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Nationwide Chinese study for establishing reference intervals for thyroid hormones and related tests

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ABSTRACT

Objective: This nationwide study aimed to establish Chinese specific reference intervals (RIs) for thyroid related tests: thyroid stimulating hormone (TSH), free thyroxine (FT4), free triiodothyronine (FT3), total thyroxine (TT4), total triiodothyronine (TT3), anti-thyroid peroxidase (TPO-Ab) and anti-thyroglobulin antibodies (TG-Ab).

Method: Apparently healthy individuals (n = 2380) were recruited from Beijing, Guizhou, Urumqi, Dongying, Shenzhen, and Qiqihar in China. All the tests were measured by immunoassay testing. Ultrasonography was performed to exclude thyroid nodules. Multiple regression analysis was performed to identify sources of variation (SVs). Standard deviation ratio (SDR) was calculated by ANOVA for judging the need to partition RI by any given SV. RIs were computed by the parametric method.

Results: TPO-Ab and TG-Ab cutoffs were determined as 5 mIU/L and 2 IU/L, respectively using probability plot analysis and extrapolation of the central linear segment. Individuals with thyroid nodule and/or autoantibodies showed altered levels of thyroid hormones, and were thus excluded (n reduced to 1828). Gender difference was observed for FT3 and TT3 with females having lower levels than males. A significant relationship between age and FT3 was observed in males by Spearman correlation analysis ($r = -0.231$, $p < .05$). Although the SDR for gender difference in TSH levels was low, the difference in the upper limits of the RI was beyond allowable bias, and thus RIs (mIU/L) were partitioned by sex: females 0.72–5.50, males 0.70–4.59.

Conclusion: By this nationwide study, RIs for thyroid hormones and cutoff values for anti-thyroid autoantibodies were established as matched to the Chinese population.

Abbreviations: AIT, autoimmune thyroiditis; ALB, albumin; ALT, Alanine aminotransferase; ANOVA, analysis of variance; BMI, body mass index; CI, confidence interval; CLSI, Clinical and Laboratory Standards Institute; Cr, creatinine; DBP, Diastolic blood pressure; ELAS, Italian Section of the European Ligand Assay Society; FT3, free triiodothyronine; FT4, free thyroxine; Glu, glucose; HDL-C, high density lipoprotein cholesterol; IFCC, International Federation of Clinical Chemistry and Laboratory Medicine; IRP, International Reference Preparation; IS, International Standard; LDL-C, low density lipoprotein cholesterol; MRA, multiple regression analysis; RI, reference interval; SBP, Systolic blood pressure; SV, source of variation; TC, total cholesterol; TG, triglyceride; TG-Ab, Thyroglobulin antibody; TH, thyroid hormone; TPO-Ab, thyroid peroxidase antibody; TSH, thyroid stimulating hormone; TT3, total triiodothyronine; TT4, total thyroxine; UA, urea

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1. Introduction

Thyroid disease, especially primary subclinical thyroid disease is the most common endocrine disorders encountered and managed by primary care providers [1,2]. However, the symptoms of primary subclinical thyroid disease are non-specific and highly prevalent in the population [3]. Thyroid hormones (THs), especially thyroid stimulating hormone (TSH), play a critical role in regulating biological processes such as growth and metabolism in humans. The measurements of THs depends on immunoassay; ensuring assay-specific harmonization between different manufacturers is difficult. Paramount to the goal of a common reference interval (RI) is the establishment of metrological traceability of in vitro diagnostic medical devices − also called standardization. Recently, the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) Committee for Standardization of Thyroid Function Tests developed a global harmonization approach for TSH, which reported that after using "the commutability calibration traceability", the harmonization increased the agreement of results from the participating immunoassays and may allow them to adopt a more uniform RI in the future. Recent promising finding showed that no between-country difference was found in reference values of TSH in the interim report of the global RI study conducted by IFCC Committee on Reference Intervals and Decision Limits (C-RIDL), after alignment of values based on test results of a commonly measured serum panel [4].

Previous reports indicate that serum TSH concentration changes by factors such as age, sex, and ethnicity [5–11]. Tozzoli et al. [12] found that the serum TSH levels progressively decreased in male and female age subgroups 0–4 to 85–104 years in the overall population, and a negative correlation was found between serum TSH and age in all groups. However, another recent study reported that the serum TSH level increases with age, which may imply a normal compensatory phenomenon in older adults aged ≥65 years [8]. There is also much debate about the impact of sex on TSH concentrations. Some studies found that females had higher TSH concentrations than males [5,6,13]. However, other studies found the opposite to be true [9]. Furthermore, our previous studies using clinical big data showed the effect of seasonal and temperature fluctuations on TSH [5,6], which was consistent with findings of other studies [14,15].

Reliable immunoassay methods for THs measurement have great clinical relevance for the detection of subclinical stages of thyroid disease [16]. Beckman Coulter recently reformulated their commercial TSH assay, and the modification of this assay was calibrated to the World Health Organization 3rd TSH International Standard (IS, International Reference Preparation [IRP] 81/565). Recent studies reported that the 3rd TSH assay by UniCel DXI 800 was precise, highly sensitive, comparable to the previous generation assay, and acceptable for clinical testing [17,18].

Dittadi et al. [19] conducted a multicenter study to evaluate the new immunoassay method for 3rd TSH measurement using the automated DXI platform and found some relevant improvements compared to the previous one because of the use of the most recent WHO 3rd IRP 81/563 standard and monoclonal antibodies (instead of polyclonal antibodies of the old method), and better analytical sensitivities and reproducibility. It is common knowledge that measurements from different manufacturers are not yet comparable; the RI that is established for each assay are considered assay-specific. Barth et al. [20] demonstrated clearly that there are marked variations in the RIs for thyroid hormones between analytical platforms. Furthermore, measurement of TSH on Beckman Coulter DXI 800 was widely performed in China and even worldwide [20]. However, available RIs for THs were established based only on Western population. Thus, there is need to establish TSH and other thyroid hormones (FT4, FT3, TT4, TT3) RIs in Chinese population.

Accordingly, this study was carried out by applying the up-to-date protocol [21] to obtain RIs of TSH and other thyroid hormones on Beckman Coulter UniCel DXI 800 using a direct sampling method based on a nationwide multicenter study.

2. Materials and methods

2.1. Study population

The study population was recruited from January to November 2017 and July to November 2018 from six representative cities in China (Beijing, Guizhou, Urumqi, Dongying, Shenzhen, and Qiqihar). In the multicenter program, a total of 2380 healthy participants were recruited from the north, east, west, south, and middle regions of the six cities in China. Samples were collected from the participants in the six cities. All participants were informed in writing of the intended use of the samples, and each provided written informed consent.

2.2. Data collection and physical examination

Data, including demographic characteristics and medical history, were collected from a representative sample of the study participants via a standard questionnaire [21]. Body weight was measured on a calibrated beam scale and height was measured. Body mass index (BMI) was calculated as body weight divided by the square of the height (kg/m^2). Blood pressure (BP) was measured after the participant rested quietly for at least 20 min. All participants underwent thyroid ultrasonography examination performed by trained technicians supervised by two experienced physicians.

2.3. Laboratory measurement

Each center was required to collect and store blood samples. We used blood collection tubes containing separation gel to collect blood samples, which were centrifuged within 30 min after the collection. Serum was separated and stored at −80 °C for up to a month before measurements in batches. The leading center performed the centralized tests including serum thyroid function tests (TSH, TT4, TT3, FT4, FT3, TPO-Ab and TG-Ab). Blood collection under the fasted state was performed for all participants between 7:00–12:00 in the morning. Participants were required to sit for > 20 min before the blood collection [22]. Following an overnight fast, blood was drawn from the antecubital vein. Blood specimens were centrifuged at 3000 rpm/min for 10 min. All samples were sent to the laboratory and stored at −80 °C until testing. All thyroid hormones were measured using an automated chemiluminescence immunoassay analyzer (Beckman Coulter UniCel DXI 800, Beckman Coulter; Brea, CA, USA) with corresponding reagents and calibrations. The levels of creatinine (Cr), urea (UA), fasting blood glucose (FBG), total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL−C), low-density lipoprotein cholesterol (LDL-C), alanine aminotransferase (ALT), and albumin (ALB) were measured using an automated chemistry analyzer (Beckman Coulter AU5800, Beckman Coulter; Brea, CA, USA).

Serum TSH, FT3, FT4, TT3, TT4, TPO-Ab, and TG-Ab concentrations were measured by the immunoassay method using an automated analyzer (Beckman Coulter DXI 800, Beckman Coulter; Brea, CA, USA). According to CLSI EP15-A3, the assay precision of THs and antibodies were evaluated using three levels of quality controls (Lyphochek® Immunoassay Plus Control); a total of four replicates of each quality control were measured for 5 consecutive days. Finally, the repeatability (CV%) and within laboratory precision (CV%) were lower than 10%. Before measuring the blood concentrations of the hormones, quality control materials were measured to ensure that they were within allowable limits.

2.4. Statistical analysis

Multiple regression analysis (MRA) was performed to identify the sources of variation (SVs) possibly affecting the THs levels, including

sex, age, city, and BMI. In the analysis of between-city differences using dummy variables, Beijing was set as the reference city. A given explanatory variable was considered to be of practical importance when its standardized partial regression coefficient (r_p) was > 0.15, which corresponds to $P < .0001$ with a large sample size of around 1500.

When MRA found notable SVs, one-way or 2 to 3 level nested ANOVA was performed to calculate the standard deviation (SD) of reference values attributable to SVs. The relative magnitude of each SV-specific SD (SD_{SV}) was expressed as SD ratio (SDR) or its ratio to between-individual SD (SD_G) that corresponds to SD comprising the RI (SD_{RI}): $SD_{RI} = (UL - LL)/3.92$, where LL and UL denote lower and upper limit of the RI.

Therefore, $SDR_{SV} = SD_{SV}/SD_{RI}$. We used $SDR_{SV} > 0.3$ as a primary guide to consider partitioning reference values (RVs) by a given SV [23]. However, SDR is sometimes insensitive to the actual difference in UL (or LL) after partitioning because SDR represents central tendency of variation by the SV, not necessarily of variation in the periphery. For example, when SDRsex < 0.3, the centers of males and females do not differ much, but ULs of males and females (UL_M, UL_F) may be appreciably different. Therefore, we set an additional criterion of "bias ratio" to cope with the problem.

Bias ratio of UL (BiasUL) = $|UL_M - UL_F| / \{(UL_{MF} - LL_{MF})/3.92\}$.

In accordance with the convention of allowable bias specification: $0.375 \times SD_G$ ($= SD_{RI}$), we regarded Bias UL > 0.375 as an auxiliary criterion for partitioning RVs by sex or age when SDR does not match to actual between-subgroup difference in ULs (or LLs).

For MRA and ANOVA, values of TSH, TPO-Ab, TG-Ab were logarithmically transformed before the analyses. RIs were calculated by parametric method with Gaussian transformation of RVs using modified Box-Cox formula [22]. The 90% confidence intervals (CI) of the LLs and ULs of RIs were predicted by the use of the bootstrap method through random resampling of the same dataset 50 times. The final LL and UL were smoothed by adopting the averages of repetitively derived LLs and ULs, respectively.

Basic characteristics of enrolled individuals was calculated by SPSS 20.0 software (IBM Inc., Armonk, NY, USA). Multiple regression analysis (MRA) and one-way and nested analysis of variance (ANOVA) were performed using StatFlex for Windows Version 7.0 (Artech, Osaka, Japan).

3. Results

3.1. Association of thyroid nodules and autoimmune thyroiditis (AIT) with abnormality in thyroid hormones

The distribution of thyroid hormones concentrations in relation to thyroid nodule detected by ultrasonography are shown in **Supplemental Fig. 1.** Mann-Whitney *U* test after partitioning of values by sex showed that subjects with thyroid nodules had significantly different serum concentrations of TSH in both males ($P = .008$) and females ($P = .02$), FT3 in females ($P < .0001$), and TT4 in males ($P = .002$).

Probability paper plot was used for determining cutoff values for TPO-Ab and TG-Ab by use of the following scheme. Logarithmically transformed values for the antibodies were plotted on the abscissa and cumulative frequency (0–100%) were plotted on the ordinate. Under the assumption that autoimmune thyroiditis (AIT) is the only pathological condition that causes elevation of the values, the linear segment of the cumulative curve, which corresponds to subjects without AIT, was extended upward and its intersection with cumulative frequency of 97.5% was read on the abscissa as a cutoff value. As shown in Fig. 1, the cutoff value was accordingly set to 5 mIU/L for TPO-Ab and 2 IU/L for TG-Ab. The prevalence of individuals with AIT (positive for either TPO-Ab or TG-Ab) were 8.3% (87/1038) in males and 17.7% (189/1067) in females. Serum TSH in subjects with AIT were significantly higher in both males ($P = .0021$) and females ($P < .0001$), Serum FT3 was slightly higher only in males with AIT ($P = .037$). FT4, TT4, and TT3 were not different with/without AIT (**Supplemental Fig. 2**).

According to these findings, individuals with thyroid nodules or AIT were excluded, and thus, a total of 1828 individuals were enrolled for the subsequent analyses.

3.2. Basic characteristics of enrolled individuals

The basic characteristics are shown in Table 1. The average age and BMI were 40.0 (22.0–71.0) years and 23.1 (17.0–29.9) kg/m2, respectively.

3.3. Sources of variation of RIs of thyroid hormones

The r_p values of the major SVs for FT3 were − 0.190 for sex and for city, 0.241 (Guizhou) and 0.147 (Dongying). Guizhou had significantly higher TT3 values than other cities ($r_p = 0.165$). BMI showed no significant association with any THs. For TSH, FT4, and TT4, there were no notable association of values with city, age, and sex (Table 2, **Supplemental Fig. 3**, and **Supplemental Fig. 4**). In order to ensure the magnitude of SVs identified by MRA, three-level nested ANOVA were performed. SDR_{sex} for FT3 was 0.393 while for others, this was lower than 0.3. SDR_{age} for all THs was lower than 0.3. A significant relationship between age and FT3 was observed in males by Spearman correlation analysis ($r = -0.231$, $p < .05$). SDR_{city} for FT3 was 0.308 and for others were lower than 0.3 (Table 3**, Supplemental Fig. 5**).

3.4. Reference interval of thyroid hormones for Chinese population

Comparison of RIs calculated by parametric and nonparametric methods are shown in **Supplemental Fig. 6**. We chose the parametric method in general because nonparametric method generally give a wider 90%CI for the RI limits and sometimes the upper limit was shifted to the higher side compared to those by the parametric method. According to the above analytical results, we chose to establish RIs for TT4, TT3, and FT4 without partitioning by sex nor age. For TSH, SDR_{sex} was < 0.3 (Table 4), but BiasUL (bias ratio of Uls) was 0.67. Thus, we considered it necessary to establish sex-specific RI of TSH. SDRcity for FT3 was above 0.3, therefore, we computed the RI for each city as shown in Supplemental Table 1.

4. Discussion

Reliable RIs for THs are important in the evaluation of thyroid function and for monitoring thyroid disease. THs measurements are assay-specific; thus, different detection methods or reagents might lead to differences in THs results. Recently, Beckman Coulter reformulated their commercial TSH assay, and the modification of this assay was primarily calibrated to the WHO 3rd TSH IS 81/565. In China, many clinical laboratories use Beckman Coulter TSH reagents; however, no RIs of THs in Chinese was established, using the latest TSH reagents. This is the only multicenter study for the derivation of RIs for the 3rd TSH and for other THs that was conducted on a nationwide scale in China.

To our knowledge, the selection of an appropriate reference population, unbiased and reproducible immunoassay method for THs measurement, and sophisticated statistical analytical method are the three key factors for reliable establishment of the RIs [24,25]. In the past, influence of thyroid nodule or thyroid autoantibodies on thyroid function tests have not been quantitatively evaluated.

In this study, we first determined the cut-offs of TPO-Ab and TG-Ab for diagnosing autoimmune thyroiditis (AIT), and evaluated whether it was necessary to exclude individuals with thyroid nodules or AIT. As a result, we found that individuals with thyroid nodules or AIT showed noticeable shift in the distributions of THs; thereby necessitating the need to exclude such individuals.

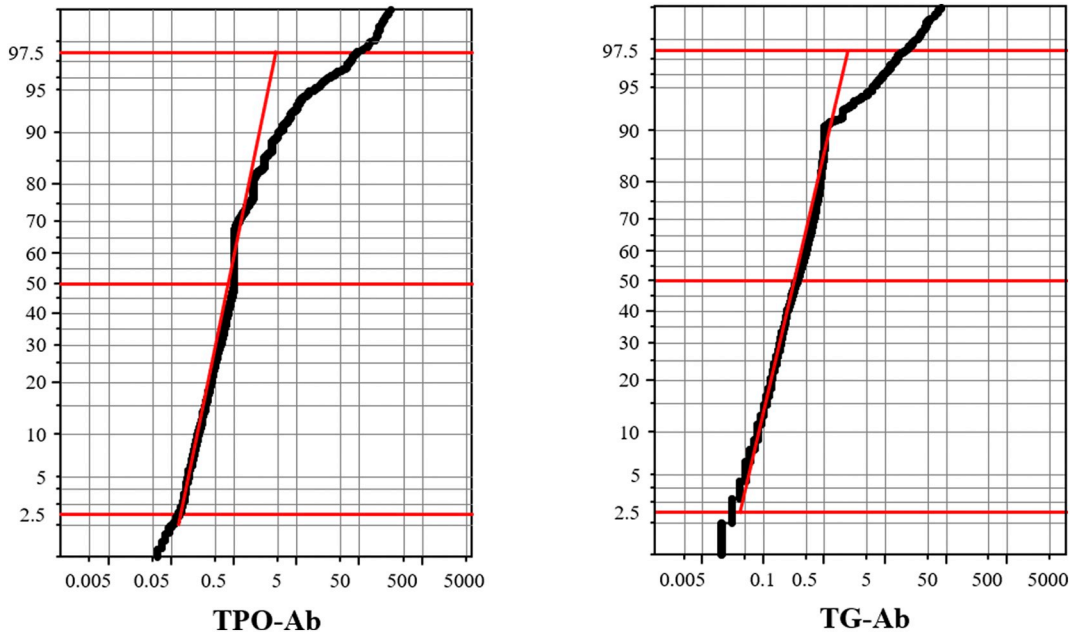

Fig. 1. Prediction of cutoffs for TPO-Ab and TG-Ab by use of probability paper plot.

Table 1
Characteristics of enrolled euthyroid participants.

	Male	Female	Total
n	951	877	1828
Age, years	40.0 (23.0–72.7)	39.0 (21.0–68.0)	40.0 (22.0–71.0)
BMI, kg/m^2	24.2 (18.1–30.6)	21.7 (17.5–28.8)	23.1 (17.0–29.9)
SBP, mmHg	123 (98–157)	110 (92–147)	120 (93–154)
DBP, mmHg	77.5 (60–104)	73.0 (54–94)	75.0 (56–100)
Alb, U/L	47.0 (40.9–53.0)	46.0 (40.0–52.0)	46.5 (40.2–52.3)
ALT, U/L	22 (9.0–70.3)	15 (7–44.2)	18 (8–59.8)
Cr, mmol/L	78.8 (556–103)	60 (430–86)	70 (45–99.7)
UA, mmol/L	367 (221–542)	275 (1720–425)	320 (183–519)
Glu, mmol/L	5.1 (4.10–7.67)	5.0 (4.1–6.1)	5.0 (4.1–6.9)
TC, mmol/L	4.7 (3.1–6.7)	4.6 (3.2–6.8)	4.7 (3.28–6.8)
TG, mmol/L	1.33 (0.53–4.95)	0.99 (0.45–3.13)	1.14 (0.47–4.11)
HDL-C, mmol/L	1.21 (0.80–1.91)	1.46 (0.94–2.20)	1.33 (0.84–2.07)
LDL-C, mmol/L	2.84 (1.48–4.53)	2.74 (1.47–4.53)	2.79 (1.48–4.53)

For each parameter, median and 95% confidence intervals (CI) in parenthesis are shown as basic statistics. The 95%CI cannot be regarded as a reference interval for each respective parameter. The statistics were calculated without any attempt for secondary exclusion.

Table 3
Results of nested ANOVA for THs.

	SDRsex	SDRage		SDRcity	
Analyte		M	F	M	F
TSH	0.143	0.050	0.000	0.192	0.253
FT4	0.161	0.097	0.000	0.192	0.000
FT3	0.393	0.250	0.000	0.362	0.326
TT4	0.196	0.000	0.136	0.178	0.078
TT3	0.259	0.147	0.000	0.274	0.056

SDRsex was derived by three-level nested ANOVA by setting sex, age, and city as grouping factors, while SDRage and SDRcity were calculated by two-level nested ANOVA for by sex. M: male; F: female.

The RI of TSH in this study differed from those in the manufacturer-provided document. However, the Italian Section of the European Ligand Assay Society (ELAS) TSH Italian Study, based on the Italian

Table 2
Results of multiple regression analysis (r_p).

	R	Guizhou	Qiqihar	Shenzhen	Urumqi	Dongying	age	sex	BMI
TSH	0.2048	0.108	-0.086	-0.044	0.062	-0.034	0.054	0.112	0.005
FT4	0.2019	-0.087	0.003	0.013	-0.039	-0.117	-0.081	-0.143	-0.041
FT3	0.3862	**0.241**	-0.010	0.036	0.048	**0.147**	**-0.187**	-0.190	0.108
TT4	0.1961	-0.029	-0.138	-0.089	-0.080	-0.066	0.026	-0.099	0.070
TT3	0.2575	**0.165**	-0.001	0.059	0.022	0.098	-0.074	-0.131	0.097

Multiple regression analyses performed analyte by analyte by setting dummy variables representing region (with Beijing set as reference category), age, sex (binary), and age as a constant explanatory variable. R represent multiple correlation coefficient. Values shown are standardized partial regression coefficients (r_p). Values of $|r_p| \geq 0.15$ which was statistically significant were marked in bold letters.

Table 4
Reference interval of thyroid hormones in Chinese population*.

Item	unit	Sex	LL-L	LL-H	LL	Me	UL	UL-L	UL-H	Between-sex bias			
										Sex	LL	Me	UL
TSH	mIU/L	MF	0.61	0.82	0.71	1.88	4.87	4.51	5.22	MF			
		M	0.60	0.81	**0.71**	**1.80**	**4.53**	4.21	4.84	M	**0.07**	**0.17**	**0.74**
		F	0.66	0.90	**0.78**	**1.99**	**5.31**	4.79	5.83	F			
FT4	pmol/L	MF	0.87	0.91	**0.89**	**1.15**	**1.50**	1.47	1.52	MF			
		M	0.89	0.92	0.91	1.16	1.52	1.49	1.55	M	**0.19**	**0.21**	**0.45**
		F	0.86	0.89	0.88	1.13	1.45	1.43	1.48	F			
FT3	pmol/L	MF	3.94	4.07	4.01	5.15	6.60	6.51	6.68	MF			
		M	4.07	4.26	**4.17**	**5.32**	**6.78**	6.67	6.89	M	**0.42**	**0.53**	**0.80**
		F	3.80	3.98	**3.89**	**4.97**	**6.25**	6.13	6.37	F			
TT4	nmol/L	MF	76	79	**77**	**107**	**144**	142	146	MF			
		M	77	80	78	110	146	144	148	M	**0.16**	**0.28**	**0.27**
		F	74	77	76	105	141	138	144	F			
TT3	nmol/L	MF	1.05	1.10	1.07	1.53	2.04	2.01	2.08	MF			
		M	1.07	1.13	**1.10**	**1.58**	**2.11**	2.06	2.15	M	**0.20**	**0.40**	**0.65**
		F	1.01	1.08	**1.05**	**1.48**	**1.95**	1.90	2.00	F			

LL, lower limit of RIs; Me, median level of RIs; UL, upper limit of RIs; M: Male; F: Female, LL-L, LL-H: 90% confidence intervals (CI) of the lower limits of the RIs; UL-L, UL-H: 90% confidence intervals (CI) of the upper limits of the RIs;. What we adopted among the RIs with/without sex partition was indicated by values in bold font. The values in red font indicate those with significant sex differences.

population, showed a TSH RI (0.362–5.280 mIU/L) similar to that suggested by the manufacturer of the Access TSH 3rd IS assay (0.45–5.33 mIU/L) [26]. Furthermore, large differences were found in TSH RI between different populations, despite the use of the same traceability reagents [20]. However, similar RIs of TSH were found with the use of different traceability reagents [27]. Most studies revealed sex-specific RIs for TSH, which is consistent with the results of this study [5,9,10,13,27,28] (Supplemental Table 2).

A new-generation TSH reagents was only just recently launched in China (December 2018), and there were relatively few laboratories using this reagent. At present, there are few articles related to the RI. From literature search, we found only two articles on 3rd TSH RI published [20,26]. The UK study showed a lower RI of TSH than that provided by the manufacturer [20]. However, an Italian study showed that the RI of TSH was similar to that provided by the manufacturer [26]. The reason for the slightly lower RI of TSH reported in the UK study than that provided by the manufacturer may be related to the calculation method. The manufacturer calculated the 97.5% CI, but this previous study calculated the 95% CI. Only this present study revealed the sex-specific RI of TSH as 0.70–4.59 mIU/L (male) and 0.72–5.50 mIU/L in Chinese population.

In our previous study, we identified statistically significant between-season differences in thyroid test results [6]. In this study, we recruited a half of the volunteers in summer and the others in winter. However, we did not obtain the consistent finding, with no appreciable between-season differences, although the findings were not included in the Results.

We regard there is some novelty to this study. First, this study was the first to establish the 3rd TSH together with other THs RIs in China on the basis of a multicenter study. Secondly, all participants underwent thyroid ultrasound to avoid influence of latentthyroid diseases. We also tried to establish the cut-offs of TPO-Ab and TG-Ab as a first step to detect and exclude those with AIT. A limitation of this study was that the iodine status was not determined because the iodine status in China is sufficient [29].

To our knowledge, this is the first nationwide multicenter study to establish RIs for THs including 3rd TSH on Beckman Coulter DXI 800 automatic analyzer. The RIs were determined from a large number of healthy euthyroid Chinese volunteers taking into account sex-difference and thyroid autoantibodies and/or nodules.

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Author contributions

DCW, XQC, SLY, LYX, and LQ designed the experiment. DCW, KI, SLY, LQ, XQC, and LYX analyzed the data. LC, HZ, LL, YRT, QQC, PCH, LAH, and DDS collected samples. DCW, LQ, KI, SLY, XQC, HLL, DDL, YCY, and SLY were involved in scientific discussions. DCW, KI, XQC, SLY, and LQ drafted and revised the manuscript. All authors have read and approved the final manuscript.

All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

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Employment or leadership

None declared.

Honorarium

None declared.

Competing interests

The funding organization(s) played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the

report; or in the decision to submit the report for publication. The authors declare no competing interests.

Appendix A. Supplemental data

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