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Fluoride exposure and thyroid function among adults living in Canada: Effect modification by iodine status

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ABSTRACT

Background: Fluoride exposure has the potential to disrupt thyroid functioning, though adequate iodine intake may mitigate this effect. This is the first population-based study to examine the impact of chronic low-level fluoride exposure on thyroid function, while considering iodine status. The objective of this study was to determine whether urinary iodine status modifies the effect of fluoride exposure on thyroid stimulating hormone (TSH) levels.

Methods: This cross-sectional study utilized weighted population-based data from Cycle 3 (2012 – 2013) of the Canadian Health Measures Survey (CHMS). Information was collected via a home interview and a visit to a mobile examination centre. The weighted sample represented 6,914,124 adults in Canada aged 18–79 who were not taking any thyroid-related medication. Urinary fluoride concentrations were measured in spot samples using an ion selective electrode and adjusted for specific gravity (UF_{SG}). Serum TSH levels provided a measure of thyroid function. Multivariable regression analyses examined the relationship between UF_{SG} and TSH, controlling for covariates.

Results: Approximately 17.8% of participants fell in the moderately-to-severely iodine deficient range. The mean (SD) age of the sample was 46.5 (15.6) years and the median UF_{SG} concentration was 0.74 mg/L. Among iodine deficient adults, a 1 mg/L increase in UF_{SG} was associated with a 0.35 mIU/L increase in TSH [95% CI: 0.06, 0.64; p = 0.01, one-tailed].

Conclusions: Adults living in Canada who have moderate-to-severe iodine deficiencies and higher levels of urinary fluoride may be at an increased risk for underactive thyroid gland activity.

1. Introduction

Fluoride is an element that occurs either naturally in the environment or can be industrialized and added artificially to public drinking water to protect against dental caries. Approximately 38.7% of the population in Canada receives artificially fluoridated drinking water (Public Health Capacity and Knowledge Management Unit; Quebec Region for the Office of the Chief Dental Officer of Canada, 2017a). Provinces with the highest proportion of fluoridated drinking water include: Ontario, Manitoba, and the Northwest Territories, while provinces with the lowest proportion include: the Yukon Territories, British Columbia, Newfoundland, and Quebec (Public Health Capacity and Knowledge Management Unit; Quebec Region for the Office of the Chief Dental Officer of Canada, 2017b). The recommended fluoride concentration for drinking water in Canada is 0.7 mg/L (Government of Canada, 2017). However, the national average tap water fluoride concentration in Canada, including both fluoridated and non-fluoridated regions, is 0.12 mg/L (Canadian Health Measures Survey, 2017). Fluoride exposure can also occur from tea, beverages made with fluoridated water, processed foods, dental products, supplements, pharmaceuticals, and foods sprayed with fluoride-containing pesticides.

Hypothyroidism, the most common thyroid disorder, is characterized by suppression of thyroid gland activity. Subclinical hypothyroidism is indicated by high serum thyroid stimulating hormone (TSH) concentrations of 4.5–9 mIU/L with normal triiodothyronine (T_3) and thyroxine (T_4) levels. However, TSH levels above 2.5 mIU/L may increase risk for subclinical and clinical hypothyroidism (Demers & Spencer, 2002; Waise & Price, 2009). Subclinical hypothyroidism is estimated to occur in 4.3–9.5% of the US adult population (Hollowell et al., 2002; Canaris et al., 2000) and is associated with various health

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problems (Gonzalez Gil & de la Sierra, 2017; Pesic et al., 2015; Jayasingh & Puthuran, 2016), including: miscarriage and preterm birth among pregnant women, and altered growth and neurodevelopment among offspring (Maraka et al., 2016; Zhang et al., 2017; Murphy et al., 2015; Vrijkotte et al., 2017). Moreover, "high normal" TSH levels of 2.0–4.0 mIU/L and 2.5–4.5 mIU/L have been associated with hypocholesterolemia (Michalopoulou et al., 1998), and an increased risk of metabolic syndrome (Ruhla et al., 2010) respectively. Thus, studying factors that contribute to low thyroid function, even at the subclinical level, is of high public health importance.

Animal studies have shown reductions in T_3 and T_4 levels due to fluoride exposure, even at low doses (Cinar, 2005; Bobek et al., 1976). In humans, fluoride in drinking water, even at levels as low as 0.3–0.5 mg/L, have predicted elevated TSH concentrations (Kheradpisheh et al., 2018; Bachinskii et al., 1985). Higher water fluoride concentrations have also predicted an increased likelihood of a hypothyroidism diagnosis among adults (Kheradpisheh et al., 2018; Peckham et al., 2015) and an increased incidence of diabetes among adults in the United States (Fluegge, 2016) and children in Canada (Chafe et al., 2018). However, these findings were from ecological studies and thus did not control for important confounders. Higher urinary fluoride and TSH levels have also been observed among children and adolescents living in endemic fluorosis areas (Singh et al., 2014; Khandare et al., 2018). In contrast, other studies have not found significant differences in thyroid hormone levels or thyroid disorder diagnoses as a function of urinary fluoride levels (Barberio et al., 2017).

Iodine deficiency can contribute to decreased thyroid hormone production and exacerbate the thyroid-disrupting effects of certain chemicals, as well as fluoride (Jiang et al., 2016; Yang et al., 1994; Xu & Zhang, 1994). Adequate iodine levels can offset adverse goitrogenic effects of fluoride (Xu & Zhang, 1994; Zhao et al., 1998). Fluoride exposures of 0.05–0.13 mg/kg/day have been associated with adverse thyroid effects among iodine sufficient people, while lower fluoride exposures of 0.01–0.03 mg/kg/day have been associated with these effects among iodine deficient people (National Research Council, 2006). Synergistic effects of high fluoride and deficient iodine have also been found among both animals (Zhao et al., 1998; Guan et al., 1988; Ge et al., 2005) and humans (Lin et al., 1991). Still, few studies have considered iodine as a moderator of fluoride's effects on thyroid function as large sample sizes are needed for assessment of effect modification. Cycle 2 (2009–2011) data from the CHMS indicated low iodine intakes among 22% of Canadians aged 3 to 79 (Statistics Canada, 2013; World Health Organization, 2013).

We examined whether the relationship between fluoride exposure and thyroid function is modified by iodine status among adults participating in a Canadian population-based survey. We hypothesized that higher urinary fluoride levels would predict higher TSH levels and that this relationship would be stronger among adults with moderate-to-severe iodine deficiencies.

2. Materials and methods

2.1. Participants

We utilized data from Cycle 3 of the Canadian Health Measures Survey (CHMS, 2012–2013) because it was the first cycle to include thyroid hormone measurements (data from Cycle 4 were not available at the outset of this study). The CHMS is an ongoing survey launched by Statistics Canada, Health Canada and the Public Health Agency of Canada in 2007 to collect health and wellness data and biological specimens on a nationally representative sample of Canadians. Cycle 3 was conducted between January 2012 and December 2013. It consisted of 5785 Canadians ages 3 to 79 recruited from 16 sites across all ten provinces with 2671 people providing urine and blood samples for fluoride and TSH analysis (out of the approximately 2950 who were asked to do so). People living in the three territories, on reserves or other aboriginal settlements in the provinces, full-time members of the Canadian forces, institutionalized people, and those living in remote areas were not sampled. The overall response rate for all aspects of Cycle 3 was 79% (Statistics Canada, 2015; Begin, 2015).

Information was collected from participants via a computer-assisted home interview and a visit to a mobile examination centre where biospecimens and physical measures were collected by trained professionals. Cases of hypo- or hyperthyroidism were identified from information gathered directly from the interview; participants were asked: "Remember we are interested in conditions diagnosed by a health professional … Do you have a thyroid condition?". Data on medication usage during the past month was also collected and photographs of medication bottles were obtained as part of the household questionnaire. Medication usage was later confirmed during mobile examination centre visits. This enabled identification of which participants were using thyroid or anti-thyroid medication. Individuals who were on these medications and/or reporting a thyroid condition diagnosed by a health professional were excluded from our analyses. Pregnant women were also excluded given that pregnancy may increase stress on the thyroid and demand for iodine intake. We limited our analyses to adults aged 18 and over (with urinary fluoride and TSH levels) due to the low prevalence of hypothyroidism in younger individuals. Finally, we excluded participants who had iodine levels above the WHO cut-off of 2.37 μmol/L for excess iodine levels (Iodine Status Worldwide: WHO Global Database on Iodine Deficiency, 2004). We removed these participants because excess iodine levels can cause abnormalities in TSH (Katagiri et al., 2017), including elevations, and we wanted to test the relationship between fluoride exposure and TSH as a function of iodine deficiency, not iodine excess. Approximately 1% of participants reported having kidney disease, and they were equally likely to have adequate or deficient iodine levels. Since these individuals could be particularly vulnerable to adverse effects of fluoride on the thyroid gland, we did not exclude them.

Approximately 1000 adults between the ages of 18 and 79 met the above inclusion criteria (exact number cannot be reported as per Statistics Canada reporting requirements). Using sampling weights provided by Statistics Canada, the weighted sample represented 6,914,124 adults. See Appendix A for frequencies of participants at each CHMS site according to fluoridation status.

The CHMS was approved by the Health Canada institutional review board and participants provided written informed consent. Detailed CHMS methodology has been published elsewhere (Statistics Canada, 2015; Labrecque, 2014) and full study details can be found at www.statscan.gc.ca. The present study also received ethics approval from the York University Research Ethics Board (Certificate e2018–233).

2.2. Fluoride measure

Fluoride was measured in urine which provides a valid measure of exposure given that urinary fluoride levels have been shown to directly correlate with water fluoride concentration levels in adults (Ahmed et al., 2012; Mansfield, 1999) Approximately 40% of absorbed fluoride is excreted in urine, while 60% is absorbed in calcified tissue (Barbier et al., 2010). Spot urine samples were collected under normal (not fasting) conditions and not standardized with respect to time of collection. Urinary fluoride concentrations were analyzed using an Orion PH meter with a fluoride ion selective electrode after being diluted with an ionic adjustment buffer (Institut National de Sante Publique du Quebec (INSPQ), 2009). To account for variations in urine dilution, urinary fluoride concentrations were adjusted for specific gravity (UF_{SG}; mg/L). A reference value for UF_{SG} among adults unexposed to community water fluoridation is geometric mean = 0.613 mg/L (95% CI of 0.21–1.6 mg/L) (Usuda et al., 2007). Analyses were performed at the Toxicology Laboratory of the INSPQ (accredited under ISO 17025) under standardized operating procedures (Statistics Canada, 2015). Fluoride concentrations in tap water were also measured via a basic anion exchange chromatography procedure.

2.3. Measure of thyroid gland function

TSH levels were utilized as the primary measure of thyroid function because they provide a sensitive index of both subclinical and clinical thyroid dysfunction. Blood samples were collected at the mobile centre examination visit by a phlebotomist using a standard venipuncture method. The time of day of blood collection was not standardized. Serum TSH levels were measured using a third generation assay analyzer equipped with a chemiluminescent detection system (Health Canada, 2012a); the reference interval for TSH was 0.55–4.78 mIU/L. Serum levels of free T4 were analyzed using a competitive chemiluminescent immunoassay (Health Canada, 2012b) and the reference interval was 11.5–22.5 pmol/L. Thyroid hormones were analyzed at the INSPQ on the Siemens ADVIA Centaur XP analyzer (Health Canada, 2012b).

2.4. Measure of iodine

Iodine level was measured in spot urine samples by colorimetric microplate assay. The limit of detection (LoD) was 0.20 μmol/L (Health Canada, 2012c). For iodine values that fell below the limit of detection, we used an imputed value of LoD/√2 (Hornung & Reed, 1990). Urinary iodine is considered a valid measure of population level iodine status and a highly sensitive measure of dietary iodine intake (Pearce & Caldwell, 2016; Zimmermann & Andersson, 2012). Moderate-to-severe iodine deficiency was defined as urinary iodine ≤0.38 μmol/L using guidelines established by the World Health Organization (Iodine Status Worldwide: WHO Global Database on Iodine Deficiency, 2004). Participants were considered to have adequate levels of iodine if the urinary iodine value fell between > 0.38 and ≤2.37 μmol/L.

2.5. Covariates

Covariates were selected a priori based on being empirically related to thyroid function and/or fluoride absorption in the body. They included: age, sex, body mass index (BMI), and serum calcium level. Serum calcium was measured by colorimetric Arsenazo III dye-binding on the Ortho Clinical Diagnostics Vitros 5,1FS analyzer. Urinary iodine was considered an effect modifier because of iodine's important role in thyroid function and potential protective effect on the thyroid from fluoride. Age, sex and BMI were included because they can influence TSH levels (Shinkov et al., 2014; Sanyal & Raychaudhuri, 2016). Lastly, serum calcium was included because dietary calcium intake may affect fluoride absorption in the body (National Research Council, 2006) and thyroid hormone regulation (Kališnik et al., 1990).

2.6. Statistical analysis

Data analysis was conducted at the Research Data Centre at York University in Toronto. As directed by Statistics Canada, all models applied survey weights to take into account aspects of the sample design, such as stratification, in order to permit generalization to the entire Canadian population. A survey weight and 500 bootstrap weights were applied to ensure more accurate model and variance estimations. Stata (V.14.X) software was used for all analyses.

Spearman correlations were used to test relationships between urinary fluoride, urinary iodine and TSH. Linear regression was used to model TSH levels as a function of urinary fluoride and iodine level while controlling for covariates. The potential modifying effect of iodine status, defined as urinary iodine deficiency ≤0.38 μmol/L (yes/no) was analyzed as an interaction term; yes (urinary iodine ≤0.38 μmol/L) = 1 and no (urinary iodine > 0.38 μmol/L) = 0. For ease of interpretability, we also recoded our model with iodine deficient participants as our reference. Assumptions pertaining to normality, homogeneity of variance and linearity were satisfied. Regression diagnostics identified a few extreme cases with high TSH levels (exact number of cases cannot be reported due to confidentiality requirements by Statistics Canada). Although these extreme cases had large standardized residuals and high Cook's distance in unweighted analyses, these TSH values were biologically plausible and thus not indicative of an error in data collection or entry. Therefore, they were included in the analyses. These few deemed outliers in the unweighted analyses were equally balanced between iodine deficient and non-deficient groups. In the weighted analysis, these few cases were found to have survey weights close to the mean of the survey weights, such that they were neither strongly weighted up nor strongly weighted down. Given our directional hypothesis, a one-tailed alpha of 0.05 was the criteria for statistical significance for the applicable variables in the regression analyses.

3. Results

3.1. Population characteristics

Missing data were < 5% in all analyses except for household income which was reported by 77% of respondents; however, Statistics Canada provided imputed estimates for these missing values. Approximately 1,346,329 people (17.8% of the overall sample weighted to the population) fell in the moderately-to-severely iodine deficient range. Demographic characteristics for the overall sample (excluding participants with excess iodine levels), adults with moderate-to-severe iodine deficiencies, and adults without iodine deficiencies or excesses are presented in Table 1.

Descriptive statistics for urinary iodine, TSH, free thyroxine, UF_{SG} and tap water fluoride concentrations as a function of iodine status are presented in Table 2. See Supplemental Table S1 for UF_{SG} concentrations according to age and sex. Tap water fluoride concentrations were low overall (median = 0.11 mg/L) with all values falling below the recommended level (≤0.70 mg/L) for community water fluoridation; water fluoride concentrations were lower among iodine deficient participants (median = 0.07 mg/L; range (10th - 90th percentile) = 0.00 - 0.27 mg/L) than non-iodine deficient participants (median = 0.12 mg/L; range (10th - 90th percentile) = 0.00 - 0.61 mg/L). Arithmetic mean UF_{SG}

Table 1
Demographic characteristics for the overall sample and according to iodine status.

	All adults[1] (N = 6,914,124)[2]	Adults with moderate/severe iodine deficiencies (N = 1346, 329)	Adults without moderate/severe iodine deficiencies (N = 5,567,795)[1]
Age (yrs.); M (SD)	46.49 (15.55)	48.21 (15.61)	46.08 (15.50)
Sex; N (%)			
Male	3,563,836 (51.54)	696,775 (51.75)	2,867,061 (51.49)
Female	3,350,288 (48.46)	649,554 (48.25)	2,700,734 (48.51)
BMI; M (SD)	26.95(4.94)	26.06 (4.05)	27.16 (5.11)
BMI Categories[3]; N (%):			
Normal		606,795 (45.14)	2,100,051 (37.90)
Overweight		569,152(42.34)	1,998,364 (36.06)
Obese		168,213(12.51)	1,442,598 (26.03)
Total household income; M (SD)	$93,659.95 ($64,183.32)	$93,881.67 ($69,655.63)	$93,606.34 ($62,788.40)

Abbreviations: BMI = Body mass index; M = Mean; SD = standard deviation.
[1] Includes descriptive statistics for the sample after the removal of participants with excess iodine levels.
[2] The total N may differ for some variables due to missing biomonitoring data. The total N for the entire sample size weighted to the Canadian population is shown.
[3] Normal: 18.50 ≤ BMI ≤ 24.99; Overweight: 25.00 ≤ BMI ≤ 29.99; Obese: BMI ≥ 30.

Table 2
Thyroid hormone, fluoride, iodine and calcium concentrations.

	Arithmetic mean	Standard deviation	Median	10th percentile[3]	90th percentile[3]
All adults					
TSH (mIU/L)	1.79	1.23	1.79	0.79	2.87
Free thyroxine (pmol/L)	14.55	2.07	14.4	12.2	17.6
UF_{SG} (mg/L)	0.94	1.05	0.74	0.34	1.73
Urinary iodine (μmol/L)	0.85	0.50	0.77	0.28	1.52
Tap water fluoride (mg/L)	0.22	0.24	0.11	0.00	0.60
Serum Calcium (mmol/L)	2.41	0.30	2.40	2.29	2.51
Iodine deficient[1] adults					
TSH (mIU/L)	1.66	1.25	1.46	0.83	2.41
Free thyroxine (pmol/L)	14.69	2.41	14.2	11.6	17.8
UF_{SG} (mg/L)	1.06	1.11	0.74	0.29	2.09
Urinary iodine (μmol/L)	0.25	0.08	0.27	0.14	0.36
Tap water fluoride (mg/L)	0.12	0.16	0.07	0.00	0.27
Serum calcium (mmol/L)	2.43	0.38	2.41	2.32	2.52
Non-iodine deficient[2] adults					
TSH (mIU/L)	1.82	1.22	1.54	0.77	2.94
Free thyroxine (pmol/L)	14.52	1.97	14.4	12.4	17.2
UF_{SG} (MG/L)	0.91	0.65	0.72	0.36	1.60
Urinary iodine (μmol/L)	0.99	0.45	0.87	0.50	1.56
Tap water fluoride (mg/L)	0.25	0.25	0.12	0.00	0.61
Serum calcium (mmol/L)	2.41	0.30	2.40	2.28	2.51

Note. Reported concentrations are for the entire sample, including outliers, but with those who had excess urinary iodine levels excluded.
UF_{SG} = urinary fluoride adjusted for specific gravity.
[1] Moderate to severe iodine deficiency defined as urinary iodine ≤0.38 μmol/L.
[2] Non-iodine deficient defined as urinary iodine > 0.38 to ≤2.37 μmol/L.
[3] Values falling at the more extreme values, such as the 5th and 95th percentiles, were unreleasable due to minimum sample size requirements set by Statistics Canada. Parametric and non-parametric analyses cannot be conducted to test differences in distributions using weighted data and thus are not reported.

concentrations however were higher among iodine deficient participants (M = 1.06 mg/L; SD = 1.11) than non-iodine deficient participants (M = 0.91 mg/L; SD = 0.65, Cohen's d = 0.165). However, geometric means for UF_{SG} concentrations were identical (0.80 mg/L) and median values were similar between the iodine deficient and sufficient participants (median values = 0.74 and 0.72 mg/L, respectively). TSH and free T4 values at the 10th and 90th percentile for the population fell within the standard reference range, while outlying TSH and T4 values did not.

Lower urinary iodine was associated with higher UF_{SG} in the entire sample ($\rho = -0.11$, $p < 0.000$). This negative association was observed among both adults with adequate iodine ($\rho = -0.11$, $p = 0.003$), and adults with moderate-to-severe iodine deficiencies ($\rho = -0.15$, $p = 0.04$). TSH levels were weakly correlated with urinary iodine levels for the entire population ($\rho = 0.09$, $p = 0.003$), but the relationship did not reach significance when participants were subset by iodine status. UF_{SG} was not significantly correlated with free thyroxine.

3.2. Regression results

After adjustment for covariates, UF_{SG} did not significantly predict TSH levels among iodine sufficient adults (UF_{SG}: B = −0.02, t = −0.19, p = 0.43), but iodine status did (B = −0.55, t = −4.43, p = 0.00). (Table 3). Serum calcium also positively predicted TSH (B = 1.87, t = 2.21, p = 0.03).

3.2.1. Evaluation of effect modification

Iodine status was found to modify the association between UF_{SG} and TSH (B = 0.36, t = 1.84, p = 0.03) (Table 3). In the recoded model (i.e. using iodine deficiency as the reference group), a 1 mg/L increase in UF_{SG} was associated with a 0.35 mIU/L increase in TSH (B = 0.35, t = 2.33, p = 0.01; [95% CI: 0.06, 0.64]) among adults with iodine deficiency (Fig. 1).

We conducted a supplemental regression analysis to determine if the results were maintained when urinary fluoride was adjusted for urinary creatinine (UF_{CR}) according to described methods (Analytical Method for the Determination of Urine Creatinine of Hitachi 917 (C-530); Condensed Veresion for the CHMS, [Press Release], 2008) instead of specific gravity. This was indeed the case (see Supplemental Table S2).

Table 3
Linear regression predicting serum TSH with UF_{SG}.

Variable	B	95% Confidence interval	Standard error	t	p
UF_{SG} [1]	−0.02	- 0.19, 0.15	0.09	−0.19	0.43
Urinary iodine	−0.55	−0.80, −0.31	0.12	−4.43	**0.00**
UF_{SG}*urinary iodine [1]	0.36	−0.03, 0.75	0.20	1.84	**0.03**
Age	0.00	−0.00, 0.01	0.00	1.17	0.24
BMI	0.02	−0.02, 0.06	0.02	1.02	0.31
Serum calcium	1.87	0.21, 3.53	0.85	2.21	**0.03**
Sex	0.04	−0.20, 0.27	0.12	0.30	0.77

Note. UF_{SG} = urinary fluoride adjusted for specific gravity; UF_{SG}*Urinary Iodine = the interaction between urinary fluoride and iodine status; BMI = Body Mass Index; UF_{SG}*Urinary Iodine and Urinary Iodine were the only analyses that considered iodine status.
[1] p values for UF_{SG} and UF_{SG}*Urinary Iodine are for one-tailed tests. All other analyses were non-directional and p values are reported for two-tailed tests.

3.2.2. Sensitivity analysis

We conducted a sensitivity analysis to determine whether UF_{SG} predicted TSH among adults with moderate-to-severe iodine deficiencies after participants with outlying TSH values were removed (Supplemental Table S3). The interaction effect remained significant (albeit weaker) if we excluded the outliers. Serum calcium was no longer a significant predictor of TSH in this analysis. These results remained consistent if we conducted our analysis using UF_{CR} instead of UF_{SG} (Supplemental Table S4).

4. Discussion

We examined the relationship between urinary biomarkers of fluoride exposure and TSH levels as a function of iodine status in a

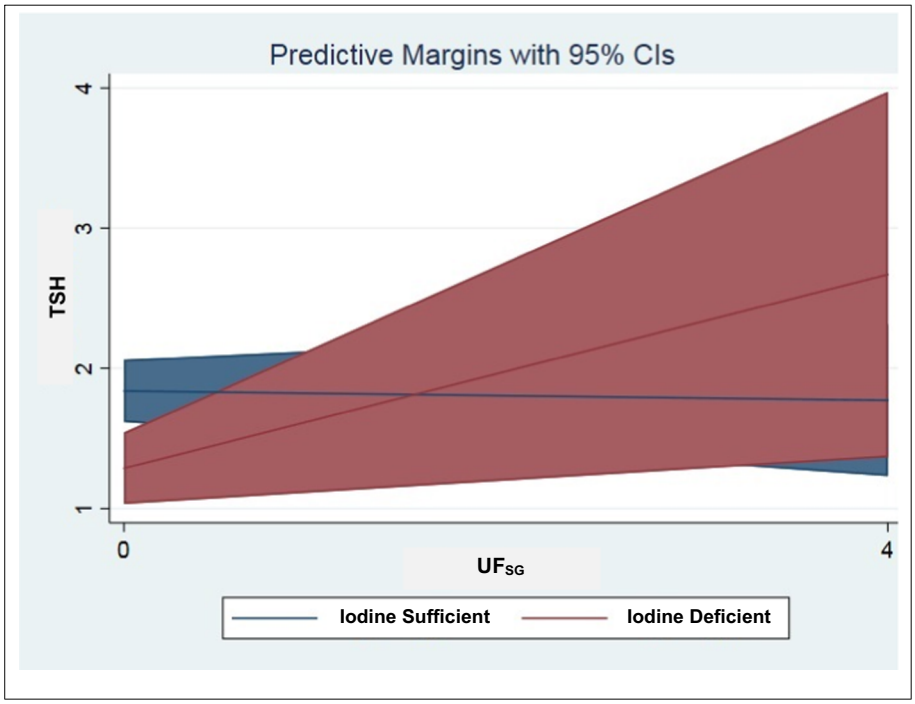

Fig. 1. The interaction between UF_{SG} and iodine status in predicting TSH levels
Note. UF_{SG} = urinary fluoride adjusted for specific gravity; TSH = thyroid stimulating hormone; iodine sufficient defined as urinary iodine > 0.38 to ≤2.37 μmol/L; iodine deficient defined as urinary iodine ≤0.38.

Canadian population exposed to artificially fluoridated and non-fluoridated drinking water. The distribution of participants in our sample living in fluoridated or non-fluoridated regions was comparable to that of the Canadian population (Public Health Capacity and Knowledge Management Unit; Quebec Region for the Office of the Chief Dental Officer of Canada, 2017a). We utilized TSH levels as the outcome since TSH is sensitive to both clinical and subclinical thyroid dysfunction. We considered iodine status given its importance for thyroid health, and its potential impact for effect modification of fluoride on thyroid function.

Higher urinary fluoride levels were not associated with higher TSH levels in the general population of adults living in Canada. Thus, we did not find evidence that fluoride exposure, when considered by itself, contributes to thyroid dysfunction among this general population. However, consistent with our hypothesis, participants' iodine status modified the relationship between urinary fluoride and TSH such that adults with moderate-to-severe iodine deficiencies who had higher urinary fluoride levels also tended to have higher TSH levels, after adjustment for covariates. Among these iodine deficient adults, a 1 mg/L increase in UF_{SG} was associated with a 0.35 mIU/L increase in TSH; and results of urine adjusted for creatinine (UF_{CR}) were very similar. To put this into context, a UF_{SG} concentration of 1.0 mg/L is approximately the 70th percentile for adults in our sample. The association between urinary fluoride and TSH is not apparent, however, in the absence of considering iodine status as an effect modifier. This is consistent with other recent research examining fluoride exposure and thyroid hormone levels among adults in Cycle 3 of the CHMS that did not consider iodine status (Barberio et al., 2017).

Consistent with Cycle 2 CHMS data (Statistics Canada, 2013), it was notable that almost 18% of our sample had moderate-to-severe iodine deficiency. Among this 18%, individuals who have higher urinary fluoride levels, and likely other risk factors for thyroid dysfunction, may be at an increased risk for underactive thyroid gland activity. Our finding that iodine status independently predicted higher TSH levels after controlling for covariates provides further support for iodine's important role in thyroid hormone regulation. Indeed, two prior epidemiological studies examining fluoride exposure and thyroid hormones comment on the importance of considering this nutrient even though neither did (Peckham et al., 2015; Barberio et al., 2017).

Interestingly, although iodine deficient individuals tended to be exposed to lower water fluoride concentrations, they had higher arithmetic mean urinary fluoride concentrations and a greater range of UF_{SG} values (explaining why the geometric means were identical between groups) compared with the non-iodine deficient group. This may reflect differences in intake of water or other sources of fluoride, or differences in fluoride excretion and retention between iodine deficient and non-deficient individuals. Moreover, since urinary fluoride concentrations irrespective of iodine status were relatively high considering overall low tap water concentrations (Massmann, 1981), it may be that drinking water is not the primary source of fluoride exposure among our sample. Indeed, a significant proportion of Canadians consume tea (Garriguet, 2008) which can provide a major dietary source of fluoride (Dabeka & McKenzie, 1995; Mostafaei et al., 2015).

There are several potential mechanisms by which fluoride and iodine may interact to affect thyroid function. First, fluoride interferes with Na/K-ATPase (Suketa et al., 1995; Murphy & Hoover, 1992) and iodothyronine deiodinase (Shashi & Singla, 2013), two enzymes that are important for thyroid function. Na/K-ATPase maintains functionality of the sodium iodide symporter which facilitates thyroidal iodide uptake (Nicola et al., 2014). Iodothyronine deiodinase catalyzes T_3 from deiodination of T_4 (Bianco & Larsen, 2005), and therefore, interference from fluoride could decrease T_3 production, and subsequently increase TSH. Fluoride has also been shown to inhibit prolactin, which promotes thyroidal iodine uptake, lowers T4 secretion and inhibits stimulatory effects of exogenous TSH (Ortiz-Perez et al., 2003; Rillema & Rowady, 1997; Grau & Stetson, 1977). Consistent with our findings, interference by fluoride with any of these mechanisms would result in more pronounced adverse effects on the thyroid gland among individuals with iodine deficiencies because their iodine stores would be more readily depleted.

There is also evidence that despite fluoride's lighter atomic weight, iodine may contribute to increased excretion of fluoride from the body

(Xu & Zhang, 1994; Zhao et al., 1998). One study conducted in China found a 40% higher prevalence of dental fluorosis among individuals living in a community with low water iodine levels than those in a community with sufficient water iodine levels, despite both being fluoridated at 0.8 mg/L (Xu & Zhang, 1994). Prevalence of dental fluorosis and bone fluoride concentrations have also been shown to be significantly higher among rats with iodine deficiencies than rats with normal or excess iodine levels, despite both having equivalent excess fluoride concentration exposures (Zhao et al., 1998). These findings imply that deficient iodine intake may lead to increased fluoride absorption. However, more research is needed to better understand the mechanism by which fluoride and iodine interact within the body to affect thyroid function.

Our study has some limitations. First, regarding our exclusion criteria, there may have been people who did not report taking thyroid hormone medications or having a thyroid condition even if they did. Furthermore, exclusion of participants who reported a thyroid condition (or taking thyroid medication) during the past month, as well as pregnant women, meant that those who may have been most sensitive to potential adverse effects of fluoride on thyroid function were excluded. Thus, the potential effect of fluoride exposure on TSH could have been underestimated in this study. Second, exposure to fluoride was assessed through spot urine samples that were not standardized with respect to time of sample collection. Due to the rapid elimination of fluoride (half-life of approximately 6 h) (Whitford, 1994), we expect temporal variation in fluoride intake because exposure may be impacted by day-to-day behaviors that were not controlled for (e.g. brushing with fluoridated toothpaste, consumption of non-fluoridated water prior to urine sampling, professionally applied fluoride, etc.). Still, exposure misclassification on the basis of the measurement technique should be non-differential and bias estimates towards the null. Third, the directionality of the association cannot be discerned due to the cross-sectional analysis. It is possible that chronic consumption of fluoridated products alters thyroid functioning in susceptible populations or that an overactive thyroid leads to excess thirst, which may result in higher urinary fluoride levels, especially among people consuming fluoridated water (McLaren, 2016). Fourth, fewer than 5% of the iodine-deficient individuals had tap water fluoride levels that fell near the recommended concentration for water fluoridation (i.e. 0.7 mg/L). Given that in some fluoridated communities, community water fluoridation accounts for 40–70% of daily fluoride intake in adults (Health and Ecological Criteria Division. Office of Water, 2010), the fluoride exposure levels observed in our iodine-deficient sample may underestimate fluoride exposure levels among individuals living in fluoridated regions. These limitations notwithstanding, spot sample urinary fluoride is a widely used exposure biomarker and an improvement over studies that do not incorporate biomonitoring data. Adjustment of urinary dilution effects (by correcting for both specific gravity and creatinine) provides a more reliable measure of internal fluoride exposure, increases the rigor of our findings and permits assessment of dose-response relationships.

5. Conclusions

Fluoride exposure among adults with moderate-to-severe iodine deficiencies living in Canada may increase risk for underactive thyroid gland activity. Additional studies are needed to clarify the mechanism by which fluoride and iodine interact in the body, as well as the effects of low level chronic fluoride exposure on thyroid function.

Declarations of interest

None.

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Appendix A. Number of participants at each CHMS Site included in Cycle 3 according to Water Fluoridation Status in 2012–2013

Site	Weighted N	% of total sample
Fluoridated		
Oshawa-Whitby	559,305	8.09
Brampton	498,143	7.20
Lethbridge	475,880	6.88
North Toronto	341,760	4.94
Halifax	239,913	3.47
Non-fluoridated		
Victoria-Saanich	593,585	8.59
Vancouver	455,034	6.58
East Montreal	364,210	5.27
Orillia	305,171	4.41
South Cent Laurentians	291,135	4.21
Mixed		
Brantford-Brant County	346,086	5.01

Kent County	254,835	3.69
West Montreal	687,586	9.94
Windsor	507,402	7.34
South West Calgary	634,261	9.17
South West Monteregie	359,821	5.20

Appendix B. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2018.09.026.

References

Ahmed, I.R.T., Hasan, S.K., Khan, N., Khan, M.H., Usmani, T.H., 2012. Correlation of fluoride in drinking water with urine, blood plasma, and serum fluoride levels of people consuming high and low fluoride drinking water in Pakistan. Fluoride 45, 384–388.

Analytical Method for the Determination of Urine Creatinine of Hitachi 917 (C-530); Condensed Veresion for the CHMS, [Press Release].

Bachinskii, P.P., Gutsalenko, O.A., Naryzhniuk, N.D., Sidora, V.D., Shliakhta, A.I., 1985. Action of the body fluorine of healthy persons and thyroidopathy patients on the function of hypophyseal-thyroid the system. Probl. Endokrinol. 31 (6), 25–29.

Barberio, A.M., Hosein, F.S., Quinonez, C., McLaren, L., 2017. Fluoride exposure and indicators of thyroid functioning in the Canadian population: implications for community water fluoridation. J. Epidemiol. Community Health 71 (10), 1019–1025.

Barbier, O., Arreola-Mendoza, L., Del Razo, L.M., 2010. Molecular mechanisms of fluoride toxicity. Chem. Biol. Interact. 188 (2), 319–333.

Begin, J., 2015. Overview of the Canadian Health Measures Survey. Statistics Canada.

Bianco, A.C., Larsen, P.R., 2005. Cellular and structural biology of the deiodinases. Thyroid 15 (8), 777–786.

Bobek, S., Kahl, S., Ewy, Z., 1976. Effect of long-term fluoride administration on thyroid hormones level blood in rats. Endocrinol. Exp. 10 (4), 289–295.

Canadian Health Measures Survey, 2017. Tap Water and Urine Fluoride Concentration Levels, 2014–2015 [Press Release].

Canaris, G.J., Manowitz, N.R., Mayor, G., Ridgway, E.C., 2000. The Colorado thyroid disease prevalence study. Arch. Intern. Med. 160 (4), 526–534.

Chafe, R., Aslanov, R., Sarkar, A., Gregory, P., Comeau, A., Newhook, L.A., 2018. Association of type 1 diabetes and concentrations of drinking water components in Newfoundland and Labrador, Canada. BMJ Open Diabetes Res. Care 6 (1), e000466.

Cinar, A.S.M., 2005. Effects of chronic fluorosis on thyroxine, triiodothyronine, and protein-bound iodine in cows. Fluoride 38 (1), 65–68.

Dabeka, R.W., McKenzie, A.D., 1995. Survey of lead, cadmium, fluoride, nickel, and cobalt in food composites and estimation of dietary intakes of these elements by Canadians in 1986–1988. J. AOAC Int. 78 (4), 897–909.

Demers, L., Spencer, C., 2002. Laboratory Medicine Practice Guidelines: Laboratory Support for the Diagnosis and Monitoring of Thyroid Disease. National Academy of Clinical Biochemistry.

Fluegge, K., 2016. Community water fluoridation predicts increase in age-adjusted incidence and prevalence of diabetes in 22 states from 2005 and 2010. J. Water Health 14 (5), 864–877.

Garriguet, D., 2008. Beverage Consumption of Canadian Adults. Statistics Canada.

Ge, Y.N.H., Wang, S., Wang, J., 2005. DNA damage in thyroid gland cells of rats exposed to long-term intake of high fluoride and low iodine. Fluoride 38 (4), 318–323.

Gonzalez Gil, L., de la Sierra, A., 2017. Prevalence of hypertension and other cardiovascular risk factors in subjects with subclinical hypothyroidism. Med. Clin. (Barc.) 148 (8), 351–353.

Government of Canada, 2017. Fluoride and Oral Health. https://www.canada.ca/en/health-canada/services/healthy-living/your-health/environment/fluorides-human-health.html, Accessed date: 1 January 2018.

Grau, E.G., Stetson, M.H., 1977. The effects of prolactin and TSH on thyroid function in Fundulus heteroclitus. Gen. Comp. Endocrinol. 33 (3), 329–335.

Guan, Z.Z., Zhuang, Z.J., Yang, P.S., Pan, S., 1988. Synergistic action of iodine-deficiency and fluorine-intoxication on rat thyroid. Chin. Med. J. 101 (9), 679–684.

Health and Ecological Criteria Division. Office of Water, 2010. Fluoride: Relative Source Contribution Analysis. United States Environmental Protection Agency.

Health Canada, 2012a. Analytic Procedure Manual: Third Generation Ultra THYROID STIMULATING HORMONE (TSH3-UL) on ADVIA Centaur XP. Division CRLNR.

Health Canada, 2012b. Analytic Procedure Manual: Free Thyroxine (FT4) on ADVIA Centaur XP: CHMS Reference Laboratory. Nutrition Research Division.

Health Canada, 2012c. In: Trans. Canada (Ed.), Urinary Iodine by Colorimetric Microplate Assay. CHMS Reference Laboratory NRD, Analytical Procedure Manual, pp. 1–8.

Hollowell, J.G., Staehling, N.W., Flanders, W.D., et al., 2002. Serum TSH, T(4), and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). J. Clin. Endocrinol. Metab. 87 (2), 489–499.

Hornung, R.W., Reed, L., 1990. Estimation of average concentration in the presence of nondetectable values. Appl. Occup. Environ. Hyg. 5, 46–51.

Institut National de Sante Publique du Quebec (INSPQ), 2009. Analytical Method for the Determination of Fluoride in Urine (M-186), Condensed Version for CHMS. Orion Research Inc, Quebec, QC.

Iodine Status Worldwide: WHO Global Database on Iodine Deficiency. Department of Nutrition for Health and Development - World Health Organization, Geneva.

Jayasingh, I.A., Puthuran, P., 2016. Subclinical hypothyroidism and the risk of hypercholesterolemia. J. Family Med. Prim. Care 5 (4), 809–816.

Jiang, Y., Guo, X., Sun, Q., Shan, Z., Teng, W., 2016. Effects of excess fluoride and iodide on thyroid function and morphology. Biol. Trace Elem. Res. 170 (2), 382–389.

Kališnik, M., Zorc-Pleskovič, R., Pajer, Z., Pavlin, K., 1990. The effect of chronic hypercalcemia or hypocalcemia on the follicular and parafollicular cells in rat thyroid gland. Am. J. Anat. 189 (3), 201–206.

Katagiri, R., Yuan, X., Kobayashi, S., Sasaki, S., 2017. Effect of excess iodine intake on thyroid diseases in different populations: a systematic review and meta-analyses including observational studies. PLoS One 12 (3), e0173722.

Khandare, A.L., Validandi, V., Gourineni, S.R., Gopalan, V., Nagalla, B., 2018. Dose-dependent effect of fluoride on clinical and subclinical indices of fluorosis in school going children and its mitigation by supply of safe drinking water for 5 years: an Indian study. Environ. Monit. Assess. 190 (3), 110.

Kheradpisheh, Z., Mirzaei, M., Mahvi, A.H., et al., 2018. Impact of drinking water fluoride on human thyroid hormones: a case- control study. Sci. Rep. 8 (1), 2674.

Labrecque, F.Q.A., 2014. Sampling Documentation for Cycle 3 of the Canadian Health Measures Survey. Internal Document. Statistics Canada, Ottawa, ON, Canada.

Lin, F.F., Aihaiti, Zhao, H.X., Lin, J., Jiang, J.Y., Maimaiti, Aiken, 1991. The relationship of a low-iodine and high-fluoride environment to subclinical cretinism in Xinjiang. IDD Newslett. 7 (3), 24–25.

Mansfield, P., 1999. The distribution of urinary fluoride concentration in the UK. Fluoride 32, 27–32.

Maraka, S., Ospina, N.M., O'Keeffe, D.T., et al., 2016. Subclinical hypothyroidism in pregnancy: a systematic review and meta-analysis. Thyroid 26 (4), 580–590.

Massmann, W., 1981. Reference values of renal excretion of fluoride. J. Clin. Chem. Clin. Biochem. 19 (10), 1039–1041.

McLaren, L., 2016. Fluoridation exposure status based on location of data collection in the Canadian health measures survey: is it valid? J. Can. Dent. Assoc. 82, g17.

Michalopoulou, G., Alevizaki, M., Piperingos, G., et al., 1998. High serum cholesterol levels in persons with 'high-normal' TSH levels: should one extend the definition of subclinical hypothyroidism? Eur. J. Endocrinol. 138 (2), 141–145.

Mostafaei, F., McNeill, F.E., Chettle, D.R., Wainman, B.C., Pidruczny, A.E., Prestwich, W.V., 2015. Measurements of fluorine in contemporary urban Canadians: a comparison of the levels found in human bone using in vivo and ex vivo neutron activation analysis. Physiol. Meas. 36 (3), 465–487.

Murphy, A.J., Hoover, J.C., 1992. Inhibition of the Na,K-ATPase by fluoride. Parallels with its inhibition of the sarcoplasmic reticulum CaATPase. J. Biol. Chem. 267 (24), 16995-700.

Murphy, N.C., Diviney, M.M., Donnelly, J.C., et al., 2015. The effect of maternal subclinical hypothyroidism on IQ in 7- to 8-year-old children: a case-control review. Aust. N. Z. J. Obstet. Gynaecol. 55 (5), 459–463.

National Research Council, 2006. Fluoride in Drinking Water: A Scientific Review of EPAs Standards Washington, DC.

Nicola, J.P., Carrasco, N., Amzel, L.M., 2014. Physiological sodium concentrations enhance the iodide affinity of the Na+/I- symporter. Nat. Commun. 5, 3948.

Ortiz-Perez, D., Rodriguez-Martinez, M., Martinez, F., et al., 2003. Fluoride-induced disruption of reproductive hormones in men. Environ. Res. 93 (1), 20–30.

Pearce, E.N., Caldwell, K.L., 2016. Urinary iodine, thyroid function, and thyroglobulin as biomarkers of iodine status. Am. J. Clin. Nutr. 104 (Suppl. 3), 898s–901s.

Peckham, S., Lowery, D., Spencer, S., 2015. Are fluoride levels in drinking water associated with hypothyroidism prevalence in England? A large observational study of GP practice data and fluoride levels in drinking water. J. Epidemiol. Community Health 69 (7), 619–624.

Pesic, M.M., Radojkovic, D., Antic, S., Kocic, R., Stankovic-Djordjevic, D., 2015. Subclinical hypothyroidism: association with cardiovascular risk factors and components of metabolic syndrome. Biotechnol. Biotechnol. Equip. 29 (1), 157–163.

Public Health Capacity and Knowledge Management Unit; Quebec Region for the Office of the Chief Dental Officer of Canada, 2017a. Table 1: provincial and territorial estimates for fluoridated water systems coverage, 2017. In: The State of Community Water Fluoridation across Canada. Public Health Agency of Canada, Canada.

Public Health Capacity and Knowledge Management Unit; Quebec Region for the Office of the Chief Dental Officer of Canada, 2017b. Table 3: provincial and territorial estimates for total community water fluoridation coverage, 2017. In: The State of Community Water Fluoridation across Canada. Public Health Agency of Canada.

Rillema, J.A., Rowady, D.L., 1997. Characteristics of the prolactin stimulation of iodide uptake into mouse mammary gland explants. Proc. Soc. Exp. Biol. Med. 215 (4), 366–369.

Ruhla, S., Weickert, M.O., Arafat, A.M., et al., 2010. A high normal TSH is associated with the metabolic syndrome. Clin. Endocrinol. 72 (5), 696–701.

Sanyal, D., Raychaudhuri, M., 2016. Hypothyroidism and obesity: an intriguing link. Indian J. Endocrinol. Metab. 20 (4), 554–557.
Shashi, A., Singla, S., 2013. Clinical and biochemical profile of deiodinase enzymes and thyroid function hormones in patients of fluorosis. Aust. J. Basic Appl. Sci. 7 (4), 100–107.
Shinkov, A., Borissova, A.M., Vlahov, J., Dakovska, L., Blajeva, E., 2014. Male gender differences in the thyroid ultrasound features, thyroid peroxidase antibodies and thyroid hormone levels: a large population-based study. J. Endocrinol. Investig. 37 (3), 269–276.
Singh, N., Verma, K.G., Verma, P., Sidhu, G.K., Sachdeva, S., 2014. A comparative study of fluoride ingestion levels, serum thyroid hormone & TSH level derangements, dental fluorosis status among school children from endemic and non-endemic fluorosis areas. Springerplus 3, 7.
Statistics Canada, 2013. USI ensures adequate iodine intake in Canada. IDD Newslett. 1.
Statistics Canada, 2015. Canadian Health Measures Survey (CHMS) Data User Guide: Cycle 3. Ottawa, ON.
Suketa, Y., Suzuki, K., Taki, T., et al., 1995. Effect of fluoride on the activities of the Na+/glucose cotransporter and Na+/K(+)-ATPase in brush border and basolateral membranes of rat kidney (in vitro and in vivo). Biol. Pharm. Bull. 18 (2), 273–278.
Usuda, K., Kono, K., Shimbo, Y., et al., 2007. Urinary fluoride reference values determined by a fluoride ion selective electrode. Biol. Trace Elem. Res. 119 (1), 27–34.
Vrijkotte, T.G., Hrudey, E.J., Twickler, M.B., 2017. Early maternal thyroid function during gestation is associated with fetal growth, particularly in male newborns. J. Clin. Endocrinol. Metab. 102 (3), 1059–1066.
Waise, A., Price, H.C., 2009. The upper limit of the reference range for thyroid-stimulating hormone should not be confused with a cut-off to define subclinical hypothyroidism. Ann. Clin. Biochem. 46 (2).
Whitford, G.M., 1994. Intake and metabolism of fluoride. Adv. Dent. Res. 8 (1), 5–14.
World Health Organization, 2013. Urinary Iodine Concentrations for Determining Iodine Status in Populations.
Xu, Y.L.C., Zhang, X., 1994. The effect of fluorine on the level of intelligence in children. Endem. Dis. Bull. 9 (2), 83–84.
Yang, Y., Wang, X., Guo, X., 1994. Effects of high iodine and high fluorine on children's intelligence and the metabolism of iodine and fluorine. Zhonghua Liu Xing Bing Xue Za Zhi 15 (5), 296–298.
Zhang, Y., Wang, H., Pan, X., Teng, W., Shan, Z., 2017. Patients with subclinical hypothyroidism before 20 weeks of pregnancy have a higher risk of miscarriage: a systematic review and meta-analysis. PLoS One 12 (4), e0175708.
Zhao, W., Zhu, H., Yu, Z., Aoki, K., Misumi, J., Zhang, X., 1998. Long-term effects of various iodine and fluorine doses on the thyroid and fluorosis in mice. Endocr. Regul. 32 (2), 63–70.
Zimmermann, M.B., Andersson, M., 2012. Update on iodine status worldwide. Curr. Opin. Endocrinol. Diabetes Obes. 19 (5), 382–387.