Changes in Thyroid Function Across Adolescence: A Longitudinal Study

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Objective: There are no large, longitudinal studies of thyroid function across adolescence. The aims of this study were to examine longitudinal trends in thyrotropin (TSH), free triiodothyronine (fT3) and free thyroxine (fT4) and determine age-specific reference ranges.

Methods: Thyroid function was assessed in 3415 participants in the Brisbane Longitudinal Twin Study at ages 12, 14, and 16, using the Abbott ARCHITECT immunoassay. Longitudinal analyses were adjusted for body mass index and puberty.

Results: In girls, mean fT4 (± SE) increased between age 12 and 14 (by 0.30 ± 0.08 pmol/L; $P < 0.001$), while remaining unchanged in boys; from age 14 to 16, fT4 increased in both girls (by 0.42 ± 0.07 pmol/L; $P < 0.001$) and boys (0.64 ± 0.07 pmol/L, $P < 0.001$). There was a slight increase in fT3 from age 12 to 14 years in girls (by 0.07 ± 0.03 pmol/L; $P = 0.042$), with a more marked increase in boys (0.29 ± 0.03 pmol/L; $P < 0.001$), followed by a decrease from age 14 to 16 in both sexes (girls, by 0.53 ± 0.02 pmol/L; $P < 0.001$; boys, by 0.62 ± 0.03 pmol/L; $P < 0.001$). From age 12 to 14, TSH showed no significant change in girls or boys, then levels increased from age 14 to 16 in both sexes (in girls, by 4.9%, 95% CI: 2.4%-10.3%, $P = 0.020$; in boys, by 7.2%, 95% CI: 3.0%-11.6%, $P = 0.001$). Reference ranges differed substantially from adults, particularly for fT4 and fT3.

Conclusions: Thyroid function tests in adolescents display complex, sexually dimorphic patterns. Implementation of adolescence-specific reference ranges may be appropriate. **(*J Clin Endocrinol Metab* 105: e1162–e1170, 2020)**

Key Words: thyroid hormones; TSH; fT3; fT4; adolescents; reference ranges

Growth and development in childhood and adolescence are dependent on normal functioning of the thyroid gland (1), with thyroid hormones essential for physical and neurological development (2). The major hormone secreted by the thyroid is thyroxine (T4), which is converted to the active hormone

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Abbreviations: BLTS, Brisbane Longitudinal Twins Study; BMI, body mass index; DZ, dizygotic; fT3, free triiodothyronine; fT4, free thyroxine; IGF-1, insulin-like growth factor 1; MZ, monozygotic; T3, triiodothyronine; T4, thyroxine; TPO, thyroid peroxidase; TPOAb, thyroid peroxidase antibodies; TSH, thyrotropin (thyroid stimulating hormone).

triiodothyronine (T3) by deiodination in extrathyroidal tissues (3, 4); the thyroid also secretes T3 directly, accounting for about 20% of total T3 production in humans. In clinical laboratory practice, pituitary-thyroid axis function is evaluated by measuring circulating concentrations of thyrotropin (TSH), free thyroxine (fT4), and free triiodothyronine (fT3). There is a complex, nonlinear inverse relationship between circulating TSH and T4, such that small changes in T4 levels result in large changes in TSH secretion, making TSH the most sensitive marker of thyroid dysfunction (5, 6).

It is well established that thyroid function tests differ between adults and children, particularly in early life. Reference ranges for TSH, fT3, and fT4 in young children are much wider than in adults, gradually approaching adult values as childhood progresses (7, 8, 9). Although numerous studies have been performed in children, a recent review found marked inconsistencies in reported reference ranges for thyroid function tests, both across and within age ranges and assays (10). Further complexity is added by evidence that puberty influences pituitary-thyroid axis function (11, 12, 13, 14, 15), resulting in differences between adolescents and children in results of thyroid function tests. Almost all published studies of thyroid function in adolescents are cross-sectional, and published longitudinal studies in adolescents are limited by small sample size (fewer than 70 participants) (16, 17). Taylor et al (12) reported a longitudinal analysis of thyroid function in 884 individuals at age 7 and 15, and noted that further studies into pituitary-thyroid axis function in adolescents were required. To our knowledge, there are no previous large, longitudinal studies of thyroid function in adolescents across multiple time points.

The Brisbane Longitudinal Twins Study includes a large cohort of individuals studied at 12, 14, and 16 years of age. The aims of this study were to examine physiological changes in pituitary-thyroid axis function across adolescence and to compare laboratory reference ranges in adolescents with those in adults.

Methods

Participants

The Brisbane Longitudinal Twins Study (BLTS) includes a large cohort of monozygotic (MZ) and dizygotic (DZ) twins and triplets, non-twin siblings and parents from Brisbane in Queensland, Australia, a region known to have adequate iodine intake (18). Phenotype data were collected when the twins and triplets were approximately 12, 14, and 16 years old (19). Blood samples were collected in the morning, without fasting. In the present study, we included data from twins, triplets, and non-twin siblings at ages 11.9 to 13 years (median 12.1 years) at the first visit, 13.9 to 15 years (median age = 14.1) at the second visit, and 15.9 to 17 years (median age = 16.1) at the final visit. Participants were recruited by media appeals, by word of mouth, and by contacting primary schools in the greater Brisbane area. The study was approved by the Human Research Ethics committee of the Queensland Institute of Medical Research (P193, P455).

Laboratory measures

Plasma fT3, fT4, TSH, and thyroid peroxidase (TPO) antibodies (TPOAb), were measured during 2017–2018 by automated immunoassay using the Abbott ARCHITECT analyser (Abbott Diagnostic, Illinois) in frozen plasma samples taken from the visits at 12, 14, and/or 16 years. Adult reference ranges, derived by local consensus and based on local population-based studies (20, 21) and laboratory data, are: TSH 0.4 to 4.0 mIU/L, fT3 3.0 to 5.5 pmol/L, and fT4 9 to 19 pmol/L. In our laboratory, adult reference ranges for TSH and fT4 are applied to children aged 11 years or older; for fT3, ranges of 3.0 to 6.5 and 3.0 to 6.0 pmol/L are applied to children aged 11 to 15 years and 15 to 19 years respectively, based on published data for this immunoassay platform (22). TPOAb positivity is defined as TPOAb > 6 IU/mL (according to the manufacturer's recommendation) at all ages.

Phenotypic measures

Height (m) and weight (kg) were measured by clinical staff with body mass index (BMI) calculated as weight divided by height squared. Pubertal status was determined by questionnaire using the Pubertal Development Scale (23) and assessment by clinical staff. Participants were classified as prepubertal or postpubertal, based primarily on a history of menarche in girls and presence of secondary sexual characteristics in boys; detailed physical examination for Tanner pubertal staging was not performed.

Statistical analysis

As this study sought to characterise normative thyroid parameters, TPOAb-positive participants were excluded, as were those with plasma TSH, fT3, or fT4 concentrations greater than 4 SD from the mean, which appeared analytically suspect or discordant. Reference ranges were calculated at ages 12, 14, and 16 years (specifically 11.9–13, 13.9–15, and 15.9–17 years) as the 2.5th and 97.5th percentiles of the relevant distribution, and were compared with the corresponding adult reference range limits using a quantile test. TSH was log transformed prior to further analysis. Longitudinal analysis was performed using a linear mixed model adjusting for age, puberty, and BMI. A nested random effects structure with separate intercepts for each subject within zygosity group within family was used to account for relatedness (within MZ twins and triplets or among DZ twins and triplets and siblings more generally) and multiple observations per individual. A symmetric correlation structure between observations at the lowest level of nesting was assumed. Results are presented separately for males and females. Due to the absence of BMI values in 1.1% of records and puberty status in 5.5% of records, these covariate data were imputed for the longitudinal analysis. The linear mixed model was applied to 100 imputed data sets, and the results were combined according to Rubin's rules. The sensitivity of regression results to missingness assumptions with

respect to the unavailable covariate data was checked via sensitivity analysis. The feedback relationship between TSH and each of ft3 and ft4 was also evaluated, allowing for sex differences by the inclusion in the model of an interaction term. To examine longitudinal trends in thyroid function variables with age as a continuous variable, generalized additive models were fitted to data from participants who attended 2 or 3 study visits, without adjustment for covariates. All analyses were performed using R version 3.5.2 (including packages nlme, mgcv, mice, miceadds and snpar).

Results

Study population and baseline characteristics

The derivation of study participant numbers for each analysis is shown in Fig. 1. Of the 3001 participants with available thyroid function test results, 128 (4%) were TPOAb-positive, comprising 1.9%, 2.9%, and 3.3% of girls and 0.4%, 0.9%, and 1.1% of boys at age 12, 14, and 16 years respectively. Four individuals with TSH, fT3, or fT4 values that were > 4 SD from the mean and appeared discordant or analytically suspect were also excluded. Demographic data for participants are shown in Table 1.

Reference ranges for thyroid function in adolescents

Fig. 2 shows the distribution of TSH, fT3, and fT4 for TPOAb-negative participants at age 12, 14, and 16 years. Table 2 shows reference ranges derived for each age group, and for subgroups of boys and girls. For all 3 age groups, reference range limits for TSH, fT3, and fT4 differed significantly from those for adults ($P < 0.0001$), with the exception of the lower limit for TSH in 14-year-old girls, which did not differ significantly from the adult equivalent by quantile testing. For TSH, the reference range at each age was narrower than the adult reference range of 0.4 to 4.0 mIU/L, and differed somewhat by age and sex; for example, at age 14, the reference range was 0.5 to 3.0 mIU/L for girls and 0.6 to 3.3 mIU/L for boys. For fT3, reference ranges were quite different from the adult range of 3.0 to 5.5 pmol/L, with substantial differences by age and sex. In girls, the largest discrepancy with the adult range was at age 12, when the fT3 reference range was 4.5 to 6.3 pmol/L. In boys, the most marked difference from adults was at age 14, when the fT3 reference range was 4.7 to 6.6 pmol/L; indeed, the mean fT3 value of 5.64 pmol/L in 14-year-old boys exceeded the adult reference range. At age 16, fT3 reference ranges were closer to the adult range, particularly in girls. For fT4, reference ranges in adolescents were narrower than the adult range of 9.0 to 19.0 pmol/L and were fairly consistent by age and sex; for example, the reference range was 10.5 to 15.5 pmol/L for 14-year-old girls and 10.2 to 15.5 pmol/L for 14-year-old boys.

If adult reference ranges were applied to adolescents, the proportions misclassified would be relatively small for TSH and fT4 (approximately 5%), because of the narrower reference intervals in adolescents (Table 2). For fT3, however, application of the adult reference range would result in substantial misclassification, particularly at age 12 and 14; for example, 35% of 12-year-old girls and 58% of

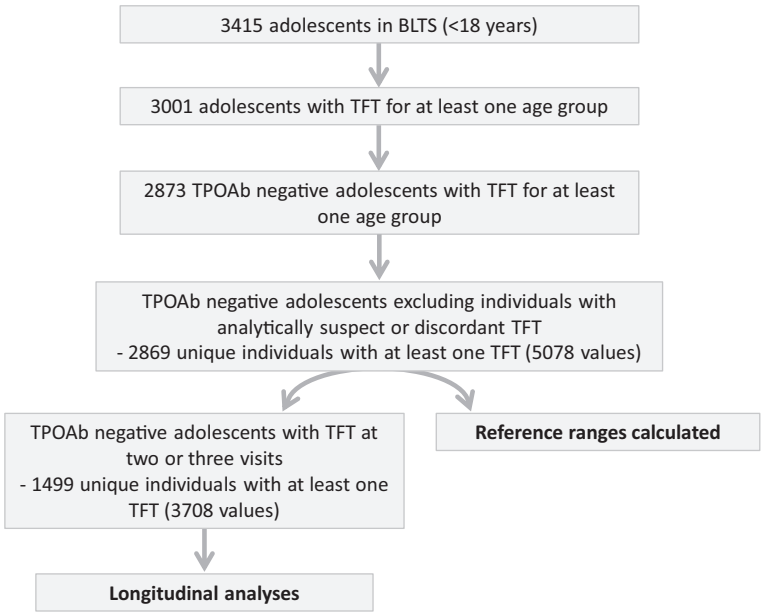

Figure 1: Chart showing derivation of study samples for each analysis. Abbreviations: TFT, thyroid function tests; TPOAb, thyroid peroxidase antibodies; BMI, body mass index.

Table 1. Demographics of Participants Included in Reference Range Analyses and Longitudinal Analysis: Data Shown for Study Visits at Age 12, 14, and 16 Years

		Female			Male		
Reference range sample	n	868	810	884	851	820	845
	Age (yr)	12.2 ± 0.2	14.2 ± 0.2	16.2 ± 0.2	12.2 ± 0.2	14.2 ± 0.2	16.2 ± 0.2
	Height (cm)	153.0 ± 7.5	161.8 ± 6.6	164.5 ± 6.4	151.3 ± 7.6	166.0 ± 8.5	175.3 ± 7.1
	Weight (kg)	44.8 ± 10.4	54.6 ± 10.8	59.7 ± 10.9	43.6 ± 10.4	57.4 ± 12.9	68.0 ± 13.7
	BMI (kg/m^2)	19.0 ± 3.5	20.8 ± 3.6	22.0 ± 3.6	18.9 ± 3.4	20.7 ± 3.7	22.0 ± 3.9
	Twin/triplet, n	858	741	807	843	748	777
	Sibling, n	10	69	77	8	72	68
	DZ, n	551	491	500	543	496	503
	MZ, n	307	250	307	300	252	274
	Prepubertal, n	639	118	7	713	235	5
	Postpubertal, n	201	684	625	121	575	494
	No puberty data	28	8	252	17	10	346
Longitudinal cohort	n	649	714	455	683	737	470
	Age (yr)	12.1 ± 0.2	14.2 ± 0.2	16.2 ± 0.2	12.1 ± 0.2	14.2 ± 0.2	16.2 ± 0.2
	Height (cm)	153.0 ± 7.7	161.9 ± 6.4	165.1 ± 6.3	151.2 ± 7.6	165.7 ± 8.4	175.6 ± 7.1
	Weight (kg)	44.3 ± 10.0	54.4 ± 10.8	59.7 ± 10.8	43.7 ± 10.3	56.9 ± 12.8	68.4 ± 13.3
	BMI (kg/m^2)†	18.8 ± 3.3	20.7 ± 3.6	21.9 ± 3.6	19.0 ± 3.3	20.6 ± 3.7	22.1 ± 3.8
	Twin/triplet, n	648	699	439	683	718	451
	Sibling, n	1	15	16	0	19	19
	DZ, n	422	459	308	440	471	322
	MZ, n	226	240	131	243	247	129
	Prepubertal, n†	501	104	4	575	217	5
	Postpubertal, n†	148	610	451	108	520	465

Values are presented as mean ± SD or count. Sibling refers to non-twin sibling.
Abbreviations: BMI, body mass index; DZ, dizygotic; MZ, monozygotic.
†Includes imputed data.

14-year-old boys would be misclassified as having elevated plasma fT3, when in fact their levels were normal for age and sex.

Longitudinal analysis of thyroid function across adolescence

Complete thyroid function data (TSH, fT3, fT4) were available for 1499 participants who attended 2 (N = 789) or 3 (N = 710) study visits. Missing covariate data, BMI information, and puberty status, were imputed for 41 and 204 records respectively. Missing puberty status data was associated with male 16-year-old adolescents more than any other subgroup. However, following sensitivity analysis, allowing for different proportions of postpubertal subjects in the missing subset, principal results were found not to vary from those reported. Fig. 3 shows a visualization of the data using generalized additive models fitted to data from twins, triplets, and non-twin siblings, which covered the full adolescent age range available (11.9–17 years), unadjusted for covariates.

Plasma TSH levels were significantly higher in boys than in girls, with median TSH being 17.7% higher (95% CI: 13.5%-22.0%, $P < 0.001$); this difference persisted after adjustment for age, puberty and BMI (17.2%, 95% CI: 13.0%-21.5%, $P < 0.001$). In girls, median TSH decreased between age 12 and 14 years by 10.0% (95% CI: –12.9% to –7.0%, $P = 0.002$), but after adjustment for puberty and BMI, the attenuated decrease of 4.1% was not significant (95% CI: –8.7% to 0.62%, $P = 0.088$). TSH in girls increased significantly between age 14 and 16 (unadjusted increase 6.2%, 95% CI: 2.4%-10.3%, $P = 0.001$; covariate-adjusted 4.9%, 95% CI: 2.4%-10.3%, $P = 0.020$). In boys, median TSH did not change significantly between 12 and 14 years, then it increased between age 14 and 16 years (unadjusted increase 9.1%, 95% CI: 5.2%-13.2%, $P < 0.001$; covariate-adjusted 7.2%, 95% CI: 3.0%-11.6%, $P = 0.001$).

Free T3 concentrations were consistently higher in boys than girls, with a mean difference of 0.39 ± 0.02 pmol/L ($P < 0.001$) (adjusted mean difference 0.38 ± 0.02 pmol/L; $P < 0.001$). Inspection of the plots in Fig. 3 suggested biphasic trajectories, particularly in boys. In girls, mean fT3 decreased by 0.27 ± 0.02 pmol/L ($P < 0.001$) between 12 and 14 years, but after adjustment for covariates a slight increase was noted (0.07 ± 0.03 pmol/L; $P = 0.042$). From 14 to 16 years, fT3 decreased by 0.54 ± 0.02 pmol/L ($P < 0.001$) in girls; this change persisted after covariate adjustment (0.53 ± 0.02 pmol/L; $P < 0.001$). In boys, fT3 increased from 12 to 14 years by 0.33 ± 0.02 pmol/L ($P < 0.001$), then decreased from age 14 to 16 years by 0.59 ± 0.03 pmol/L ($P < 0.001$). These shifts were

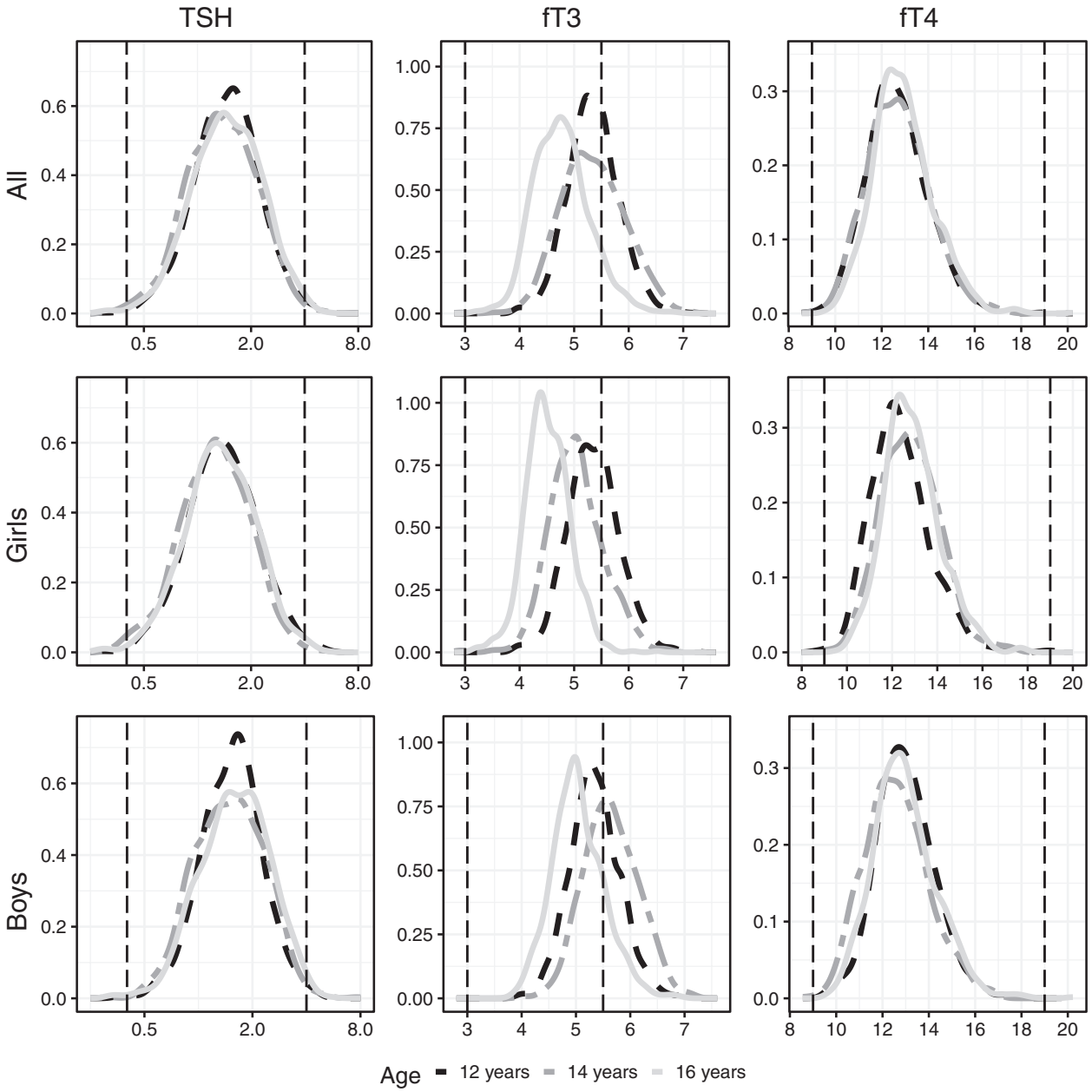

Figure 2: Density plots showing distribution of logTSH, fT3, and fT4 at age 12, 14, and 16 years. Vertical lines refer to adult reference range.

modified only slightly by covariate adjustment (12 to 14 years: 0.29 ± 0.03 pmol/L, $P < 0.001$; 14 to 16 years: −0.62 ± 0.03 pmol/L, $P < 0.001$). There was a positive relationship between logTSH and ft3 ($\beta = 0.11$; $P = 0.002$), which differed by sex, with evidence of a stronger relationship in females than males ($P_{interaction} < 0.001$; $\beta_F = 0.16$ vs $\beta_M = 0.059$).

Free T4 concentrations were slightly higher on average in boys than girls, with a mean difference of 0.21 ± 0.05 pmol/L ($P < 0.001$) (adjusted difference 0.20 ± 0.05 pmol/L; $P < 0.001$). In girls, mean fT4 increased by 0.50 ± 0.06 pmol/L ($P < 0.001$) between 12 and 14 years, which after adjustment for covariates moderated to 0.30 ± 0.08 pmol/L ($P < 0.001$). From 14 to 16 years, mean fT4 increased by a further 0.17 ± 0.07 pmol/L ($P = 0.011$); after covariate adjustment this change was more marked (0.42 ± 0.07 pmol/L; $P < 0.001$). In boys, fT4 decreased from 12 to 14 years by 0.26 ± 0.05 pmol/L ($P < 0.001$), then increased from age 14 to 16 years by 0.48 ± 0.06 pmol/L ($P < 0.001$). The 12 to 14 year shift was not significant after covariate adjustment (0.04 ± 0.07 pmol/L; $P = 0.603$), whereas the 14 to 16 year increase became more marked (0.64 ± 0.07 pmol/L; $P < 0.001$). There was a weak inverse relationship between logTSH and ft4 ($\beta = -0.017$; $P = 0.002$), which did not differ by sex ($P_{interaction} = 0.364$).

Table 2. Reference Ranges for TSH, fT3, and fT4 at Ages 12, 14, and 16

		Age, yr	N	Mean/ Median	2.5th-97.5th percentile	Misclassified by adult reference range	
						Misclassified as abnormal, %	Misclassified as normal, %
All	TSH (mIU/L)	11.9–13	1717	1.48	0.61–3.35	0	4.0
		13.9–15	1630	1.36	0.53–3.12	0	3.9
		15.9–17	1729	1.46	0.58–3.43	0	3.8
	fT3 (pmol/L)	11.9–13	1719	5.35	4.47–6.30	34.4	2.6
		13.9–15	1630	5.34	4.31–6.49	36.1	2.5
		15.9–17	1729	4.77	3.89–5.91	6.2	2.5
	fT4 (pmol/L)	11.9–13	1717	12.66	10.32–15.38	0	5.0
		13.9–15	1630	12.71	10.32–15.52	0	5.0
		15.9–17	1729	12.92	10.61–15.68	0	5.0
Girls	TSH (mIU/L)	11.9–13	867	1.37	0.57–3.30	0	3.8
		13.9–15	810	1.25	0.48–2.96	0	4.1
		15.9–17	884	1.35	0.54–3.26	0	4.1
	fT3 (pmol/L)	11.9–13	868	5.35	4.47–6.31	35.3	2.5
		13.9–15	810	5.04	4.13–6.09	14.2	2.3
		15.9–17	884	4.51	3.71–5.30	1.7	2.6
	fT4 (pmol/L)	11.9–13	868	12.35	10.23–15.14	0	5.1
		13.9–15	810	12.81	10.49–15.53	0	5.3
		15.9–17	884	12.88	10.64–15.60	0	5.2
Boys	TSH (mIU/L)	11.9–13	850	1.55	0.66–3.33	0	4.2
		13.9–15	820	1.50	0.63–3.31	0	4.4
		15.9–17	845	1.62	0.66–3.55	0	4.0
	fT3 (pmol/L)	11.9–13	851	5.35	4.48–6.30	33.7	2.6
		13.9–15	820	5.64	4.71–6.64	57.9	2.7
		15.9–17	845	5.04	4.18–6.05	14.1	2.6
	fT4 (pmol/L)	11.9–13	849	12.98	10.63–15.68	0	5.1
		13.9–15	820	12.61	10.18–15.51	0	5.1
		15.9–17	845	12.96	10.61–15.75	0	5.2

Median values shown for TSH, mean values shown for fT3 and fT4. For comparison, the reference ranges in adults are: TSH 0.4–4.0 mIU/L, fT3 3.0–5.5 pmol/L and fT4 9–19 pmol/L. *Misclassified as abnormal* refers to individuals with values within the age-specific reference range, but outside the adult reference range. *Misclassified as normal* refers to individuals with values outside the age-specific range, but within the adult reference range. Abbreviations: fT3, free triiodothyronine; fT4, free thyroxine; TSH, thyrotropin (thyroid stimulating hormone).

Discussion

In this, the first large longitudinal study of thyroid function of adolescents across multiple time-points, we demonstrate complex, dynamic patterns of pituitary-thyroid axis function. We found that fT4 increased from age 14 to 16 in both sexes. Free T3 concentrations were higher in adolescent boys than girls, with trajectories that appeared sexually dimorphic; in girls fT3 levels showed minimal change from age 12 to 14 then declined sharply to age 16, whereas in boys fT3 increased from age 12 to 14 then declined by age 16. TSH levels also differed by sex, being higher in boys, and increasing from 14 to 16 years in both sexes.

In the only previous well-powered, longitudinal study to include adolescents, participants were studied at age 7 and 15 years (12). In that study, fT3 was higher in girls than boys at age 7, then declined in girls but not in boys to become significantly lower in girls than boys at by age 15. This sex difference at age 15 is broadly consistent with our data, but the design of the previous study did not allow detection of the dynamic changes in fT3 between age 12 and 15. In this regard, our results are consistent with cross-sectional data from Gunapalasingham et al (9), who reported that fT3 declined from age 12 to 16 in girls, whereas in boys, fT3 increased from age 12 to 14, then fell from 14 to 16 years. Taylor et al (12) also reported that fT4 was higher in girls than boys at age 7, then declined significantly in girls but not boys to give similar levels at age 15. In our study, the fT4 curves for boys and girls cross between age 15 and 16, making a detailed comparison of the studies difficult, but the results are broadly consistent. With regard to TSH, Taylor et al (12) reported higher levels in boys than girls at age 7, which increased in both sexes to remain higher in boys at age 15 years, consistent with our data.

The physiological basis for the changes observed in fT4 and fT3 across adolescence is uncertain, but could arise from changes in activity in one or more of the iodothyronine deiodinase enzymes, which convert T4 to T3 and other metabolites, and also inactivate T3 (3). Estradiol increases the activity of type 3 but not type 2

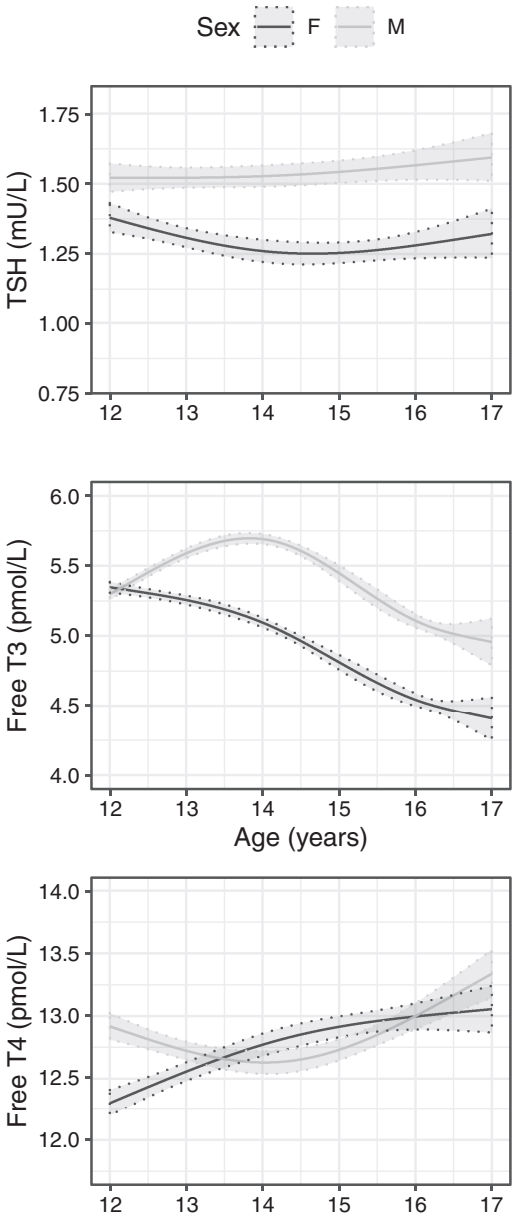

Figure 3: Longitudinal changes in TSH, fT3, and fT4 by age. Results are visualized using generalized additive models fitted to the unadjusted data. Mean values and 95% confidence bands are illustrated by solid and dotted lines respectively.

deiodinase in vitro (3, 24) and hepatic type 1 deiodinase activity shows sex differences in rats, being higher in gonad-intact (but not castrate) males than females (25). Growth hormone is also reported to affect deiodinase activity (3, 25, 26), and in animal studies sex steroids affect TSH secretion (27, 28). Therefore, the changes observed may reflect a complex interplay between the growth hormone-insulin-like growth factor 1 (IGF-1), hypothalamo-pituitary-gonadal and hypothalamo-pituitary-thyroid axes during pubertal development.

The present study, describing the physiological changes in pituitary-thyroid axis function which occur during adolescence, clarifies findings of previous cross-sectional studies of age-dependency of thyroid function tests, which gave inconsistent results (7, 10, 11, 29, 30, 31, 32, 33). The sex differences we found for TSH and fT3 in adolescents are consistent with most previous studies (6, 10, 30, 34), but not all (35, 36, 37, 38).

An important finding of our study was that reference ranges for thyroid function in adolescents differ substantially from those for adults. For TSH and fT4, the reference intervals were narrower than those for adults, and broadly similar in boys and girls. For fT3, however, reference ranges were substantially higher than those for adults, and showed marked differences across age groups (differing only by 2 years of age) and between males and females. As noted by Önsesveren et al (10), there is marked inconsistency in the literature regarding reference ranges for thyroid function in childhood and adolescence. Our data strengthen the case for the use of age- and sex-specific reference ranges for thyroid function tests in adolescents.

Strengths of this study include the large size of the cohort, with detailed assessment of thyroid function by TSH, fT3, and fT4 at 3 time points across adolescence, allowing both derivation of age- and sex-specific reference ranges, and longitudinal analysis to describe physiological changes in pituitary-thyroid axis function. Measurement of TPOAb allowed exclusion of individuals with evidence of thyroid autoimmunity. Our study also has limitations. Assessment of pubertal status was limited to prepubertal/postpubertal, and the data did not allow detailed assessment of relationships between pubertal stages and thyroid function, or associations with other parameters such as growth velocity, body composition, or circulating levels of IGF-1 or sex hormone levels. Samples were collected without participants fasting, and it is known that thyroid function tests are affected by fasting and feeding (39). Thyroid function tests were performed using a single platform (Abbott ARCHITECT), and reference intervals may differ from other immunoassay methods. Most of the participants were twins, but there is no evidence that pituitary-thyroid axis regulation differs between twins and singletons, and previous studies have shown that twins are representative of the general population for a range of phenotypes (40). Our study population was predominantly white and residing in an iodine-sufficient region, and the findings may not be fully applicable to other populations.

In conclusion, we report results of a longitudinal study demonstrating complex, dynamic and sexually dimorphic changes in thyroid function across adolescence. This advances understanding of hypothalamo-pituitary-thyroid axis physiology in this age group. The results suggest that close scrutiny of clinical results from adolescents may be warranted, and strengthens the

case for implementation of age- and sex-specific reference intervals. Further studies to elucidate the physiological mechanisms underlying the observed changes are indicated.

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Data Availability: The datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

References

1. Grüters A. Diagnostic tests of thyroid function in children and adolescents. In: Ranke MD, ed. *Diagnostics of endocrine function in children and adolescents*. Basel: Karger; 2003:93–106.
2. LaFranchi S. Disorders of the thyroid gland. In: Kliegman RM, ed. *Neslon textbook of Pediatrics*. 20th ed. Philadelphia: Elesvier; 2018.
3. Bianco AC, Dumitrescu A, Gereben B, et al. Paradigms of dynamic control of thyroid hormone signaling. *Endocr Rev*. 2019;**40**(4):1000–1047.
4. Taylor PN, Peeters R, Dayan CM. Genetic abnormalities in thyroid hormone deiodinases. *Curr Opin Endocrinol Diabetes Obes*. 2015;**22**(5):402–406.
5. Brown SJ, Bremner AP, Hadlow NC, et al. The log TSH-free T4 relationship in a community-based cohort is nonlinear and is influenced by age, smoking and thyroid peroxidase antibody status. *Clin Endocrinol (Oxf)*. 2016;**85**(5):789–796.
6. Hadlow NC, Rothacker KM, Wardrop R, Brown SJ, Lim EM, Walsh JP. The relationship between TSH and free T_4 in a large population is complex and nonlinear and differs by age and sex. *J Clin Endocrinol Metab*. 2013;**98**(7):2936–2943.
7. Verburg FA, Kirchgässner C, Hebestreit H, et al. Reference ranges for analytes of thyroid function in children. *Horm Metab Res*. 2011;**43**(6):422–426.
8. Lazarus J, Brown RS, Daumerie C, Hubalewska-Dydejczyk A, Negro R, Vaidya B. 2014 European thyroid association guidelines for the management of subclinical hypothyroidism in pregnancy and in children. *Eur Thyroid J*. 2014;**3**(2):76–94.
9. Gunapalasingham G, Frithioff-Bøjsøe C, Lund MAV, et al. Reference values for fasting serum concentrations of thyroid-stimulating hormone and thyroid hormones in healthy Danish/North-European white children and adolescents. *Scand J Clin Lab Invest*. 2019;**79**(1-2):129–135.
10. Önsesveren I, Barjaktarovic M, Chaker L, et al. Childhood thyroid function reference ranges and determinants: a literature overview and a prospective cohort study. *Thyroid*. 2017;**27**(11):1360–1369.
11. Kratzsch J, Schubert G, Pulzer F, et al. Reference intervals for TSH and thyroid hormones are mainly affected by age, body mass index and number of blood leucocytes, but hardly by gender and thyroid autoantibodies during the first decades of life. *Clin Biochem*. 2008;**41**(13):1091–1098.
12. Taylor PN, Sayers A, Okosieme O, et al. Maturation in serum thyroid function parameters over childhood and puberty: results of a longitudinal study. *J Clin Endocrinol Metab*. 2017;**102**(7):2508–2515.
13. Marwaha RK, Tandon N, Desai AK, et al. The evolution of thyroid function with puberty. *Clin Endocrinol (Oxf)*. 2012;**76**(6):899–904.
14. Radicioni AF, Tahani N, Spaziani M, et al. Reference ranges for thyroid hormones in normal Italian children and adolescents and overweight adolescents. *J Endocrinol Invest*. 2013;**36**(5):326–330.
15. Giannakopoulos A, Lazopoulou N, Pervanidou P, Kanaka-Gantenbein C. The impact of adiposity and puberty on thyroid function in children and adolescents. *Child Obes*. 2019;**15**(6):411–415.
16. Dunger DB, Perkins JA, Jowett TP, et al. A longitudinal study of total and free thyroid hormones and thyroxine binding globulin during normal puberty. *Acta Endocrinol (Copenh)*. 1990;**123**(3):305–310.
17. Schulte S, Gohlke B, Schreiner F, et al. Thyroid function in monozygotic twins with intra-twin birth weight differences: a prospective longitudinal cohort study. *J Pediatr*. 2019;**211**:164–171.e4.
18. Li M, Eastman CJ, Waite KV, et al. Are Australian children iodine deficient? Results of the Australian National Iodine Nutrition Study. *Med J Aust*. 2006;**184**(4):165–169.
19. Wright MJ, Martin NG. Brisbane adolescent twin study: outline of study methods and research projects. *Aust J Psychol*. 2004;**56**:65–78.
20. O'Leary PC, Feddema PH, Michelangeli VP, et al. Investigations of thyroid hormones and antibodies based on a community health survey: the Busselton thyroid study. *Clin Endocrinol (Oxf)*. 2006;**64**(1):97–104.
21. Panicker V, Wilson SG, Walsh JP, et al. A locus on chromosome 1p36 is associated with thyrotropin and thyroid function as identified by genome-wide association study. *Am J Hum Genet*. 2010;**87**(3):430–435.
22. Chan MK, Seiden-Long I, Aytekin M, 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**(9):885–891.
23. Petersen AC, Crockett L, Richards M, Boxer A. A self-report measure of pubertal status: reliability, validity, and initial norms. *J Youth Adolesc*. 1988;**17**(2):117–133.
24. Larsen PR, Zavacki AM. The role of the iodothyronine deiodinases in the physiology and pathophysiology of thyroid hormone action. *Eur Thyroid J*. 2012;**1**(4):232–242.
25. Bianco AC, Salvatore D, Gereben B, Berry MJ, Larsen PR. Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. *Endocr Rev*. 2002;**23**(1):38–89.
26. Glynn N, Kenny H, Quisenberry L, et al. The effect of growth hormone replacement on the thyroid axis in patients with

hypopituitarism: in vivo and ex vivo studies. *Clin Endocrinol (Oxf)*. 2017;**86**(5):747–754.
27. Christianson D, Roti E, Vagenakis AG, Braverman LE. The sex-related difference in serum thyrotropin concentration is androgen mediated. *Endocrinology*. 1981;**108**(2):529–535.
28. Donda A, Reymond F, Rey F, Lemarchand-Béraud T. Sex steroids modulate the pituitary parameters involved in the regulation of TSH secretion in the rat. *Acta Endocrinol (Copenh)*. 1990;**122**(5):577–584.
29. Cioffi M, Gazzerro P, Vietri MT, et al. Serum concentration of free T3, free T4 and TSH in healthy children. *J Pediatr Endocrinol Metab*. 2001;**14**(9):1635–1639.
30. Kapelari K, Kirchlechner C, Högler W, Schweitzer K, Virgolini I, Moncayo R. Pediatric reference intervals for thyroid hormone levels from birth to adulthood: a retrospective study. *BMC Endocr Disord*. 2008;**8**:15.
31. Boucai L, Hollowell JG, Surks MI. An approach for development of age-, gender-, and ethnicity-specific thyrotropin reference limits. *Thyroid*. 2011;**21**(1):5–11.
32. Kahapola-Arachchige KM, Hadlow N, Wardrop R, Lim EM, Walsh JP. Age-specific TSH reference ranges have minimal impact on the diagnosis of thyroid dysfunction. *Clin Endocrinol (Oxf)*. 2012;**77**(5):773–779.
33. Ehrenkranz J, Bach PR, Snow GL, et al. Circadian and circannual rhythms in thyroid hormones: determining the TSH and Free T4 reference intervals based upon time of day, Age, and Sex. *Thyroid*. 2015;**25**(8):954–961.
34. Päkkilä F, Männistö T, Surcel HM, et al. Maternal thyroid dysfunction during pregnancy and thyroid function of her child in adolescence. *J Clin Endocrinol Metab*. 2013;**98**(3):965–972.
35. Elmlinger MW, Kühnel W, Lambrecht HG, Ranke MB. 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*. 2001;**39**(10):973–979.
36. Djemli A, Van Vliet G, Belgoudi J, Lambert M, Delvin EE. Reference intervals for free thyroxine, total triiodothyronine, thyrotropin and thyroglobulin for Quebec newborns, children and teenagers. *Clin Biochem*. 2004;**37**(4):328–330.
37. Lim HH. Korean pediatric reference intervals for FT4, TSH, and TPO Ab and the prevalence of thyroid dysfunction: a population-based study. *Clin Biochem*. 2017;**50**(18):1256–1259.
38. Cui T, Wang W, Chen W, et al. Serum iodine is correlated with iodine intake and thyroid function in school-age children from a sufficient-to-excessive iodine intake area. *J Nutr*. 2019;**149**(6):1012–1018.
39. Basolo A, Begaye B, Hollstein T, et al. Effects of short-term fasting and different overfeeding diets on thyroid hormones in healthy humans. *Thyroid*. 2019;**29**(9):1209–1219.
40. Andrew T, Hart DJ, Snieder H, de Lange M, Spector TD, MacGregor AJ. Are twins and singletons comparable? A study of disease-related and lifestyle characteristics in adult women. *Twin Res*. 2001;**4**(6):464–477.