

Attn: Perchlorate_Comments_for OIG@epa.gov

From: Steven H. Lamm, MD, DTPH* and Ryan Monroe BA⁺

Date: March 10, 2009

Submission of Vanderver, et al. (2007)

Greetings,

I am submitting to the record the attached published paper by Vanderver et al. (2007) entitled "Cigarette Smoking and iodine as Hypothyroxinemic Stressors in U.S. Women of Childbearing Age: A NHANES III Analysis (Thyroid, August 2007; 17(8):741-746). EPA-OIG has identified thiocyanate exposure as the predominant NIS inhibitor exposure

Clinical Research Papers

Cigarette Smoking and Iodine as Hypothyroxinemic Stressors in U.S. Women of Childbearing Age: A NHANES III Analysis

G. Bruce Vanderver,¹ Arnold Engel,² and Steven Lamm²

Background: Exposure to environmental thiocyanate through smoking has been suggested to lead to hypothyroxinemia, which potentially impairs brain development in the fetuses of affected women, though studies are conflicting. It was hypothesized that iodine status might modulate the effects of thiocyanate exposure on the prevalence of hypothyroxinemia in women of childbearing age. *Design:* The study population comprised 6967 women (age range: 15–44 years) from the National Health and Nutrition Examination Survey (NHANES) III database. Smoking status was stratified into nonsmokers and those who smoked 1–10, 11–20, 21–30, and 31+ cigarettes a day. Iodine status was stratified based on urinary iodine excretion as <50, 50–99, 100–199, 200–299, and 300+ $\mu\text{g/L}$. Hypothyroxinemia was defined as the lower fifth percentile of total thyroxine levels among nonsmokers, adjusted for age and race/ethnicity. Univariate, multivariate, and regression analyses were conducted to evaluate the impact of smoking and urinary iodine excretion on the prevalence of hypothyroxinemia. *Results:* Increasing levels of cigarette smoking are associated with increasing prevalence of hypothyroxinemia [$\chi^2(4) = 14.15, p = 0.007$]. When analyzed by urinary iodine level, the hypothyroxinemic effect of smoking was limited to the highest two urinary iodine strata [$\chi^2(4) = 41.48, p < 0.001$; and $\chi^2(4) = 40.62, p < 0.001$]. A significant interaction effect between smoking and urinary iodine was noted, underscoring the relationship between high levels of urinary iodine excretion and smoking with respect to hypothyroxinemia. *Conclusions:* Heavy smoking was associated with a higher prevalence of hypothyroxinemia. The impact of thiocyanate exposure from smoking on the prevalence of hypothyroxinemia is limited to those women of childbearing age with the highest urinary iodine excretion. Iodine supplementation should be cautiously considered in women of childbearing age who are smokers.

Introduction

RESIDENTS OF THE UNITED STATES are exposed to a variety of goitrogens that have the potential to adversely affect the thyroid by interfering with either the production or the metabolism of thyroid hormones (1). Many environmental goitrogens, including thiocyanate, act by decreasing the iodine uptake of the thyroid by competitively inhibiting the sodium/iodide symporter present on the surface of the thyroid epithelial cell. The combination of iodine deficiency and heavy exposure to environmental goitrogens has been shown to have an adverse effect on thyroid status (2).

Most cells utilize both triiodothyronine (T3) and thyroxine (T4) as a source of thyroid hormone with T3 being the intranuclear active form. The developing brain, however,

depends specifically on T4, especially during early development. Further, the fetal brain is dependent on the maternal supply of T4 to meet its own needs; maternal T4 is then metabolized intracellularly by the fetus to T3. As such, maternal thyroidal insufficiency may impact fetal neurodevelopment (3).

Severe and moderate iodine insufficiency is associated with significant fetal intellectual impairment, most notably cretinism (4,5). Further, dietary exposure to environmental goitrogens (e.g., cassava) can aggravate the effects of moderate and severe iodine deficiency on intellectual development (2). Maternal hypothyroxinemia more generally has been suggested as a cause of fetal intellectual impairment (6,7). Correction of maternal hypothyroxinemia during pregnancy may eliminate its effects on intellectual development (8).

¹Preventive Medicine Residency, Johns Hopkins University Bloomberg School of Public Health, Baltimore, Maryland.

²Consultants in Epidemiology and Occupational Health, LLC, Washington, District of Columbia.

Cigarette smoking is the most ubiquitous exposure to thiocyanate, both in terms of frequency and dosage. Foss and Lund-Larsen (9) showed the dose dependency of serum thiocyanate levels with cigarette dosage among Swedish women. The mean serum thiocyanate level in nonsmokers was $33.5 \mu\text{mol/L}$, that for women smoking 5–9 cigarettes per day was $70.9 \mu\text{mol/L}$, and that for women smoking 25 or more cigarettes per day was $99.7 \mu\text{mol/L}$. Secondhand smoking was not shown to have a significant effect on serum thiocyanate levels. No differences were noted among nonsmokers with and without ambient exposure to environmental smoke, though some have found a weak correlation between environmental exposure and thiocyanate levels (10).

Smoking as the main nondietary environmental exposure to thiocyanate has been well studied. Several studies have shown a decrease in T4 levels among smokers (11–13), though other studies have failed to reproduce this finding (14,15). The lack of a consistent relationship between smoking and hypothyroxinemia may be dependent on other factors, including iodine status. It was hypothesized that environmental exposure to thiocyanate might modulate those at risk for hypothyroxinemia, particularly those with iodine insufficiency. Since hypothyroxinemia presents a potential risk to the developing fetus, this hypothesis was tested in women of childbearing age.

Methods

Population

The dataset of the third round of the National Health and Nutrition Examination Survey (NHANES III) contains interview and laboratory information on over 40,000 noninstitutionalized U.S. subjects who participated between 1988 and 1994. Informed consent was obtained from all study participants by the NHANES III administrators. The NHANES III study was approved by the Centers for Disease Control and Prevention (CDC)/National Center for Health Statistics (NCHS) Institutional Review Board (IRB). The study population for this analysis was selected as women of childbearing age (15–44 years) with data on serum T4 levels (total T4) and urinary iodine ($\mu\text{g/L}$), as well as on race/ethnicity and cigarette-smoking status. Among 8443 women of childbearing age (15–44 years) with identifiable race/ethnicity (non-Hispanic white, non-Hispanic black, or Mexican-American), 7053 had data on urinary iodine, serum T4 (total T4), and cigarette-smoking status. Eighty-six women taking levothyroxine were excluded from the study population, which resulted in a study cohort of 6967 subjects. 434 women were recorded as being pregnant.

Outcome measure

Serum total T4 levels were measured using an immunoassay for T4, with a reference range of $4.5\text{--}13.2 \mu\text{g/dL}$. Hypothyroxinemia was defined as the lower fifth percentile of total T4 levels for the study population stratified by age and ethnicity. Total T4 levels had been shown to be significantly affected by race/ethnicity and age (16). The study population was stratified by age (15–24, 25–34, and 35–44 years) and by race/ethnicity (non-Hispanic white, non-Hispanic black, or Mexican-American). The lower fifth percentile levels for each age and race/ethnicity stratification for the nonsmokers were used in these analyses to define hypothyroxinemia. The fifth

percentile values for total T4 were also calculated for the total subject population (i.e., smokers and nonsmokers combined).

Independent variables

Both iodine nutrition (urinary iodine excretion in $\mu\text{g/L}$) and cigarette smoking (in cigarettes per day) were used as independent variables in the analyses. Urine iodine levels, using the Sandell–Koltoff reaction, were measured on fasting urine samples obtained at the same time in the morning for all patients. Urine iodine levels were categorized as <50 , 50–99, 100–199, 200–299, and $300+ \mu\text{g/L}$. Current cigarette smoking was based on patient self-report and stratified by the number of cigarettes smoked per day (nonsmoker, 1–10, 11–20, 21–30, and 31+).

Statistical methods

Kruskal–Wallis tests were performed to assess the relationships between exposures and outcome. Chi-squared tests were performed to further delineate the relationships within the stratifications. The number of degrees of freedom is given in parentheses. Analysis of variance was conducted to evaluate the effect of independent variable interactions. Alpha values were set at 0.05, with a correction being made using the Bonferroni Step Down (Holm) correction method to account for multiple tests and Dunn's procedure for *post hoc* analyses. All analyses were performed using STATA 9.

Results

The study population comprised 6967 women (age range: 15–44 years) with reported urinary iodine excretion and cigarette-smoking status, and without thyroid medication. Among the 6967 women, 1703 were smokers and 5264 were nonsmokers (Table 1). Measuring serum cotinine levels is an alternative method for classifying participants as smokers and nonsmokers; however, these levels do not provide a satisfactory estimate of magnitude of smoking. The serum cotinine levels (stratified as >15 and $\leq 15 \text{ ng/mL}$) correlated well with the self-reported smoking status ($r = 0.86$). Elevated cotinine levels were only found in 254 (4.8%) of the subjects reporting themselves as being nonsmokers, thus confirming that self-reported smoking history was a good predictor of true smoking status.

Smoking prevalence among non-Hispanic whites and non-Hispanic blacks was significantly higher than among Mexican-Americans (31% vs. 27% vs. 13%, respectively) [$\chi^2(2) = 204.8, p < 0.001$]. The average age of the subjects was 30.5 years; however, smokers were significantly older than nonsmokers [$t(6965) = -3.55, p < 0.001$]. This was attributable to the significant difference in age among non-Hispanic blacks [$t(2358) = -8.7, p < 0.001$]; no difference in ages between smokers and nonsmokers was noted for Mexican-Americans and non-Hispanic whites.

Average total T4 levels were significantly lower among smokers than among nonsmokers [$t(6531) = 4.13, p < 0.001$]. While the difference between total T4 levels of smokers and nonsmokers was significantly different when stratified by race/ethnicity [$F(3, 6529) = 24.69, p < 0.001$], *post hoc* testing showed this to be significant for non-Hispanic blacks only [$t(2163) = 3.37, p = 0.002$]. Figure 1 demonstrates the variation in average total T4 levels by race/ethnicity and by age.

TABLE 1. CHARACTERISTICS OF THE SUBJECT POPULATION

	Subject numbers		
	Total	Nonsmokers	Smokers
All race/ethnicity	6967	5264 (76%)	1703 (24%)
Non-Hispanic white	2507	1727 (69%)	780 (31%)
Non-Hispanic black	2360	1721 (73%)	639 (27%)
Mexican-American	2100	1816 (87%)	284 (13%) ^a
		Average age (years)	
All race/ethnicity	30.5	30.4	31.1
Non-Hispanic white	31.1	31.6	29.9
Non-Hispanic black	30.7	29.8	33.1 ^a
Mexican-American	29.7	29.6	30.1
		Average thyroxine level µg/dL	
All race/ethnicity	9.34	9.41	9.13 ^a
Non-Hispanic white	9.31	9.34	9.25
Non-Hispanic black	9.09	9.19	8.81
Mexican-American	9.66	9.68	9.51
	<i>F</i> (2, 6530) = 32.08, <i>p</i> < 0.001	<i>F</i> (2, 4966) = 19.58, <i>p</i> < 0.001	<i>F</i> (2, 1564) = 11.34, <i>p</i> < 0.001

^a*p* < 0.001.

The study population was stratified by race/ethnicity and by age, and the lower fifth percentile of the serum T4 measures was determined (Table 2). The lower fifth percentile levels for the nonsmokers that were used in this analysis differed little from those for the nonsmokers and smokers combined.

The hypothyroxinemia prevalence was further examined in a multivariate analysis for both smoking habit (Fig. 2A) and urinary iodine excretion level (Fig. 2B). The prevalence of hypothyroxinemia was not constant across smoking strata [$\chi^2(4) = 14.15, p = 0.007$], with *post hoc* analysis demonstrating prevalence significantly elevated for those smoking 31+ cigarettes per day [$\chi^2(2) = 11.40, p = 0.014$]. There was a trend toward differing prevalence of hypothyroxinemia across the urinary iodine stratification, though it was not significant [$\chi^2(4) = 9.3, p = 0.055$]. *Post hoc* analysis suggested a trend toward higher prevalence of hypothyroxinemia for those with urinary iodine levels 200+ µg/L [$\chi^2(2) = 8.23, p = 0.08$].

The hypothyroxinemia prevalence was further examined in a multivariate analysis for both smoking habit and urinary

iodine excretion simultaneously (Table 3). Figure 3 demonstrates that the increasing prevalence of hypothyroxinemia across smoking strata was limited to the subjects with urinary iodine levels 200–299 µg/L and 300+ µg/L. Each of the top two strata for urinary iodine excretion showed a significant difference in hypothyroxinemia prevalence as smoking status increased [$\chi^2(4) = 41.48, p < 0.001$; and $\chi^2(4) = 40.62, p < 0.001$, respectively] after correction for multiple tests. Further testing demonstrated a significant interaction effect [*F*(24, 6492) = 5.54, *p* < 0.01 for the model] between urinary iodine level and smoking level [*F*(16, 6492) = 6.8, *p* < 0.001 for the interaction].

These analyses indicate that smoking is associated with hypothyroxinemia. However, this effect appears to be limited to subjects with more than adequate urinary iodine excretion levels (200 µg/L or more).

Discussion

This study was designed to assess the effect of smoking on the thyroid hormone level of U.S. women of childbearing age. It had been hypothesized that women with low urine iodine levels would be particularly sensitive to thiocyanate

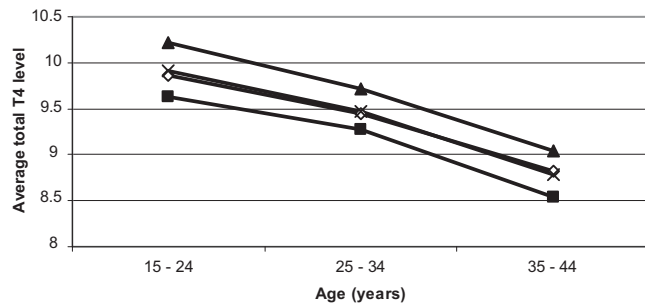
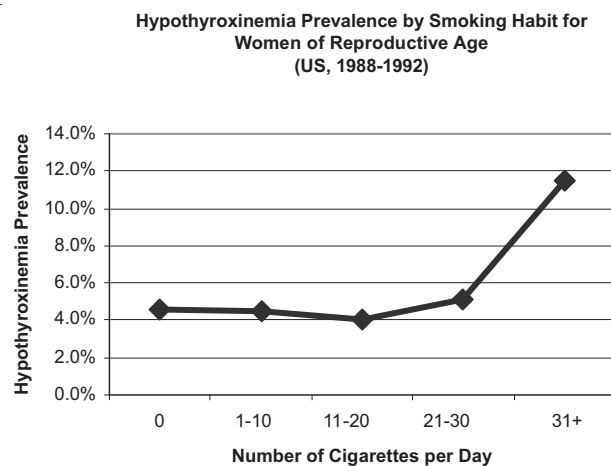


FIG. 1. Average total thyroxine level among women (age range: 15–44 years) stratified by race/ethnicity. ◇: Non-Hispanic white; ■: non-Hispanic black; ▲: Mexican-American; ×: total.

TABLE 2. LOWER FIFTH PERCENTILE VALUES FOR TOTAL THYROXINE STRATIFIED BY RACE/ETHNICITY AND BY AGE FOR NONSMOKERS IN µg/dL (WITHIN PARENTHESES ARE VALUES FOR SMOKERS AND NONSMOKERS, COMBINED)

Race/ethnicity	Age group		
	15–24 years	25–34 years	35–44 years
Non-Hispanic white	6.5 (6.5)	6.2 (6.3)	6.3 (6.3)
Non-Hispanic black	6.5 (6.5)	6.0 (5.9)	5.2 (5.0)
Mexican-American	6.8 (6.8)	6.4 (6.4)	6.2 (6.1)

A



B

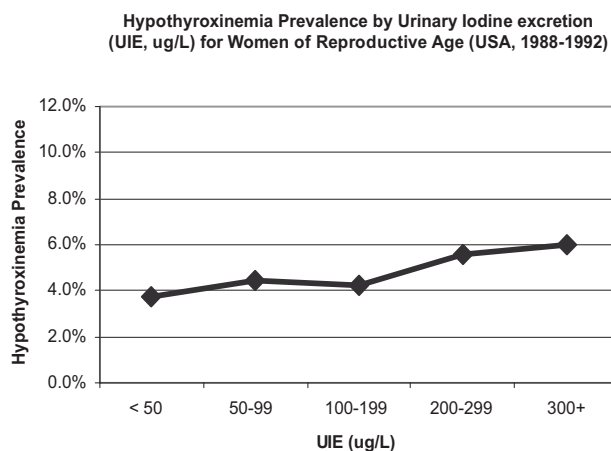


FIG. 2. Prevalence of hypothyroxinemia in women (age range: 15–44 years) stratified by (A) smoking status and (B) urinary iodine excretion.

exposure of cigarette smoking. However, our analysis demonstrated on the contrary that hypothyroxinemia related to smoking exposure was observed solely among those women with high iodine levels rather than among those with low urine iodine levels. Hypothyroxinemia prevalence in women with sufficient iodine or with low urinary iodine on a spot urine was not affected by the degree of smoking.

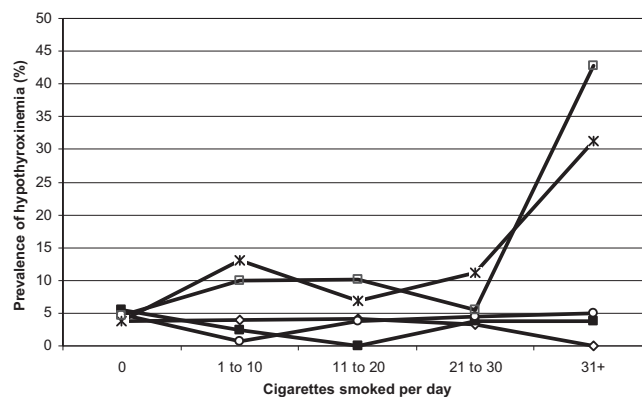


FIG. 3. Prevalence of hypothyroxinemia in women (age range: 15–44 years) stratified by urine iodine excretion. \diamond : Urine iodine <50 $\mu\text{g/L}$; \blacksquare : urine iodine 50–99 $\mu\text{g/L}$; \circ : urine iodine 100–199 $\mu\text{g/L}$; \star : urine iodine 200–299 $\mu\text{g/L}$; \square : urine iodine 300+ $\mu\text{g/L}$.

We found that smoking more than 30 cigarettes a day was associated with a higher prevalence of hypothyroxinemia, and this is consistent with several earlier studies (11,12). There was a trend toward increasing prevalence of hypothyroxinemia with increasing urinary iodine level, though this was not significant. While previous studies had indicated that very high levels of iodine are goitrogenic (17–20), it should be noted that those studies involved populations having much higher median urinary iodine levels than did our subject population (143 $\mu\text{g/L}$).

This study was dependent on several of the variables measured in the NHANES III survey. The level of smoking was determined by subject self-report. While it is known that cigarette smoking increases thiocyanate levels, the potential goitrogenic impact of other chemicals in cigarette smoke is not known. Nicotine and cotinine, the major products of smoking, do not appear to have goitrogenic potential (21). While smoking self-report may be subject to both recall and social desirability bias, the strong correlation between reported smoking and cotinine levels indicates that the smoking-status reports were reasonably accurate.

This report uses spot urine iodine excretion ($\mu\text{g/L}$) to represent iodine nutrition status. Analysis using the spot urine iodine/creatinine ratio ($\mu\text{g/g}$ creatinine) yielded similar results. Since urinary iodine levels are more commonly gathered and reported, both in the United States and internationally, it

TABLE 3. NUMBER OF SUBJECTS AND PREVALENCE OF HYPOTHYROXINEMIA STRATIFIED BY SMOKING STATUS AND URINE IODINE EXCRETION

Urine iodine excretion ($\mu\text{g/L}$)	Smoking status—cigarettes smoked per day (prevalence of hypothyroxinemia)					p (trend)
	0	1–10	11–20	21–30	31+	
<50	609 (3.78%)	77 (3.9%)	119 (4.2%)	30 (3.33%)	15 (0%)	ns
50–99	989 (5.46%)	210 (2.38%)	130 (0%)	26 (3.85%)	26 (3.85%)	ns
100–199	1912 (4.81%)	315 (0.63%)	211 (3.79%)	44 (4.55%)	60 (5%)	ns
200–299	919 (3.81%)	137 (13.14%)	87 (6.9%)	18 (11.11%)	16 (31.25%)	$p < 0.001$
300+	835 (4.67%)	101 (9.9%)	49 (10.2%)	18 (5.56%)	14 (42.86%)	$p < 0.001$

ns: nonsignificant.

was felt that this measure would be more useful for comparison across studies. Age- and sex-adjusted iodine/creatinine ratios (22) were not used since the analysis was age and race stratified and limited to women of childbearing age. Spot urine levels, particularly at low levels, do not take into account either day-to-day variation or within-day variation (23–25).

Serum T4 levels were reported as total T4. Patients taking levothyroxine were excluded from the analysis, as their T4 levels were no longer controlled by physiological factors. Analysis excluding subjects who report use of amiodarone, carbamazepine, estrogens, glucocorticoids, lithium, and phenytoin did not significantly alter the findings. Our analyses have not been weighted to reflect the complex NHANES III sampling strategy.

Our findings suggest that the major risk for the development of cigarette-smoking–induced hypothyroxinemia among women of childbearing age in the United States would be in individuals with excess iodine and not in those with lower urinary iodine levels. Previously, Belin *et al.* (26) reported for the NHANES III data the influences of cigarette-smoking exposure on thyroid-stimulating hormone (TSH), antithyroid peroxidase antibody (anti-TPOAb), and thyroglobulin antibody (TgAb) levels. They reported that the mean T4 levels were not different for nonsmokers, passive smokers, or active smokers and that the urine iodine/creatinine distributions were grossly similar for active smokers and nonsmokers. The present analysis has examined the tail ends of the distributions and found an effect at high, but not low, urinary iodine levels. The work of Knudsen *et al.* (13), which shows increased serum thyroglobulin levels and thyroid volumes with moderate and heavy smoking in areas of mild (median urinary iodine = 61 $\mu\text{g}/\text{L}$ without iodine supplementation) and moderate (median urinary iodine = 45 $\mu\text{g}/\text{L}$ without iodine supplementation) iodine deficiency, led us to expect to observe an effect among those with low urinary iodine levels. It is likely that the low urinary iodine measures among women in the iodine-sufficient U.S. population do not reflect individual women who are iodine deficient, but rather the tail-end distribution of a group of women who are iodine replete but with a low measure on that particular day. Prior analyses had shown that among women with urinary iodine levels less than 50 $\mu\text{g}/\text{L}$, there was no change in the mean T4 levels as viewed in 5 $\mu\text{g}/\text{L}$ strata across the range of 0–50 $\mu\text{g}/\text{L}$ (27). The NHANES I dataset, which related to when the U.S. median urinary iodine level was 329 $\mu\text{g}/\text{L}$, would provide a source for re-examining the analytic results of this paper among a population for whom a large proportion are at high urinary iodine levels. Our initial expectation was that smoking would show its effect on thyroid function in competition to iodine uptake at low iodine levels. It may be that the mechanism at high iodine level is related to inhibition of iodine organification or other mechanisms (28). Both NHANES III and NHANES I are Web accessible (29).

The finding that hypothyroxinemia due to smoking in U.S. women of childbearing age appears to be limited to those women with high iodine nutritional status is a novel hypothesis that should be further studied. If validated, it would suggest that iodine supplementation in pregnant U.S. women who smoke should be considered cautiously. Further, it suggests that environmental exposures to goitrogens may be

differentially affected by iodine status, and that future analyses of these exposures should take iodine status into consideration.

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Address reprint requests to:
G. Bruce Vanderver, M.D., M.P.H.
3401 38th Street, Suite 615
Washington, DC 20016
E-mail: gbgvanderver@gmail.com

Attn: Perchlorate_Comments_for_OIG@epa.gov

From: Ryan Monroe BA⁺ and Steven H. Lamm, MD, DTPH*

Date: March 10, 2009

Submission of Lamm, et al. (under review and 2007)

Greetings,

We are submitting to the record the paper by Lamm et al. (Thyroid, under review) entitled "Perchlorate, Thyroxine, and Low Iodine Association is not seen among Women of Child-bearing Age when Urine Iodine is Adjusted for Creatinine Concentration" (currently under review at Thyroid) and its published abstract (Thyroid, 2007 Sept; 17 (sup 1) S-51) .

This analysis of the NHANES 2001-2002 data is analogous to that of Blount et al., 2006 with the following three differences:

1. The study population is limited to women of child-bearing age (15-44 y/o) - This is the age group of interest;
2. The metric of iodine nutrition is the creatinine-adjusted urinary iodine level (UICr) – This eliminates the problems of dilution, the variability in the clearance of free water, and provides a metric that is reasonably constant over the 24-hour cycle;
3. The spectrum of iodine is presented as tertiles - This allows the full range to be observed and for the cut-points to be data driven. The Blount (2006) use of 100 ug/L used a population measure as a critical value for individualized data, which is not appropriate. Our lower tertile cut-point is near that of Blount, but for different reasons. The use of tertiles produced sub-sets of about 215 people each, the number that the Blount article suggested was sufficient for analytic stability.

Tables 1- 4 in the resultant analyses demonstrate quantitatively the effects of the specific iodine-uptake inhibitors at both low and high iodine levels:

1. Urinary thiocyanate levels were consistently found to have a significant negative association with serum thyroxine level at high iodine level, whether creatinine-adjusted or not, whether population-adjusted or not.
2. Urinary perchlorate levels show an effect on serum thyroxine at low iodine level in the absence of population and creatinine adjustment and show no effect on thyroxine level at low iodine level under the circumstance of adjustment for population and/or creatinine.
3. Urinary perchlorate levels show a negative effect on serum thyroxine at high iodine level using creatinine-adjusted urinary perchlorate data and whether population adjusted or not.
4. Urinary nitrate levels show a negative association with serum thyroxine only with mid-range iodine levels and in the population-adjusted analyse. The modeled associations for nitrate show no consistent pattern at low or high iodine level.
5. The sizes of the beta-coefficient for perchlorate and thiocyanate are similar at high iodine levels in the creatinine-adjusted analyses.

These data are publically available at the CDC website, and their analyses can be confirmed, validated, and extended by EPA staff scientists.

Cordially,

Perchlorate and Thyroxine: Creatinine and Population Adjustment

Perchlorate, Thyroxine, and Low Urine Iodine Association is not seen among Women of Child-bearing Age When Urine Iodine is Adjusted for Creatinine Concentration

Authors:

Steven H. Lamm, MD [Correspondent]

Joseph G. Hollowell, Jr, MD

Arnold Engel, MD

Rusan Chen, PhD

Abstract:

Objective: A significant negative association between serum thyroxine and urine perchlorate levels had been reported from NHANES 2001-2002 by Blount et al. (2006) for women age 12-85 with low iodine nutrition expressed as urine iodine (UI) less than 100 ug/l. We have reanalyzed the data using creatinine-adjusted urine iodine (UICr) as the measure of iodine nutrition and focused on women of childbearing age (age 15-44). UICr (creatinine-adjusted urine iodine) may better reflect 24-hour urine iodine excretion than does the UI (Manz, 2000; Thomson, 2001). Additionally, we have included urinary measures of the other two major iodine uptake inhibitors, nitrate and thiocyanate.

Design: Of 2091 women of child-bearing age [WCBA], 625 had serum measurements for thyroxine and urinary measurements for iodine, creatinine, and the three iodine-uptake inhibitors. Additional exclusions were made for thyroid history and extreme values. Multiple regression analyses on the thyroxine level were performed for each tercile of the UI distribution and of the UICr distribution, both with and without population-weighting, and including the three iodine-uptake inhibitors.

Main Outcome: With iodine nutrition expressed as creatinine-adjusted urinary iodine, a significant negative association with serum thyroxine was found at high iodine levels rather than at low iodine levels for both urine perchlorate and urine thiocyanate levels.. Additionally, urinary thiocyanate levels were consistently found to have a significant negative association with serum thyroxine level at high iodine level, whether creatinine-adjusted or not, whether population-adjusted or not.

Conclusion: A variety of analytic methods, including Sudaan-weighting, consistently revealed that the negative association of serum thyroxine and urine perchlorate found for WCBA with low iodine defined by UI was not found for those with low iodine defined by UICr (creatinine-adjusted).

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Word count: 275

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Introduction:

Perchlorate is a known iodine uptake inhibitor [IUI] that came to public attention in 1997 when a new drinking water assay for perchlorate detected perchlorate in much of the drinking water in southern California and found moderate levels in scattered areas. Occupational, epidemiological, and clinical studies were carried out to determine the human effects from perchlorate exposure. These studies indicated that the threshold level for perchlorate effect on iodine uptake was at an exposure of about 0.5 mg/day [500 ug/day or 250 ug/liter, assuming 2 liters per day] or 0.007 mg/kg-day [7 ug/kg-day, assuming 70 kg body weight]. The EPA reference dose [No effect level / 10-fold uncertainty factor] was set at 0.70 ug/kg-day.

The National Health and Nutrition Examination survey for 2001-2002 [NHANES 2001-2002] included data on 11,039 subjects. Urinary perchlorate was measured on a representative random subsample of 2,820 male and female participants 6 years of age or older. Based on these data, the perchlorate exposure for the US population (2001-2002) was estimated to be 0.07 ug/kg-day (geometric mean) with a median of 0.06 ug/kg-day and a 95th %ile of 0.23 ug/kg-day [Blount et al., 2007]. Thus, the EPA reference dose was found to exceed the 95th %ile for US exposure by a factor of about three.

Blount et al. (2006) further explored the dataset examining the association between urine perchlorate level and serum thyroxine levels for both men and women aged twelve or older and separated into those with and without low iodine . They defined low iodine individuals as those with a urine iodine level less than 100 ug/L, a value that the World Health Organization value uses as a population median to define a low iodine population. Their analysis showed a significant negative association (beta-coefficient = -0.89 for women age 12-85) between urine perchlorate level and serum thyroxine level (total T4) limited to women with urine iodine levels less than 100 ug/L. [Blount et al., 2006]. This study has generally been interpreted as showing an adverse relationship between perchlorate exposure and thyroid hormone level among women with low iodine nutrition.

The “gold standard” for measuring iodine nutrition in an individual is the 24-hour urine iodine excretion (measured in micrograms of iodine per day [ug/day]). However, these are not easy to obtain on either an individual basis or a population basis. Thus, the common metrics for assessing iodine nutrition for a population (or an individual) is usually either the urinary iodine concentration (ug/L) or the creatinine-adjusted urinary iodine level (micrograms of iodine per gram creatinine [ug/g]). The 2001-2002 NHANES data analysis has only been reported using the unadjusted urine iodine concentrations as the metric of iodine nutrition. Since a number of authors had reported that the creatinine-adjusted urinary iodine level (ug/g) is a better measure of iodine nutrition, we sought to replicate the Blount et al. (2006) analysis using the creatinine-adjusted urinary iodine level as the metric of iodine nutrition. Our population of interest is women of child-bearing age [WCBA], age 15-44.

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Materials and Methods:

There are 2091 women of childbearing age [WCBA] (15-44 y/o) with data in the NHANES 2001-2002 dataset. The basic NHANES (National Health and Nutrition Examination Survey) dataset contains data from medical histories, physical examinations, and laboratories on a statistically structured sample of the US population. Because of the concern on perchlorate exposure, a special chemical analysis was performed by the CDC laboratories (Centers for Disease Control and Prevention, Atlanta, GA) on surplus urine specimens for approximately one-third of the participants (weighted and unweighted). These analyses included urinary measurements for iodine, creatinine, and three iodine uptake inhibitors [IUI] - perchlorate, thiocyanate, and nitrate. Exclusions for thyroidal disease and medications and thyroidal outliers yielded an analytic dataset of 625 women of childbearing age (15-44 y/o) with serum thyroxine levels, IUI analyte measurements (urine perchlorate, thiocyanate, and nitrate) and measures of iodine nutrition (urine iodine and creatinine).

Multiple regression analysis was carried out with serum thyroxine as the dependent or outcome variable. The IUI analytes (urine perchlorate, thiocyanate, and nitrate) were the primary independent or explanatory variables and entered the regression as logarithmic functions along with the logarithm of the creatinine concentration and the logarithm of the urinary iodine concentration. Age and ethnicity were also included as co-variables as was c-reactive protein.

Two metrics were used for defining iodine status – the urinary iodine level (UI) and the urinary iodine-creatinine ratio (UICr). Additionally, each of these metrics have been separated into terciles (Low, Mid, and High) in order to examine both upper and lower tails of the distributions. The urine iodine to creatinine ratio (UI/Cr) was used as the preferred metric for iodine nutrition with the unadjusted UI analyses being presented for comparison.

Results:

The NHANES 2001-2002 data for women of childbearing age were separated into low, medium, and high terciles of urine iodine (ug/L) with cut-points at 102 ug/L and 216 ug/L [Table 1].

As had been previously reported from the same dataset by Blount et al. (2006) for women age 12 and above, we found among WCBA a significant negative association between serum thyroxine level and urine perchlorate level (log) for those with urine iodine levels in the lowest tercile. A similar significant negative association was also seen between serum thyroxine level and urine thiocyanate level (log) for those with urine iodine levels in the highest tercile. No association was seen between serum thyroxine and urine perchlorate level (log) for those in the middle or high urine iodine tercile. No associations were seen for urine nitrate levels (log).

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Population weighting using Sudaan yielded little change in the analytic results, though the regression coefficient for urine perchlorate in the low tercile was no longer statistically significant and the regression coefficient for urine nitrate in the middle tercile was negative and now statistically significant [Table 2].

The NHANES 2001-2002 data for women of childbearing age were also separated into low, medium, and high terciles of creatinine-adjusted urine iodine (ug/g) with cut-points at 95.7 ug/L and 167.4 ug/L [Table 3].

In contrast to the analysis by urine iodine tercile, we found among WCBA no association ($p = 0.72$) between serum thyroxine level and urine perchlorate level (log) for those with creatinine-adjusted urine iodine levels in the lowest tercile. However, a significant negative association was also seen between serum thyroxine level and both urine perchlorate level (log) and urine thiocyanate level (log) for those with creatinine-adjusted urine iodine levels in the highest tercile. A nearly significant positive association was seen between serum thyroxine and urine nitrate level (log) for those with creatinine-adjusted urine iodine levels in the highest tercile.

Population weighting of the analysis (Sudaan) had little effect on the analytic results for urine perchlorate (log) or for urine thiocyanate (log) though the results for urine nitrate (log) changed markedly. Whether the analysis was weighted for population or not, no association was seen between serum thyroxine level and urine perchlorate level (log) for the WCBA in the low tercile for creatinine-adjusted urine iodine (ug/g) level.

Discussion:

The analysis of the NHANES 2001-2002 dataset that contained serum thyroxine (T4), urine perchlorate, and urine iodine levels reported finding a significant negative association between serum thyroxine and urine perchlorate level (log) for women age 12 and above whose urine iodine level was less than 100 ug/L (Blount et al., 2006). This association was not found for men or for women with higher urine iodine levels.

We have examined two factors in this analysis. Blount et al. (2006) defined iodine status on the basis of the urinary iodine level and dichotomized the study population into a low iodine group and a non-low iodine group at 100 ug iodine per liter urine. The cut-point of 100 ug/L was based on the WHO definition of iodine sufficiency for a population as having a median urinary iodine level of 100 ug/L. We have chosen to analyze the study population in three groups, using terciles as the cut-points to separate those with low, mid, and high iodine levels. This has the advantage that separation is data driven, examines both tails simultaneously, and has a mid group that approximates +/- one standard deviation for what would be a normalized data distribution.

Secondly, we defined iodine status on the basis of the creatinine-adjusted urinary iodine level and trichotomized the study population into low, mid and high iodine groups as terciles. Urinary iodine concentration (UI in ug/L) in a spot urine sample is a

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confounded measure of iodine excretion, as it is the iodine excretion (ug iodine) relative to the free water excretion (liter) during an unknown sampling time. Great variability is introduced by the wide range of factors influencing water elimination. The creatinine-adjusted urinary iodine as a measure has reduced variability as it is the ratio of the iodine excretion (ug iodine/liter) to the creatinine excretion (g creatinine/liter), where the rate of creatinine excretion is understood to be steady over time.

Manz et al. (2000) had reported from the Euro-growth Study that “the urinary iodine-creatinine ratio appears to be a much better parameter for assessing iodine supply than urine iodine concentration.” Thompson et al. (2001) reported from New Zealand that thyroid volume correlated significantly with the 24-hour urinary iodide excretion (ug/day) and the creatinine-adjusted urinary iodine (the iodine/creatinine ratio in ug/g) but not with the urine iodide concentration (ug/L). Hoption Cann et al. (2007), based on the prospective NHANES Epidemiologic Follow-up Study (NHEFS) of the first National Health and Nutrition Examination Survey (NHANES I), assessed prostate cancer risk with respect to baseline iodine status. They reported that “measurement of the urinary iodine/creatinine ratio is one of the most widely used methods of estimating iodine intake and was used as the surrogate measure of iodine status.” They produced their risk estimates using the urinary iodine-creatinine ratio in terciles as we have. We consider that the use of the iodine/creatinine ratio as the metric of iodine supply reduces the individual variability and provides a more reliable measure of iodine status.

Barr et al. (2005) has recommended in the multiple regression analysis of biomonitoring data of population groups that both the urinary creatinine and the urinary analyte concentration (unadjusted for creatinine) should be included as independent variables. Blount et al. (2006) has followed this recommendation and has dealt with creatinine by forcing it to remain in the regression model, even if the p-value is high. This methodology deals with creatinine as an explanatory variable. However the urine creatinine level also has a role in defining the study population or group. After age and gender separations, Blount et al. (2006) define their study population on the basis of iodine status. While Barr et al. (2005) state that “urinary biomonitoring data typically are adjusted to a constant creatinine concentration to correct for variable dilutions among spot samples,” this role of creatinine as a measure has not been incorporated into the Blount et al. (2006) analysis. We propose that it be included by stratifying on the creatinine-adjusted urinary iodine level.

The definition of “low iodine” is influential, as individuals defined as low iodine on the basis of urinary iodine level have an incomplete overlap with the individuals defined as low iodine on the basis of the creatinine-adjusted urinary iodine level. Forty percent of the women defined as low iodine (i.e., low tercile) on the basis of the urine iodine level would not have been defined as low iodine (i.e., low tercile) on the basis of creatinine-adjusted urinary iodine, and forty percent of the women defined as low iodine (i.e., low tercile) on the basis of the creatinine-adjusted urine iodine level would not have been defined as low iodine (i.e., low tercile) on the basis of the urine iodine level.

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It is presumed that the pharmacological mechanism by which perchlorate might affect the serum thyroxine level is by partial inhibition of the sodium-iodide symporter (NIS) protein. Other measured urinary analytes that have similar mechanism are thiocyanate and nitrate.

Cigarette smoking is thought to be the major source of thiocyanate. Vanderver et al. (2007) have examined the relationship between cigarette smoking, iodine status, and hypothyroxinemia in US women of childbearing age (NHANES III). They have demonstrated that heavy smoking was associated with a higher prevalence of hypothyroxinemia and that this was an observation limited to women with high iodine levels and not seen among women with low iodine level. In contrast, Steinmaus et al. (2007) had shown that the significant effect of perchlorate on serum thyroxine was particular strong for high thiocyanate level (> 1800 ug/ml) and low iodine level (< 100 ug/L). They reported that the effect in women with low urine iodine was greater in self-reported cigarette smokers than in non-smokers and limited to the women with high urine cotinine levels or high urinary thiocyanate levels.

Our results are similar to those of Vanderver et al. (2007) in that we consistently found a significant negative association between thyroxine and thiocyanate only at high iodine levels and not at low iodine levels, whether analyzed with or without creatinine-adjustment and with or without population weighting. The set of relationships between thyroxine, thiocyanate, and iodine is not clear. Charnley (2008) has suggested that inconsistencies in the analyses reflect the failure to include environmental analytes that affect thyroxine level by a non -IUI mechanism, such as PCBs, PCDD, PCDFs, and other TEQs.

While the free thyroxine (fT4) level is the metric of biological activity and thus would be the outcome variable of choice, this dataset only contained the serum total thyroxine level (TT4), which includes both the free thyroxine and the protein-bound thyroxine, primarily bound to thyroxine-binding globulin (TBG). As such it serves as a surrogate for the fT4, recognizing that multiple factors – e.g., estrogens, medications, disease states, and age – all influence the TBG level. Their inclusion in the regression analyses is an incomplete adjustment for their effects.

Finally, as our particular interest has historically been women of childbearing age and their children, we have chosen from this dataset to examine the data for WCBA. There are likely to be fewer sources of variability in this narrower age range than in the 12-85 (+) age range. Creatinine metabolism is both age-related and influenced by chronic diseases, with falls in urine creatinine levels at age 50 and above. Issues of menarche, menopause, and estrogen use are less in the 15-44 year age group than in the 12-85 (+) age group.

Our analyses show no effect of urine perchlorate level on serum total thyroxine levels among women of childbearing age with low iodine status when defined as the low tercile of the distribution of the creatinine-adjusted urinary iodine level (UICr < 102 ug

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iodine/gr creatinine). On the contrary, we find a negative association in the high UICr tercile for urinary perchlorate as well as for urinary thiocyanate.

Having made these adjustments in our analytic approach, we are surprised and concerned with the difference in the analytic outcomes. We find in the examination of the association between serum thyroxine and urine perchlorate levels (log) a significant negative association among WCBA in the high tercile of iodine supply when the metric of iodine supply is the creatinine-adjusted urine iodine (ug/g) level and in the low tercile iodine supply when the metric of iodine supply is the unadjusted urine iodine level.

We also find that a negative association between serum thyroxine and urine thiocyanate level (log) is found in the high tercile of iodine supply, whether measured as the creatinine-adjusted or the unadjusted urine iodine. There is additional variability in the associations to lead to caution in their interpretation. These questions should be revisited in other databases.

Author disclosure statement: No competing financial interests exist for any of the authors.

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Table 1: Regression Analyses of Serum Thyroxine (T₄) Level by Urine IUI (log) Levels for WCBA by Tercile of Urinary Iodine (ug/L) [NHANES 2001-2002]

UI	Low (<u><= 101.6 ug/L</u>)	Med <u>101.6-215.8 ug/L</u>	High <u>> 215.8 ug/L</u>
Perchlorate	-0.91 (p = 0.04)	0.70 (p = 0.16)	-0.62 (p = 0.17)
Thiocyanate	-0.24 (p = 0.56)	-0.01 (p = 0.97)	-0.94 (p = 0.02)
Nitrate	0.72 (p = 0.26)	-0.71 (p = 0.31)	0.96 (p = 0.14)

Table 2: Regression Analyses of Serum Thyroxine (T₄) Level by Urine IUI (log) Levels for WCBA by Tercile of Urinary Iodine (ug/L) with population weighting (Sudaan) for NHANES 2001-2002

UI	Low (<u>< 86.7 ug/L</u>)	Med <u>86.7-187.4 ug/L</u>	High <u>> 187.4 ug/L</u>
Perchlorate	-0.84 (p = 0.14)	0.78 (p = 0.51)	-0.07 (p = 0.89)
Thiocyanate	-0.37 (p = 0.24)	0.42 (p = 0.63)	-0.61 (p = 0.04)
Nitrate	0.25 (p = 0.67)	-1.97 (p = 0.02)	-0.71 (p = 0.16)

Table 3: Regression Analyses of Serum Thyroxine (T₄) Level by Urine IUI (log) Levels for WCBA by Tercile of Creatinine-adjusted Urinary Iodine (ug/g) NHANES 2001-2002

UICR	Low (<u>< =95.7 ug/g</u>)	Med <u>(95.7-167.4 ug/g)</u>	High <u>> 167.4 ug/g</u>
Perchlorate	0.25 (p = 0.72)	-0.49 (p = 0.42)	-1.09 (p = 0.03)
Thiocyanate	0.02 (p = 0.94)	-0.04 (p = 0.91)	-1.21 (p = 0.01)
Nitrate	0.42 (p = 0.52)	-0.63 (p = 0.48)	1.14 (p = 0.06)

Table 4: Regression Analyses of Serum Thyroxine (T₄) Level by Urine IUI (log) Levels for WCBA by Tercile of Creatinine-adjusted Urinary Iodine (ug/g) with population weighting (Sudaan) for NHANES 2001-2002

UICR	Low (<u><= 92.0 ug/g</u>)	Med <u>(92.0-163.7 ug/g)</u>	High <u>> 163.7 ug/g</u>
Perchlorate	-0.13 (p = 0.89)	-0.35 (p = 0.67)	-1.093 (p = 0.01)
Thiocyanate	-0.30 (p = 0.31)	0.71 (p = 0.29)	-0.98 (p = 0.02)
Nitrate	1.55 (p = 0.06)	-2.31 (p = 0.02)	0.06 (p = 0.94)

Perchlorate and Thyroxine: Creatinine and Population Adjustment

Corresponding author:

Steven H. Lamm, MD

Attn: Perchlorate_Comments_for_OIG@epa.gov

From: Steven H. Lamm, MD, DTPH* and Joseph G. Hollowell, MD, MPH⁺

Date: March 10, 2009

Submission of Lamm, et al. (2005)

Greetings,

We are submitting to the record the abstract “The effect of iodine supplementation on urine iodine in an iodine-sufficient population of pregnant US women”. This was published in Thyroid 2005 (S 177). This is responsive to your interest of the effect of iodine supplementation on pregnant women. This shows the effect on urine iodine levels (creatinine-adjusted) but does not examine for an effect on serum thyroxine levels. It is noteworthy that this analysis of the NHANES III data was presented in terms of creatinine-adjusted urinary iodine levels in 2005, prior to the Blount paper.

Cordially,

Steven H. Lamm, MD, DTPH
Joseph G. Hollowell, MD, MPH

Objective: to compare iodine nutrition and serum thyroid hormone levels of primary Chilean school children from an area with high (Calama) and normal (Santiago) urinary iodine excretion.

Subjects: Thyroid glands were palpated in 696 children from Calama detecting a goiter prevalence of 11.6% and in 762 from Santiago with a 6.4%. **Methods:** In children with goiter, aged 6 to 14 years old, urinary iodine excretion and iodine concentration in salt were measured by spectrophotometry; T₃, T₄ and TSH by chemiluminescence, and TPO antibody (Ab) by ELISA.

Results: iodine concentration in salt was 33.3 ± 4.1 µg/g in Calama and 32.7 ± 6.1 µg/g in Santiago, adequate for the present Sanitary Regulation. According to the results shown in the table, children from Calama had significantly lower T₃ and T₄ and higher urinary iodine excretion, TSH and TPO Ab in comparison with children from Santiago.

	Urinary iodine µg/g creatinine	TSH mIU/L	T ₃ ng/dL	T ₄ µg/dL	TPO Ab % positivity
Calama (n= 59)	487± 256	3.3± 1.8	104± 33	7.1± 1.5	31.7
Santiago (n= 76)	253± 169	2.3± 1.2	156± 62	8.3± 2.4	3.3
p by t-test or Chi ²	<0.001	<0.001	<0.001	<0.002	<0.001

CONCLUSION: Chilean children with goiter from a high urinary iodine excretion area, present findings of autoimmune thyroid disease supported by higher serum TPO Ab and TSH, that may be explained by an immunogenic effect of iodine excess, and a trend towards hypothyroidism demonstrated by lower serum T₃ and T₄ and higher TSH levels.

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EFFECT OF IODINE SUPPLEMENTATION ON URINE IODINE IN IODINE-SUFFICIENT POPULATION - PREGNANT US WOMEN

LAMM, STEVEN H.; CHEN, RUSAN; ENGEL, ARNOLD; HOLLOWELL, JOSEPH G.
Consultants in Epidemiology and Occupational Health, LLC; Georgetown University; Johns Hopkins University, Bloomberg School of Public Health; University of Kansas Medical Center, USA

The importance of adequate iodine nutrition and maternal thyroxine during pregnancy on fetal neurologic development is now known and has been widely demonstrated in populations with sub-optimal iodine nutrition. However, the benefits of iodine supplementation in iodine-sufficient populations have not been clearly elucidated.

US iodine sufficiency is seen in the urinary iodine excretion (UIE) levels measured in a national population survey of US (NHANES III, 1988-94) that included 334 pregnant women, 29.9 % of whom reported 150 µg/day iodine supplementation during the prior month. The median UIE for the pregnant US women was 141 µg/g, with a median of 120 µg/g for those without supplementation and of 181 for those with supplementation.

The median UIE for pregnant women rose 61 µg/g with iodine supplementation. The 10th % ile rose 26 µg/g; the 20th % ile rose 32 µg/g; the 75th % ile rose 112 µg/g (48 µg/L); and the 95th % ile rose 92 µg/g (19 µg/L). Pregnant women at the lower tail of the distribution seemed to have retained a considerable amount of the iodine supplementation, and pregnant women at the upper tail seemed to have retained little of the iodine supplementation. With supplementation the proportion below 50 µg/g fell from 6.4 % to 3.1% and the proportion below 100 µg/g fell from 36 % to 21 %.

Supplemented	10 % ile	20 % ile	50 % ile	75 % ile	95 % ile
No	59 µg/g	79 µg/g	120 µg/g	202 µg/g	448 µg/g
Yes	84 µg/g	111 µg/g	181 µg/g	314 µg/g	540 µg/g
Difference	25 µg/g	32 µg/g	61 µg/g	112 µg/g	92 µg/g

CONCLUSION: Iodine supplementation in this iodine-sufficient population seems to have improved the UIE distribution among pregnant women when measured in µg/g creatinine. The degree to which this may reduce the frequency of early maternal thyroidal insufficiency has yet to be determined.

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THE AUSTRALIAN NATIONAL IODINE NUTRITION STUDY (NINS)

LI, MU; MA, GARY; WAITE, KAY; EASTMAN, CRESWELL
Australian Centre for Control of Iodine Deficiency Disorders (ACCIDD), ICPMR, Westmead Hospital, on behalf of the National Iodine Nutrition Study Steering Committee, Sydney, Australia

Recent studies in the states of New South Wales, Victoria and Tasmania indicate that iodine deficiency is reemerging in Australia. To investigate the iodine nutrition status of the whole population and provide evidence for public health policy, a national iodine nutrition survey was carried out in 2003 and 2004 in mainland Australia. The island State of Tasmania was not included in the study as a voluntary iodine fortification program is ongoing in that state. In each state, 8-10 year old schoolchildren were selected by a randomization process of Year 4 classes from both government and non-government schools. A ThyroMobil was imported for the study. Morning spot urine samples were collected, children were weighed, measured and thyroid volumes were determined by ultrasound. Thyroid volume of each lobe (ml) = length (mm) × width (mm) × thickness (mm) × 0.479/1,000. Thyroid volume results were compared with the international standards by age and body surface area (BSA) (Zimmermann et al, 2003).

Over 1,700 schoolchildren from 88 schools were examined; this was the largest study of the kind ever conducted in Australia. Urinary iodine excretion (UIE) results showed children in New South Wales (89 µg/L) and Victoria (73.5 µg/L) are mildly iodine deficient, while those in Western Australia (142.5 µg/L) and Queensland (136.5 µg/L) are iodine replete. South Australian children are borderline iodine deficient (101 µg/L). Furthermore, about half of the children from New South Wales, Victoria and South Australia had UIE level in the range of mild (50-99 µg/L) to moderate (20-49 µg/L) iodine deficiency. The thyroid volumes of Australian children were larger than the international standards for BSA and more so for age in both boys and girls.

CONCLUSION: The results confirm inadequate iodine nutrition in the Australian population and call for implementation of mandatory salt iodisation.

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STUDY ON SUPPLYING IODINE ON THYROID FUNCTION AND THYROID DISEASES IN IODINE DEFICIENCY SUBJECTS

GUO, XIAOWEI; LIU, YUAN; ZHAI, LIPING; WANG, XIN; QIN, QILIANG
Shandong Institute for Endemic Disease Control and Research (GXW,LY,ZLP,WX,QQ), Jinan, China

To observe the effect of supplying iodine on thyroid function and thyroid disease in iodine deficiency area. The levels and abnormal