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Gestational changes of thyroid function and urinary iodine in thyroid antibody-negative Japanese women

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Abstract. Iodine is an essential nutrient for thyroid hormone synthesis, and iodine deficiency especially in pregnant and lactating women results in serious damage to their infants. To characterize iodine nutrition throughout gestation by using a food frequency questionnaire (FFQ) and urinary iodine concentration (UIC) measurement, and to establish appropriate gestational age-specific reference ranges for serum TSH and FT_4 in thyroid autoantibody (ThAb) negative euthyroid Japanese women, a total number of 563 pregnant women including 422 subjects with negative ThAbs, 105 postpartum women and their 297 newborn infants were included in the study. Dietary iodine intake (DII) was evaluated by FFQ. Serum TSH, FT_4 and UIC were sequentially determined in the three trimesters of pregnancy and at the 31st postpartum day. The overall median UICs throughout pregnancy and in the postpartum period were 224.0 and 135.0μg/L, respectively, suggesting sufficient iodine nutrition. The median DII was 842.4μg/day in pregnant women. In the longitudinal study, the median UIC in the first trimester (215.9μg/L) significantly decreased in the second trimester (136.0μg/L). The prevalence of pregnant women with a UIC below 150μg/L was 31.6% and that in lactating women with a UIC below 100μg/L was 33.3%. The pattern of gestational change in serum TSH and FT_4 was comparable to that in iodine-sufficient areas. A substantial percentage of women might be at risk for iodine deficiency if there is a restriction of iodine-rich foods. However, iodine supplementation for pregnant women must be carefully balanced against the risk of iodine excess particularly in Japan. Further research in larger samples is needed.

Key words: Iodine, TSH, FT_4, Food frequency questionnaire, Pregnancy

IODINE is an essential nutrient for thyroid hormone synthesis, and iodine deficiency remains a major global threat to health and development especially in fetuses, newborns and young infants [1]. In 2013, as defined by a national or subnational median urinary iodine(UI) concentration of 100–299μg/L in school-aged children, 111 countries have sufficient iodine intake and 30 countries remain iodine-deficient [2].

The major source of iodine intake is daily foods and more than 90 percent of all ingested iodine is excreted in urine [3]. Therefore, the median urinary iodine concentration (UIC) is a reliable biomarker for recent iodine intake and used to classify a population's iodine status [4]. An alternative method that is considered to be the 'gold standard' to directly assess nutrient intake is the diet study including 24-hour dietary recall, food record method, duplicate meal, dietary history and food frequency questionnaire (FFQ) [5]. There are limited data regarding dietary iodine intake using FFQ [6-9], and in pregnant women several studies have been carried out in the UK [7], Italy [8] and Norway [9]. The approximate daily iodine intake estimated by FFQ was well correlated with either the 24-hour urinary iodine excretion (UIE) [9] or the iodine intake by 24-hour recall [7].

During and after pregnancy, daily iodine requirements are higher than those in non-pregnant adults because of increased thyroid hormone production, increased renal iodine excretion, iodine requirement for a fetus and iodine secretion into breast milk. In iodine-deficient areas, total body iodine stores decline gradually during pregnancy and results in impaired maternal and fetal thyroid hormone synthesis [10-12]. Recently iodine intake during gestation is reported to be insufficient, even in the areas that have been iodine sufficient for several decades [13-14] or where there

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are iodine prophylaxis programs to promote the consumption of iodized salt [15-18]. The World Health Organization (WHO) [19], United Nations Children's Fund (UNICEF) [20] and the International Council for Control of Iodine Deficiency Disorders (ICCIDD) Global Network [21] have endorsed the policy of iodine supplementation in pregnant and breast-feeding women. According to new guidelines of both the American Thyroid Association (ATA) [22] and the Endocrine Society [23] daily intake of 250μg iodine has been advised for all pregnant and breast-feeding women, not only in iodine-deficient areas but also iodine-sufficient countries. However it is not clear if this policy is appropriate and applicable in Japan, a long-term iodine-sufficient country.

From the early 1990s to the present, there have been a number of reports on the iodine status during pregnancy and lactation in the countries or regions with various iodine statuses. However, few longitudinal studies on gestational changes of UIC and thyroid parameters have been carried out in iodine-replete regions. We have recently reported the reference range and changing pattern of trimester-specific UIC during and after pregnancy in Japanese women [24]. Our results suggested that the iodine status in Japanese pregnant and lactating women assessed by median UIC was regarded as adequate according to WHO/UNICEF/ICCIDD-recommended criteria.

The purpose of the present study is to assess the nutritional status of iodine and gestational change of UIC as well as iodine intake by using FFQ and UI measurement, and to determine a trimester-specific reference range of serum thyrotropin (TSH) and the free thyroxine (FT_4) value in serum thyroid autoantibody (ThAb) negative Japanese pregnant women.

Materials and Methods

Subjects

As part of a larger study assessing iodine status in Japanese, healthy pregnant and lactating women without known thyroid disease were consecutively recruited from Yamaguchi Hospital in Funabashi City, Chiba Prefecture between 2005 and 2006. The total population of this city was 570,000-590,000 in this period. This hospital is a private women's hospital and the average number of deliveries was 2,024 annually during the study period.

Study design

All the participants completed an iodine-specific FFQ and provided one spot urine sample at the first visit. In pregnant women blood and spot urine samples were collected in the first, second and third trimesters. Urinary iodine, serum TSH and FT_4 concentrations as well as two ThAbs, *i.e.* thyroperoxidase antibody (TPOAb) and thyroglobulin antibody (TgAb) were measured and the subjects with thyroid autoantibodies detected during the study were excluded from the reference interval analysis for serum TSH and FT_4.

Serum and casual urine samples were kept frozen at -30˚C before analysis. A heel-prick blood sample was taken at 4.0±0.1 postnatal days from all infants. Gestational dates were confirmed by ultrasound in the first trimester of pregnancy.

Ethical approval

The study was approved by the local ethics committee, and written informed consent was obtained from each of the participants at the initial study visit.

Daily dietary iodine intake assessed by FFQ

We have developed a semi-quantitative FFQ to estimate daily dietary iodine intake (DII) for Japanese. The reproducibility and validity of iodine intake measurement by the FFQ used in this study were described in detail elsewhere [25]. It contained 50 different iodine-rich food items classified in 10 categories with a specified serving size (Table 1). For each food item, participants indicated their average frequency of consumption in the previous months by checking 1 of 10 frequency categories ranging from "almost never" to "3 times/day." The selected frequency category for each food item was converted to a daily intake. Daily iodine intake from each food was calculated on the basis of frequency of consumption and iodine content of the specific food and given in μg iodine per day. The iodine content in food stated in the Japanese Food Composition Table 2010 [26] was used, except for processed food where new figures were used [27]. In our preliminary study, daily DII and UIC were determined in 28 non-pregnant healthy women without known thyroid disease using the same method. Their mean (SD) age was 32.6 (4.6) years (range, 22 to 39 years), and the median with interquartile range (IQR) values of daily DII and UIC were 764.3 (545.4 to 1421.0) μg/day and 196.0 (115.3 to 334.8) μg/L, respectively (unpublished data).

Table 1 Foods and iodine content in FFQ to estimate the iodine intake

Foods of food groups		Food items	Reference portion size	Iodine content (μg) / Portion size
Algae	Kombu (Laminariaceae)	1. *Tsukudani* [1]	10g	1100.0
		2. *Kobumaki* [2]	30g	3240.0
		3. *Tororo Kombu* (tangle flakes)	3g	6848.6
		4. Soup with Kombu extract	200mL	252.0
	Wakame (Undaria pinnatifida)	5. Wakame Salad or Vinegarded Wakame	20g	1143.4
		6. Miso or clear soup	10g	778.8
		7. Fruit-bearing leaves (*Mekabu*)	40g	156.0
	Nori (Seaweed)	8. Purple laver, Seasoned and toasted	2g	121.9
		9. Purple laver, Toasted	1.5g	31.5
		10. *Hitoegusa Tsukudani* [3]	10g	18.1
	Agar jelly	11. *Mitsumame* [4]	30g	6.3
		12. *Tokoroten*[5]	50g	120.0
	Other brown algae	13. *Hijiki*	20g	9400.0
		14. *Mozuku*	40g	56.0
Fishes & Shell fishes		15. Horse mackerrels	65g	13.7
		16. Sardines	50g	16.5
		17. Eel	80g	61.6
		18. Skipjack	80g	20.0
		19. Salmons	80g	4.0
		20. Mackerels	80g	16.8
		21. Pacific saury	75g	16.1
		22. Shishamo (spirinchus lanceolatus)	30g	16.7
		23. Sea breams	80g	6.4
		24. Cod fishes	100g	260.0
		25. Walleye pollack (*Tarako*)	30g	39.0
		26. Yellowtail	100g	24.0
		27. Tunas	80g	9.6
		28. Oysters	15g	9.8
Soup		29. Miso-soup	150mL	0 (53.2)*
		30. Clear soup	150mL	94.5 (103.9)*
Noodle		31. Noodles in hot broth (*Kakeudon*[6], *Kakesoba*[7])	250mL	157.5 (258.3)*
		32. Dipping noodles (*Tsukemen*[8])	100mL	63.0 (143.5)*
One-pot dish (*Nabemono*)		33. *Oden*[9]	200mL	126 (109.3)*
		34. One-pot dish (*Yosenabe*[10], *Sukiyaki*, *etc*.)	250mL	157.5 (268.8)*
Prepared foods		35. Instant one-pot dish	one meal	216.1
		36. Instant clear soup		51.4
		37. Instant soup		51.4
		38. Instant miso-soup		43.7
		39. Instant noodle		7.8
		40. *Chazuke*[11]		23.2
		41. *Furikake*[12]		23.2
Seasonings & Spices		42. Vinegar with Kombu extract	5mL	22.5
		43. Soy sauce with Kombu extract	5mL	100.0
		44. Salt with Kombu powder or extract	6g	270.6
Eggs		45. Hen's egg	50g	24.0
		46. Japanese quail's egg	10g	14.0
		47. Iodine-riched egg[13]	50g	650.0
Confectioneries		48. Potato chips	30g	78.0
		49. Rice cracker (*Shio-senbei*)	15g	16.5
Tea		50. *Kobu-cha* (Kombu powder for drink)	2g	576.3

* one portion using homemade soup stock (ready-made soup stock)

[1], Kombu boiled in soy sauce; [2], a roll of tang containing dried fish in it cooked with sugar and soy sauce; [3], Nori simmered in soy sauce and sugar; [4], a mixture of gelatin cubes, boiled beans and fruit topped with molasses; [5], a jelly-like food made from red seaweed (*tengusa*); [6], Udon noodles in broth; [7], Buckwheat noodles in soup; [8], Cold noodles accompanied by soup for dipping; [9], a dish containing all kinds of ingredients cooked in a special broth of soy sauce, sugar, sake, *etc*.; [10], a mixed stew of chicken, seafood and vegetables cooked at the table; [11], a quick dish of boiled rice with tea poured on it; [12], a tastily seasoned dried food for sprinkling on rice; [13], Eggs produced by hens raised on feed containing seaweed

Analytical methods

Serum TSH and FT_4 were measured by electrochemiluminescence immunoassay (ECLIA) using ECLusys TSH and FT_4 (Roche Diagnostics K.K., Tokyo, Japan). Detection limit and reference ranges for Japanese given by the manufacturers were: TSH, 0.0022mU/L, 0.50 to 5.00mU/L; FT_4, 0.01ng/dL, 0.90 to 1.7ng/dL. Neonatal blood TSH was measured in the dried heel blood spot samples by ELIZA using Enzaplate N-TSH (detection range; 0.5-80mU/L, Bayer Medical Ltd. Tokyo, Japan). TPOAb and TgAb were measured in maternal serum by RIA using TPOAb Cosmic II (detection limit and range; 0.04U/mL, 0.3 to 60U/mL) and TgAb Cosmic II (0.15U/mL, 0.3 to 100U/mL) (RSR Limited, Cardiff, UK), respectively. The TPOAb and TgAb values above the manufacturer's reference limit (0.3U/mL) were considered positive. Iodine in urine was measured by the ammonium persulfate digestion on microplate (APDM) method based on the Sandell Kolthoff reaction [28]. The analytical sensitivity for iodine was 1.39μg/dL and the intra-assay and inter-assay coefficients of variation were 4.4 and 3.9%, respectively. The creatinine (Cr) concentration in urine was estimated by colorimetric enzymatic assay. All urine samples were assayed in duplicate. Urinary iodine concentration was expressed relative to creatinine excretion (μg/gCr) or as a concentration (μg/L) and SI units (μmol/L) in parentheses. (For conversion to SI units: 1μg/L=0.0079μmol/L).

Statistics

The results were presented as median, geometric mean, mean with SD, range or percentile. The urinary iodine and serum TSH concentrations were distributed asymmetrically and their logarithmically transformed values were therefore used to normalize the distribution. The normality of the transformed data was tested using the Kolmogorov-Smirnov test. For the reference interval analysis means and standard deviations of logarithm of the concentration was then used as parameters to fit a normal distribution, and the 25th and 75th percentiles (IQR, Interquartile range) were calculated based on the standard normal distribution by transforming back to the linear scale. Differences between paired data or groups were examined using one-way analysis of variance (ANOVA) with Tukey's multiple comparison test, the Kruskal-Wallis test and Dunn's Multiple Comparison Test. Differences between two unmatched groups for normally and non-normally distributed data were tested using the unpaired *t* test and Mann-Whitney test, respectively. Simple linear regression analysis was used to test for correlations between urinary iodine and serum TSH or FT_4 concentrations. A *p*-value less than 0.05 was considered significant. All data were processed and statistically analyzed using GraphPad Prism version 6.00 for Windows (GraphPad Software, San Diego, CA, U.S.A.).

Epidemiological criteria of IDD in pregnant and lactating women

The median UIC expressed as micrograms of iodine per liter (μg/L) is used for better comparison of the population's iodine status with the WHO-defined deficiency grades, and the iodine intake in pregnant and lactating women is regarded as follows: insufficient, below 150μg/L; adequate, 150 to 249μg/L; more than adequate, 250 to 499μg/L; excessive, 500μg/L or higher for pregnant women and insufficient, below 100μg/L; adequate, 100μg/L or higher for lactating women [19].

Results

A total number of 563 pregnant and 105 lactating women agreed to complete the study. All the lactating women were not identical with individuals from the study group of pregnant women. The mean±SD age of pregnant women was 30.9±4.2 (range, 19 to 43) years and that of lactating women at 31.7±1.3 postpartum days was 30.7±4.4 (range, 20 to 40) years.

Venous blood samples were obtained from 484 of the 563 pregnant women and after excluding 62 pregnant women with positive ThAb, 422 pregnant women and their 297 newborn infants comprised the study sub-group and their mean±SD age was 30.1±4.2 years (range, 19 to 43 years). In a cross-sectional study a total of 695 blood samples from 422 subjects included multiple measurements from the same subjects (221 women provided single samples, 129 women provided two samples, and 72 women provided three samples).

The mean±SD gestational age and birth weight of the infants were 39.8±1.2 weeks (range, 36.7 to 43.7 weeks) and 3101.0±344.5g (range, 2260 to 4140g), respectively, and the male/female ratio was 1.02. There was no infant classified as a small-for-age neonate that was defined as a birth weight below the mean minus 2.5SD on the growth curves of Japanese children reported in 2010 by the Japan Pediatric Society.

A total of 65 ThAb negative pregnant women serially

Table 2 Dietary iodine intake, urinary iodine and creatinine concentrations during pregnancy and in postpartum

Category of Gestational Period	First Trimester	Second Trimester	Third Trimester	Postpartum
Number of subjects	166	221	176	105
Gestational weeks (mean±SD)	8.9 (1.6)	21.9 (4.6)	34.2 (3.3)	31.7 (1.3)[a]
Dietary Iodine Intake (μg/day)				
Median (IQR)	743.1* (434.4-1482)	945.9 (572.8-1556)	876.2 (516.6-1420)	1071 (690.5-1708)
Geometric mean (95%CI)	740.2* (646.3, 847.8)	916.2 (820.9, 1023)	859.9 (766.2, 965)	1052 (919.1, 1204)
UIC (μg/L)				
Median (IQR)	226.5* (144.8-434.5)	259.0* (120.5-660.0)	204.5* (116.0-459.0)	135.0 (82-258)
Geometric mean (95%CI)	285.5* (239.8, 340.0)	277.8* (239.1, 322.9)	257.3* (218.6, 302.9)	161.3 (133.7, 194.7)
UIC below 150μg/L,%	25.9	32.1	36.4	(33.3)[b]
UIC exceeding 1000μg/L,%	13.9	14.5	13.1	4.8
Urine Creatinine (g/L)				
Mean (SD)	1.38[†] (0.73)	0.99 (0.62)	0.92 (0.53)	0.92 (0.53)
UIC (μg/gCr)				
Median (IQR)	186.6[‡] (116.8-349.7)	272.6* (161.6-658.3)	259.0* (163.4-548.3)	159.2 (105.3-406.0)
Geometric mean (95%CI)	247.9[‡] (208.8, 294.4)	344.2* (298.4, 397.0)	339.4* (289.7, 397.6)	209.6 (173.1, 253.7)

The total number of subjects is 668. [a], Postpartum days; [b], Percentage less than 100μg/L; IQR, Interquartile range (25-75th percentiles); SD, Standard Deviation; CI, Confidence Interval; Cr, Creatinine
*, Different from postpartum (p<0.05); [†], Different from the second and third trimester and postpartum (p<0.05); [‡], Different from the second and third trimester (p<0.05)

provided three samples for TSH, FT_4 and UI measurements in each trimester and comprised the longitudinal study group. Their mean±SD age was 31.8±4.2 years.

Iodine status and gestational change of UIC in pregnant and lactating women

The UIC values in pregnant women were highly variable (18 to 16300μg/L) and the median (IQR) value for the overall UI samples throughout pregnancy (mean±SD gestational week, 21.9±10.5) was 224.0 (131.0 to 466.0) μg/L or 243.1 (149.0 to 531.7) μg/gCr. In lactating women the median (IQR) UIC was 135.0 (82.0 to 258.0) μg/L or 159.2 (105.3 to 406.0) μg/gCr. The percentage of subjects with a UIC below 150μg/L or above 1000μg/L was 31.6% and 13.9%, respectively, while in postpartum samples the rates of UIC below 100μg/L or above 1000μg/L were 33.3% and 4.8%, respectively.

The median UIC values during pregnancy were significantly higher than that in postpartum women (204.5 to 259.0μg/L *vs.* 135.0μg/L). There were no significant differences in the median and mean UIC values among the three trimesters. The rate of the subjects with a UIC below 150μg/L increased from 25.9 to 36.4% in the third trimester (Table 2).

The median value of creatinine-adjusted UIC was lowest in the first trimester (186.6μg/gCr) reflecting the highest creatinine excretion (1.38g/L); it increased to 272.6μg/gCr in the second trimester and subsequently decreased slightly to 259.0μg/gCr in the third trimester but remained above those in the first trimester and postpartum (159.2μg/gCr). A similar pattern was observed for the mean UIC values (Table 2).

Dietary iodine intake during pregnancy and postpartum

The overall DII calculated by FFQ in pregnant and lactating women ranged from 35.7μg to 8.7mg/day. The distribution of daily DII of pregnant women is shown in Fig. 1. The median (IQR) values were 842.4 (495.7 to 1469.0) μg/day during pregnancy and 1071.0 (690.5 to 1708.0) μg/day in the postpartum period. The percentage of subjects with a daily DII below 250μg/day or above 1100μg/day was 7.9% and 36.4%, respectively, in the pregnant women, while those of the lactating women were 2.8% and 49.5%, respectively. There was no significant difference in the median DII value among the four groups; however, the median value in the first trimester group was significantly lower than that in the postpartum group (743.1 *vs.* 1071.0μg/day) (Table 2).

Daily DII was positively correlated with UIC, both expressed as μg/L (*Pearson's r=0.09, p<0.0214*) or μg/gCr (*Pearson's r=0.11, p<0.0089*) during pregnancy. In each trimester there was no significant correlation

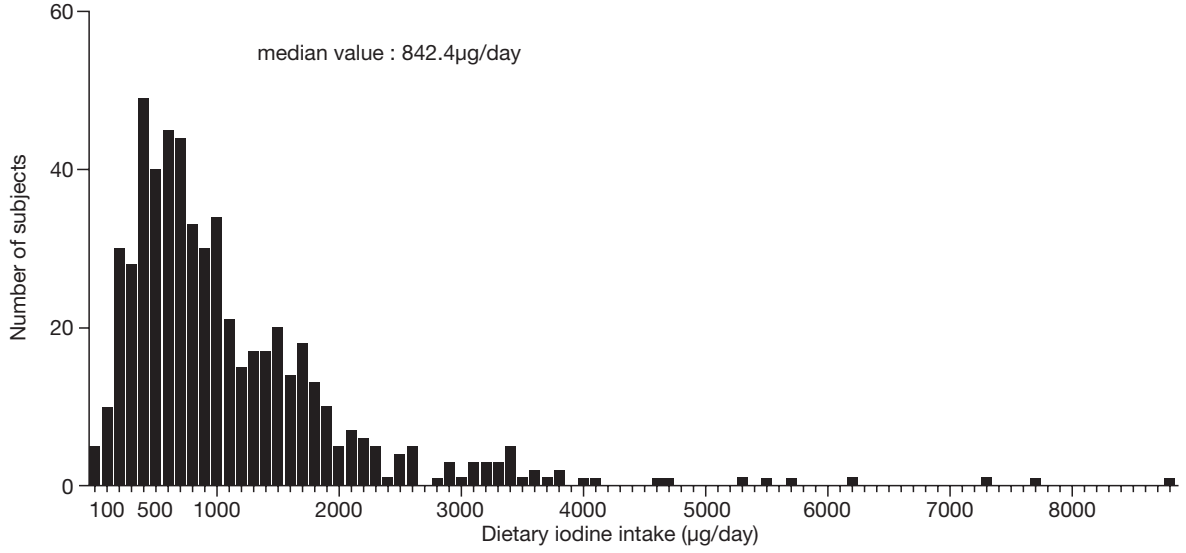

Fig. 1 Frequency distribution of daily DII during pregnancy
The number of subjects is 563. The median dietary iodine intake determined by FFQ was 842.4μg/day.

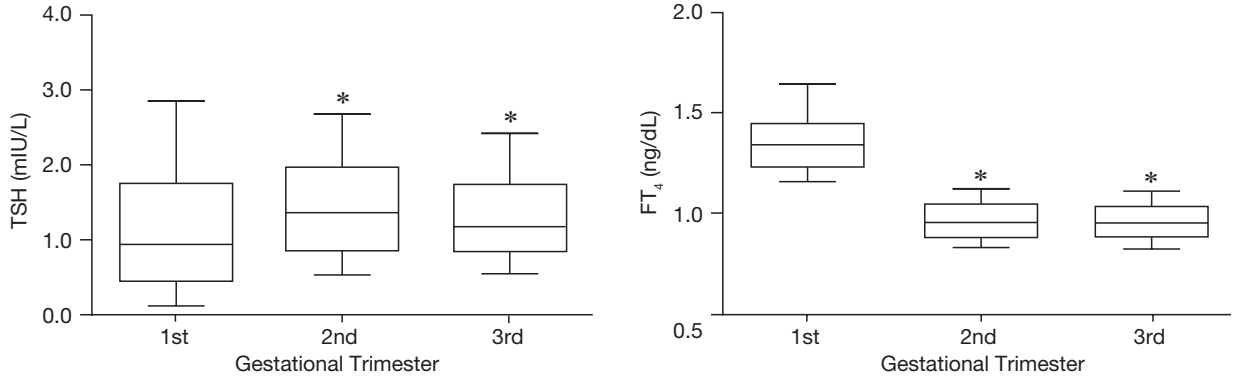

Fig. 2 Changes in serum TSH and FT_4 concentrations during pregnancy
The boxes represent median TSH and FT_4 concentrations with IQR. Whiskers denote 10 to 90 percentile. The number of blood samples is 181 in the first trimester, 181 in the second trimester and 333 in the third trimester. The mean (SD) gestational ages are 8.7 (1.2) weeks in the first trimester, 26.9 (4.1) weeks in the second trimester and 34.3 (2.3) weeks in the third trimester. *, $p<0.05$ *vs*. first trimester.

of daily DII with maternal TSH, FT_4 or neonatal TSH (data not shown).

Gestational change of serum TSH and FT_4 in ThAb negative pregnant women

The gestational change of serum TSH and FT_4 concentrations is depicted in Fig. 2. Serum TSH concentration ranged from less than 0.005 to 7.34mU/L and the median value increased from 0.92mU/L (IQR, 0.04 to 3.93) in the first trimester to 1.35mU/L (IQR, 0.43 to 3.01) in the second trimester and subsequently decreased slightly to 1.17mU/L (IQR, 0.40 to 2.92) in the third trimester. Serum FT_4 concentration ranged from 0.62 to 5.8ng/dL and the median FT_4 concentration in the first trimester was 1.34ng/dL and decreased to 0.98ng/dL in the second trimester and remained stable. A similar pattern was observed for the mean TSH and FT_4 values (data not shown).

Gestational trimester-specific reference range of serum TSH, FT4 and UIC (Repeated measurement)

In 65 pregnant women the pattern of gestational change in serum TSH, FT_4 and UIC was essentially comparable to that in our present cross-sectional study in

Table 3 Gestational trimester-specific reference range of serum TSH, FT_4 and UI concentrations in ThAb negative pregnant women (Repeated measurement)

Category of Gestational Period	First Trimester	Second Trimester	Third Trimester
Gestational weeks, Mean (SD)	9.1 (1.7)	25.5 (5.2)	36.0 (0.6)
TSH (mU/L)			
Median (IQR)	0.95 (0.7-1.35)	1.11 (0.96-1.33)	1.12 (0.99-1.33)
Mean (95%CI)	0.90 (0.78, 1.03)	1.10* (1.03, 1.17)	1.12* (1.05, 1.2)
FT_4 (ng/dL)			
Median (IQR)	1.34 (1.12-1.76)	0.94 (0.78-1.11)	0.91 (0.82-1.12)
Mean (SD)	1.43 (0.58)	0.94* (0.10)	0.94* (0.10)
UIC (μg/L)			
Median (IQR)	215.9 (149.0-314.5)	136.0* (73.0-271.5)	147.9 (80.9-221.4)
Mean (95%CI)	243.2 (184.0, 321.8)	143.3* (107.4, 191.1)	155.9 (116.2, 208.9)
UIC (μg/gCr)			
Median (IQR)	187.5 (110.7-327.0)	204.6 (117.4-372.0)	196.2 (124.8-468.7)
Mean (95%CI)	240.8 (177.5, 327.0)	238.2 (184.2, 308.0)	237.7 (182.4, 310.1)

The number of subjects is 65. Serum TSH and FT_4 were measured by using ECLusys TSH and FT_4 (Roche Diagnostics K.K., Tokyo, Japan). IQR, Interquartile range (25-75th percentiles); SD, Standard Deviation; CI, Confidence Interval; Cr, Creatinine *, Different from first trimester ($p<0.05$)

Fig. 2; however, there was a significant decrease of UIC in the second trimester (215.9 *vs.* 136.0μg/L) (Table 3).

Relationship between serum TSH and FT_4 concentrations

There was a significant negative correlation between TSH and FT_4 in all serum samples (*Pearson r=-0.51, p<0.0001*) and in each trimester (1st trimester, *Pearson r=-0.63, p<0.0001*; 2nd trimester, *Pearson r=-0.21, p=0.007*; 3rd trimester, *Pearson r=-0.42, p<0.0001*).

Relationship between UIC and serum TSH or FT_4 concentrations

The overall UIC during pregnancy was correlated positively with serum TSH (*Spearman r=0.1365, p=0.0006*) and negatively with FT_4 concentrations (*Spearman r=-0.1446, p=0.0003*). There were significant direct correlations of UIC with serum TSH concentrations in the third trimester (*Spearman r = 0.2724, p=0.0009,* n=145) and with FT_4 concentrations in the first trimester (*Spearman r = -0.2074, p= 0.007,* n=168).

Relationship between maternal TSH, FT_4, UIC and neonatal TSH

Neonatal TSH concentration in 297 infants ranged from 0.02 to 11.03mU/L and the median (IQR) and geometric mean (95%CI) of TSH values were 2.28mU/L (1.43 to 3.56) and 2.09mU/L (1.90 to 2.29), respectively. The recall rate was 0.67% (2 of 297 infants) and no infant was diagnosed as being congenitally hypothyroid.

There was significant correlation between neonatal TSH and maternal UIC exclusively in the third trimester (*Pearson r=0.1579, p=0.007, n=290*), while neonatal TSH was not correlated with maternal TSH nor FT_4.

Discussion

The present study confirmed the previous findings by others [29] and us [24] that iodine intake in Japanese pregnant and lactating women is regarded as adequate according to WHO criteria. However, 31.6% of pregnant women and 33.3% of lactating women have UIC less than 150μg/L or 100μg/L, respectively, which indicate insufficient iodine intake, although the median UICs for all the subjects were 224.0μg/L in pregnant and 135.0μg/L in lactating women. This observation suggests that even in an iodine-sufficient area, an additional iodine supply might be necessary in pregnant and lactating women who do not consume iodine-rich foods. The Endocrine Society recommends and emphasizes the need for routine supplementation of all pregnant women with adequate iodine in the form of prenatal vitamins containing 150-200μg iodine. However, in the present study more than two thirds of the pregnant women (36.4%) were ingesting iodine exceeding 1100μg daily, which is double the recommended nutrient intake for iodine in the US. Therefore, iodine supplementation for pregnant women must be carefully

balanced against the risk of iodine excess particularly in Japan, an iodine-sufficient area. It is important to evaluate accurately the iodine status and the signs of iodine deficiency in pregnant women and small children [30].

The primary sources of iodine in the United States and European countries are dairy products, iodized salt, bread with iodate dough conditioners, eggs, and iodine-containing supplements [6, 8-9, 31-32]. In Japan iodine fortification of household salt is prohibited and major food categories that contribute to dietary iodine include marine products and processed foods that contain seaweed extracts. Our recent study of healthy adults indicated that 80.6% of total iodine resources were seaweeds such as kelps and *kombu*, while 5.9 and 13.5% were fish and processed foods, respectively [25]. Japanese iodine intake from seaweeds is highest in the world [33-34]. However, national survey data for iodine intake is not currently available because iodine is not included as a target nutrient in the national health and nutrition survey. According to our collaborative studies with WHO in Tokyo and Hokkaido, the median UI value in school-aged children is 281 to 288μg/L, suggesting a sufficient iodine status with more than adequate iodine intake [35-37]. In addition, children with a high UI excretion and increased thyroid gland volume were observed in a coastal area of Hokkaido [37].

In Japanese pregnant and lactating women the median DII determined by FFQ was 842.4 and 1071.0μg/day, respectively, and far higher than their median UIC values (224.0 and 135.0μg/L). Although the exact reason for this discrepancy between the UIC and daily DII by FFQ is not clear, there may be some factors other than the complexity of iodine physiology during pregnancy [11-12]. Firstly, in healthy non-pregnant adults approximately 90% of iodine of dietary origin is excreted in urine and 10% of iodine intake in faces. Secondly, the UIC in a spot urine sample may vary up to three-fold in an individual during a single day [30]. Thirdly, the median UIC given as μg/L will be lower than the median iodine excretion per 24 hours because the average urine volume passed by adults is more than one liter in 24 hours. Therefore the UI excretion of groups of healthy adults measured as μg/24h is often equal to UI measured as μg/L×1.5 [30]. Fourthly, the FFQ retrospectively evaluates daily iodine intake over a period of time, whereas the UIC reflects iodine intake over the recent few days rather than usual intake. Lastly, it has been reported that the level of iodine intake measured by the FFQ tends to be overestimated and the reason is not clear [6]. However, the FFQ can be used to classify subjects into low and high iodine intake groups [6]. Further studies are necessary to improve the accuracy of dietary intake by FFQ.

The ATA and Endocrine Society recommended that a sustained iodine intake of 500 to 1100μg daily should be avoided during pregnancy due to concerns on the potential for fetal hypothyroidism [22-23]. In the US and Europe the tolerable upper intake limits of iodine for pregnant and lactating women are 1100 and 600μg/day, respectively [1], while in Japan it is determined as 2200μg/day [38]. Supporting data for this value is a case report of neonates with thyroid dysfunction born to mothers who daily ingested approximately 2.3-3.2mg of iodine during pregnancy [39]. In our study the 90th percentile value of daily DII was 2126μg/day and the ratio of pregnant women with more than 1000μg of UIC was 13.9%, suggesting no possible risk of adverse health effect due to iodine excess in our subjects as a population.

The median UIC in our cross-sectional study was highest as early as in the first trimester, then decreased and remained stable until term without showing significant differences among the three trimesters. However, the longitudinal analysis of data revealed a significant decrease of UIC at mid-gestation. The gestational change of UIC has been reported from various countries and the results are discordant irrespective of the iodine status of the study subjects or areas [24, 40-53]. The peak UIC value is observed at the first trimester in Ireland [40-41], Switzerland [45], Bosnia and Herzegovina [48], Iran [46], Sri Lanka [41], Tasmania (Australia) [47] and Japan [24], at the second trimester in Iran [44] and Mexico [50] or at the third trimester in Sweden [42], France [53], Sudan [42] and Hong Kong [43]. With the progression of pregnancy, the median UIC either declined in Ireland [41], the United Kingdom [41], Switzerland [45], Bosnia and Herzegovina [48], Iran [46], India [52], Tasmania (Australia) [47] and Japan [24] or increased in iodine-deficient areas from Spain [49], France [51, 53] and Hong Kong [43]. However, a statistically significant change was observed only in one study from Hong Kong [43], and the median UICs were not different between gestational stages in the studies from Sweden [42], northern Paris (France) [51], Sudan [42], Iran [44], Sri Lanka [41], Mexico [50] and Japan [24]. All regions except Sudan and northern Paris are regarded as optimal iodine status suggesting that under the situation of sufficient iodine intake the increased renal

UI excretion steadily decreases throughout gestation. These variable results on UIC may reflect the difference in regional and/or individual iodine status *i.e.*, initial thyroid iodine content and daily dietary iodine intake, in alteration of renal threshold for iodine excretion, or in study design and sample size.

In normal pregnancy, as the direct consequence of the markedly increased serum TBG levels by estrogen stimulation, total serum thyroid hormone levels increase significantly from early gestation, reach plateau levels by 20 weeks gestation and remain stable until term. Serum FT_4 substantially decreases with the progression of gestation. The serum TSH level remains stable and comparable to pre-gestation levels after the transient decrease in the first trimester due to the thyrotrophic effect of high hCG secretion and its reference range is lower throughout pregnancy [10-11]. In our longitudinal study, TSH values in ThAb negative Japanese are generally comparable to the reference range for serum TSH during pregnancy defined in populations with optimal iodine intake recently reported by the ATA [22]. Although slight but significant ethnic differences in serum TSH concentration have been reported [54], our median TSH values are not lower than in Caucasian women. Free T_4 reference intervals in pregnancy varied widely between methods and were also influenced by the iodine status of the population. The ATA recommended that method-specific and trimester-specific reference ranges of serum FT_4 are required in each laboratory. Our results regarding the gestational change of FT_4 and TSH values and negative correlation between serum TSH and FT_4 during pregnancy are consistent with the previous report in Japanese [55] using the same analytical method for TSH and FT_4 as ours. A similar correlation was reported in an area with insufficient iodine intake from France [56], suggesting the contribution to the maternal thyroid adaptation by serum TSH in any iodine status.

There was a direct correlation of UI values with FT_4 negatively and with TSH positively during pregnancy. This finding is consistent with the recent report on UIC in early gestation from Japan [29].

Data on the association of maternal UIC during pregnancy and postpartum with neonatal thyroid parameters are conflicting [24, 56-59]. In our study there was a positive direct correlation between maternal UIC and neonatal TSH concentration exclusively in late pregnancy. However, no direct correlation of maternal UIC with neonatal TSH concentration was observed in several studies including our previous study [24], those in the mildly iodine-deficient areas from Australia [57-58], and in the pregnant women with extremely high UI excretion (1.2mg/L) in China [59], whose UIC positively correlated with neonatal FT_4 concentration. The exact reason for the discrepancies is not clear and further large epidemiological studies are necessary in different iodine statuses.

The strengths of the study include the assessment of iodine intake by FFQ and the longitudinal measurement of urinary iodine and serum TSH and FT_4 concentrations in ThAb negative euthyroid subjects during pregnancy. The study was limited by a local data with the relatively small sample size of subjects, therefore iodine supplementation to all pregnant and breastfeeding women should be determined with caution in Japan.

In conclusion, this study is the first report regarding the iodine status throughout gestation by using diet study and UIC in an iodine-sufficient area and also provides the local reference range for serum TSH and FT_4 values during pregnancy. Although Japan has been regarded as an iodine-sufficient or even excessive country, a substantial percentage of pregnant and lactating women have a UIC below 100μg/L and might be at risk for iodine deficiency if there is a restriction of iodine-rich foods. Currently there are no national estimate of iodine status among Japanese women of reproductive age as well as in infants and children. Further research on the iodine status of vulnerable populations in larger samples and the development of reliable methods to select individuals with low iodine intake are needed.

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Disclosure

None of the authors have any potential conflicts on interest associated with this research.

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Erratum

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Gestational changes of thyroid function and urinary iodine in thyroid antibody-negative Japanese women
Yozen Fuse, Yoshimasa Shishiba and Minoru Irie

Table 3 contains some typographical errors. Median (IQR) of FT_4 should be median (5-95th percentile) and the median (IQR) values of TSH are replaced with median (5-95th percentile) values.
The correct version of Table 3 is given below:

Table 3 Gestational trimester-specific reference range of serum TSH, FT_4 and UI concentrations in ThAb negative pregnant women (Repeated measurement)

Category of Gestational Period	First Trimester	Second Trimester	Third Trimester
Gestational weeks, Mean (SD)	9.1 (1.7)	25.5 (5.2)	36.0 (0.6)
TSH (mU/L)			
Median (5-95th percentile)	0.95 (0.44-1.69)	1.11 (0.81-1.48)	1.12 (0.81-1.55)
Mean (95%CI)	0.90 (0.78, 1.03)	1.10* (1.03, 1.17)	1.12* (1.05, 1.2)
FT_4 (ng/dL)			
Median (5-95th percentile)	1.34 (1.12-1.76)	0.94 (0.78-1.11)	0.91 (0.82-1.12)
Mean (SD)	1.43 (0.58)	0.94* (0.10)	0.94* (0.10)
UIC (μg/L)			
Median (IQR)	215.9 (149.0-314.5)	136.0* (73.0-271.5)	147.9 (80.9-221.4)
Mean (95%CI)	243.2 (184.0, 321.8)	143.3* (107.4, 191.1)	155.9 (116.2, 208.9)
UIC (μg/gCr)			
Median (IQR)	187.5 (110.7-327.0)	204.6 (117.4-372.0)	196.2 (124.8-468.7)
Mean (95%CI)	240.8 (177.5, 327.0)	238.2 (184.2, 308.0)	237.7 (182.4, 310.1)

The number of subjects is 65. Serum TSH and FT_4 were measured by using ECLusys TSH and FT_4 (Roche Diagnostics K.K., Tokyo, Japan). IQR, Interquartile range (25-75th percentile); SD, Standard Deviation; CI, Confidence Interval; Cr, Creatinine *, Different from first trimester ($p<0.05$)