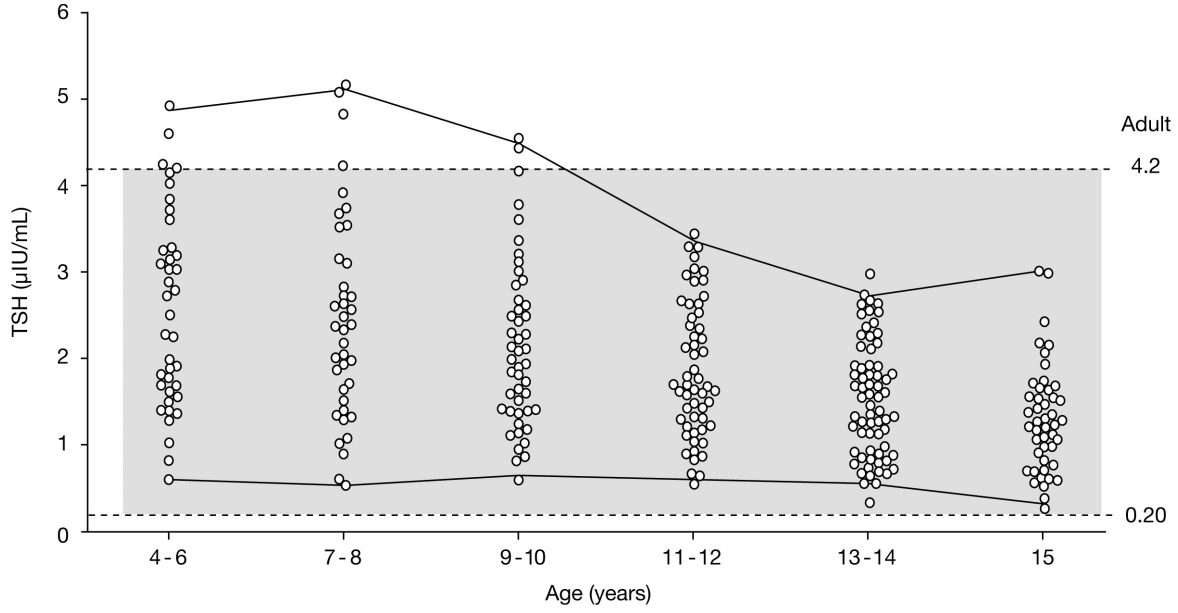

Fig. 1 Reference range of TSH in consecutive age groups
Both the upper and lower limits of reference range declined with advancing age. Significant inter-group differences were observed ($p \leq 0.0001$ by Wilcoxon/Krukal-Wallis test).

the lower limit also decreased with advancing age, with the peak value in the 9-10 years age group (Fig. 1). Pertinent data were tested for significant differences in individual parameters, and statistically significant differences were found at $p \leq 0.0001$ (Wilcoxon/Krukal-Wallis test) for all.

Discussion

We made it our principal objective to strictly define normal thyroids while setting the reference values for FT3, FT4 and TSH in children and adolescents. In the study reported herein, we based our definition of normal thyroids on the following comprehensive crite-

Table 3 Review of the reference ranges of the thyroid hormones in children reported from previous studies

Country	Cases	Sampling process	Sample	Examination Kit or analyzer system	First author Publication year	
Germany	656	Data base	FT3, FT4, TSH	Immulite 2000 (Siemens, Healthcare Diagnostics GmbH, Eschborn, Germany)	Verburg FA [5]	2011
Canada	1769	Data base TgAb (-) TPOAb (-)	FT4, TSH	Roche modular analytics E 170 (Roche Diagnostics GmbH, Mannheim, Germany)	Henderson MP [6]	2011
Northern Iran	243	euthyroid	T3, T4, TSH	ACS:180 system (Bayer Health Care, Leverkusen,Germany)[8]	Mansourian AR [7]	2010
India	5343	no goiter US normal TgAb (-) TPOAb (-)	T3, T4, TSH	IRMA (Immunotech, Beckman, Coulter)	Marwaha RK [9]	2010
USA	6023	Data base	FT4, TSH	Abbott Architect ci8200	Soldin SJ [10]	2010
USA	FT3 1107 FT4 1426	Data base	FT3, FT4	T3,T4:Sigma (St. Louis, MO). Deuterium-labeled T4 :IsoSciences (King of Prussia, PA)	Soldin OP [11]	2009
Austria	2194	Data base	FT3, FT4, TSH	Advia® Centaur™ (Bayer Health Care Diagnostika, Vienna, Austria)	Kapelari K [12]	2008
Canada	366	Data base	FT4, T3, TSH, Tg	Access 2 immunoassay system (Beckman-Coulter, Chaska, MN, USA)	Djemli A [13]	2004
Spain	371	Data base	FT3, FT4, TSH	Advia® Centaur™ (Bayer Health Care Diagnostika, Vienna, Austria)	García Cuartero B [14]	2003
Germany	460	Data base	FT3, FT4, TSH, T3, T4	Advia® Centaur™ (Bayer Health Care Diagnostika, Vienna, Austria)	Hübner U [15]	2002
Germany	762	Data base	FT3, FT4, TSH, T3, T4, TBG	Immulite® (DPC Los Angeles, USA)	Elmlinger MW [16]	2001
USA	5817	Data base	T3, T4, TSH, FT4	DELFIA immunofluorometric system according to the manufacturer's instructions (Wallac Oy)	Zurakowski D [17]	1999
Belgium	1050	Data base TSH normal	FT3	Amerlex MAB (Ortho Clinical Diagnostics, Amersham, UK; sold in Belgium by Orange, Brussels, Belgium)	Verheecke P [18]	1997
Japan	TSH 1425 FT3, FT4 778	Data base	FT3, FT4, TSH	TSH:TSH-RIABEAD II (Dinabot. Co) FT3, FT4 Amerlex-MAB kits (Ortho-Clinical Diagnostics CO)	Kawai T [3]	1996
USA	1137	Data base	FT3, FT4, TSH, T3, T4	Abbott IMx® (Abbott Laboratories,Abbott Park, IL)	Soldin SJ [19]	1995
Japan	203	normal children	FT3, FT4, T3, T4, rT3, TBG	FT3, FT4:Amersham plc, T3, rT3:Dainabot CO, LTD T4:Torabenoru CO, LTD, TBG:Hoechst AG	Y. Shiki [2]	1986

ria: absence of any congenital anomalies or underlying disorders, absence of palpable goiter as assessed by a physician with rich clinical experience in the clinical diagnosis/management of thyroid disorders, absence of abnormalities of the thyroid gland on ultrasonographic examination, and seronegativity for antithyroid antibodies (TgAb, TPOAb). Only subjects meeting all of these criteria were enrolled in this study.

Subjects whose parents had a current or previous history of chronic thyroiditis were judged as having currently normal thyroids if the above criteria were met, even though the possibility of the subject developing chronic thyroiditis at a future time could not be ruled out, since, as shown in Table 1, such subjects showed no statistically significant differences in the thyroid hormone levels in the present study. On the other hand, when reference ranges of thyroid hormones in children established in the past, shown in Table 3, were referred to, the reference ranges were found to be rather unreliable for the definition of normal thyroids, as compared with the criteria used in our study. The reference ranges of FT3, FT4 and TSH established in our study, even though the number of subjects studied was fewer, are considered to be more reliable [2, 5-8, 10-19].

We conducted this study using data accumulated over the past more than 9 years. The results indicated

that the reference values of FT3 and FT4 in children gradually declined with advancing age, thereby approximating the trend observed in adults. Concerning TSH, however, the upper limit of the reference range of TSH in children aged 11 years or older was lower than that in adults, although there was the same trend of decrease of the upper limit of the reference range with age as that seen for FT3 and FT4. As for this deviation of the upper limit of TSH from the upper limit of the reference range for adults, the results were similar to those for FT3 and FT4 showing a progressive decrease with advancing age, thereby approximating the reference range for adults, when viewed with reference to the true normal TSH value of <2.5 μU/mL from the third National Health and Nutrition Examination Survey (NHANES III) reported by Surks MI *et al*. [20]. The results of this study were reviewed in comparison with the most recently conducted large-scale study by the Pediatric Reference Range Study Group in Japan, showing that the upper limit of the reference range of FT3 declined with age after reaching its peak in male children around 11-12 years of age and female children at 9 years of age, the age at which the peak values were attained and which higher than those in our study. Furthermore, while the upper limit was higher and the lower limit was lower in all age groups as compared with those in the reference range for adults in the large-scale study, both the upper and lower limits were higher in all age groups in our study as compared with those in the reference range for adults. In regard to FT4, our results were generally consistent with those of the large-scale study, in that both the upper and lower limits decreased progressively with age, with the peak in male children at 6-8 years of age and female children at 6 years of age. As for TSH, both the upper and lower limits remained practically unchanged throughout all age groups of 4 years or older; the results are noticeably different from those of our study despite the subject age group is same. However, according to past overseas reports listed in Table 3, except for the study reported by Marwaha RK *et al*. [9], the reference range of TSH decreased more or less uniformly with advancing age, consistent with our finding. The results were comparable between iodine-sufficient regions and iodine-deficient regions. The following two reports of observations of changes with time in the same subjects, *i.e.*, a 5-year follow-up survey of serum TSH levels over time without treatment in Israel, an iodine-sufficient region, conducted by Lazar L *et al*. [21], and a 24-month follow-up of spontaneous progress in pediatric patients with idiopathic subclinical hypothyroidism in Italy, a modestly iodine-deficient region, conducted by Wasniewska M *et al*. [22], also demonstrated a decline of the serum TSH level with age. Thus, our finding of a decline of the serum TSH with chronological age is considered to be credible.

As described above, the serum FT3, FT4 and TSH levels in children differ from those in adults. It is, therefore, of importance to carry out evaluation of the thyroid function in children using reference values appropriate for the chronological ages, because misdiagnosis of hypothyroidism or SITSH, especially in children aged 10 years or younger, and oversight of mild subclinical hypothyroidism which could occur at an increased incidence among children aged 11 years or older could occur if the diagnosis is made using reference values for adults.

References

1. Sato H, Inomata H, Sasaki N, Niimi H, Nakajima H, *et al*. (1986) Measurement of serum TSH concentration by highly sensitive radioimmunometric assay in children. *Horumon To Rinsho* 34: 333-337 (in Japanese).
2. Shiki Y (1986) A study on the changes with age in thyroid function during infancy, childhood and adolescence. *Nihon Naibunpi Gakkai Zasshi* 62: 169-187 (in Japanese).
3. Kawai T (1996) Pediatric Standard Value Study Group (ed), Standard clinical test values for Japanese children. Japan Public Health Association, Tokyo, 375-378, 383-386, 391-394 (in Japanese).
4. The Japan Society of Ultrasonics in Medicine (2000) Current Textbook of Ultrasonics in Medicine Vol.4. Igakushoin, Tokyo: 348-366 (in Japanese).
5. Verburg FA, Kirchgässner C, Hebestreit H, Steigerwald U, Lentjes EG, *et al*. (2011) Reference ranges for analytes of thyroid function in children. *Horm Metab Res* 43: 422-426.
6. Henderson MP, Grey V (2011) Establishing and evaluating pediatric thyroid reference intervals on the Roche Modular Analytics E 170 using computational statistics and data-mining techniques. *Clin Biochem* 44:767-470.
7. Mansourian AR, Ahmadi AR, Saifi A, Bakhshandehnosrat S (2010) The children reference range of thyroid hormones in Northern Iran. *Pak J Biol Sci* 13: 862-865.

8. Moncayo R, Moncayo H, Virgolini I (2005) Reference values for thyrotropin. *Thyroid* 15: 1204-1205.
9. Marwaha RK, Tandon N, Desai AK, Kanwar R, Aggarwal R, *et al*. (2010) Reference range of thyroid hormones in healthy school-age children: country-wide data from India. *Clin Biochem* 43: 51-56.
10. Soldin SJ, Cheng LL, Lam LY, Werner A, Le AD, *et al*. (2010) Comparison of FT4 with log TSH on the Abbott Architect ci8200: Pediatric reference intervals for free thyroxine and thyroid-stimulating hormone. *Clin Chim Acta* 411: 250-252.
11. Soldin OP, Jang M, Guo T, Soldin SJ (2009) Pediatric reference intervals for free thyroxine and free triiodothyronine. *Thyroid* 19: 699-702.
12. Kapelari K, Kirchlechner C, Högler W, Schweitzer K, Virgolini I, *et al*. (2008) Pediatric reference intervals for thyroid hormone levels from birth to adulthood: a retrospective study. *BMC Endocr Disord* 8: 15.
13. Djemli A, Van Vliet G, Belgoudi J, Lambert M, Delvin EE (2004) Reference intervals for free thyroxine, total triiodothyronine, thyrotropin and thyroglobulin for Quebec newborns, children and teenagers. *Clin Biochem* 37: 328-330.
14. García Cuartero B, García Lacalle C, Jiménez Lobo C, Nebreda Pérez V, Calvo Rey C, *et al*. (2003) Values of thyrotropin, free triiodothyronine and free thyroxine using chemiluminescence in children and adolescents in the Autonomous Community of Madrid. *An Pediatr (Barc)* 58: 222-227.
15. Hübner U, Englisch C, Werkmann H, Butz H, Georgs T, *et al*. (2002) Continuous age-dependent reference ranges for thyroid hormones in neonates, infants, children and adolescents established using the ADVIA Centaur Analyzer. *Clin Chem Lab Med* 40: 1040-1047.
16. Elmlinger MW, Kühnel W, Lambrecht HG, Ranke MB (2001) Reference intervals from birth to adulthood for serum thyroxine (T4), triiodothyronine (T3), free T3, free T4, thyroxine binding globulin (TBG) and thyrotropin (TSH). *Clin Chem Lab Med* 39: 973-979.
17. Zurakowski D, Di Canzio J, Majzoub JA (1999) Pediatric reference intervals for serum thyroxine, triiodothyronine, thyrotropin, and free thyroxine. *Clin Chem* 45: 1087-1091.
18. Verheecke P (1997) Free triiodothyronine concentration in serum of 1050 euthyroid children is inversely related to their age. *Clin Chem* 43: 963-967.
19. Soldin SJ, Morales A, Albalos F, Lenherr S, Rifai N (1995) Pediatric reference ranges on the Abbott IMx for FSH, LH, prolactin, TSH, T4, T3, free T4, free T3, T-uptake, IgE, and ferritin. *Clin Biochem* 28: 603-606.
20. Surks MI, Goswami G, Daniels GH (2005) The thyrotropin reference range should remain unchanged. *J Clin Endocrinol Metab* 90: 5489-5496.
21. Lazar L, Frumkin RB, Battat E, Lebenthal Y, Phillip M, *et al*. (2009) Natural history of thyroid function tests over 5 years in a large pediatric cohort. *J Clin Endocrinol Metab* 94: 1678-1682.
22. Wasniewska M, Salerno M, Cassio A, Corrias A, Aversa T, *et al*. (2009) Prospective evaluation of the natural course of idiopathic subclinical hypothyroidism in childhood and adolescence. *Eur J Endocrinol* 160: 417-421.