The Effect of Environmental Perchlorate on Thyroid Function in Pregnant Women from Cordoba, Argentina, and Los Angeles, California

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Abstract

Objective: Thyroid hormone, requiring adequate maternal iodine intake, is critical for neurodevelopment *in utero.* Perchlorate decreases uptake of iodine into the thyroid gland by competitively inhibiting the sodium/iodide symporter (NIS). It is unclear whether environmental perchlorate exposure adversely affects thyroid function in first trimester pregnant women.

Methods: Urinary iodine and perchlorate measurements and thyroid function tests were obtained in 134 pregnant women from Los Angeles, CA (mean ± SD 9.1 ± 2.2 weeks gestation) and 107 pregnant women from Córdoba, Argentina (mean 10.0 ± 2.0 weeks).

Results: Median urinary iodine values were 144 μg/L in California and 130 μg/L in Argentina. Urinary perchlorate levels were detectable in all women: median (range) in California 7.8 (0.4 – 284) μg/L; and in Argentina 13.5 (1.1 – 676) μg/L. Serum thyroperoxidase antibodies (TPO Ab) were detectable in 16% of women from California and 17% of women from Argentina. Using Spearman rank correlation analyses, there was no association between urinary perchlorate concentrations and serum TSH, free T_4 index, or total T_3 values, including within the subset of women with urinary iodine values less than 100 μg/L. In multivariate analyses using the combined Argentina and California data sets adjusting for urinary iodine concentrations, urine creatinine, gestational age, and TPO Ab status, urine perchlorate was not a significant predictor of thyroid function.

Conclusions: Low-level perchlorate exposure is ubiquitous, but is not associated with alteration in thyroid function tests among women in the first trimester of pregnancy.

Introduction

Thyroid hormone is essential for normal neurodevelopment of the fetus, especially during the first trimester, and during the early neonatal period. Thus, mild hypothyroidism during the first trimester has been reported to adversely affect pregnancy and neurocognitive outcomes in children (1,2,3). Perchlorate is a competitive inhibitor of the sodium iodide symporter (NIS) located on the basolateral membrane of the thyroid epithelial cell and, in large concentrations, decreases the active transport of iodine into the thyroid and, thus, could decrease thyroid function (4). In the past, perchlorate was employed to treat hyperthyroidism but early reports of toxicity resulted in its abandonment until the early 1980's when Wenzel et al safely employed perchlorate to treat Graves' disease (5). We and others have used doses of up to 600 mg perchlorate daily to treat resistant type I amiodarone iodine induced thyrotoxicosis (6).

Small quantities of perchlorate, far less than that used in the treatment of thyrotoxicosis, are ubiquitous in the environment. Perchlorate is used in solid propellants for rockets and missiles, road flares, fireworks, airbag inflation systems, Chilean fertilizers (widely used in the U.S.), a wide variety of foodstuffs including cows' and human breast milk, prenatal vitamins, and in the environment due to natural causes (4,7,8,9,10).

Small quantities of perchlorate have been detected in the urine since 1996 and was detected in all 2,820 urine specimens from the 2001-2002 NHANES survey with a median value of 3.6 μg/liter (11). In a subsequent report, Blount et al. reported that these low levels of perchlorate were inversely correlated with the serum TSH and directly so with the serum T_4 values in 348 women with mild iodine deficiency (<100 μg I/liter)

(12). No effect on thyroid function due to these low levels of perchlorate was observed in men, irrespective of their iodine status (12). A recent analysis of the 104 pregnant women in the NHANES 2001-2002 sample found no relationship between urine perchlorate concentrations and serum TSH or T4, regardless of urinary iodine values (13). We have very recently reported that 1,002 first trimester, iodine deficient pregnant women residing in Cardiff, Wales and Turin, Italy had urinary iodine concentrations less than 100 μg/liter and similar urine perchlorate concentrations (3.6 μg/liter) to those reported in the U.S. In this large cohort of pregnant women, we found no association between perchlorate exposure and serum TSH and FT_4 concentrations, including both mildly hypothyroid and TPO positive women (14).

We now report the results of urinary perchlorate and iodine values and thyroid function tests in first trimester pregnant women residing in Los Angeles, CA and Cordoba, Argentina which represents the largest such study in pregnant women residing in the Western Hemisphere.

Methods

Subjects

First trimester pregnant women were recruited from prenatal clinics in the Los Angeles County Hospital, Los Angeles, CA and Hospital Universitario de Maternidad dependent Universidad Nacional de Cordoba, Cordoba, Argentina between 2004 and 2007. Women with known thyroid dysfunction, including those on L-T_4 therapy, those recently exposed to radiographic contrast media, and those ingesting medications known to contain iodine were excluded. Spot urines were obtained during the clinic visit and

5cc samples frozen until batch shipped to Boston Medical Center. Blood was obtained at the same visit. Sera were frozen in Cordoba and batch shipped to the thyroid function test laboratory at the Keck School of Medicine for measurement. The Institutional Review Boards at the UNC and the Keck School of Medicine approved the study.

Laboratory Methods

Perchlorate content of urine samples was measured using ion chromatography-mass spectrometry (15). Urine iodine concentrations were measured by the method of Benotti et al (16). Urine creatinine was measured using Jaffé's alkaline picrate method (17). TSH, TT4, TT3, and a thyroid hormone-binding ratio (THBR) estimate of TBG were all measured using the Elecsys (Roche, IN). Free hormone index corrections for TBG effects (FT_4I) were calculated by dividing total T4 by the THBR result. Thyroid peroxidase (TPO) antibodies were measured using the Nichols Advantage (San Juan Capistrano, CA) platform. All samples were measured in duplicate. The reference ranges were: TSH, 0.3-3.0 mIU/L; TT4, 4.5-12.5 µg/dL; THBR, 0.72-1.24; FT_4I, 4.5-12.5 µg/dL, and TT3 80-180 ng/dL.

Statistical Analyses

All values are reported as the mean +/- SD and, where appropriate, as the median. Analyses were carried out for both cohorts separately, and combined. Wilcoxon rank sum tests or independent sample t tests were used as appropriate to assess for differences in median urinary iodine and urinary perchlorate concentrations and thyroid function tests in Cordoba vs. Los Angeles. Individual Spearman rank correlation analyses were used to

separately examine the associations between urine perchlorate concentrations and serum T4, FT4I, T3, and TSH in each cohort and in the combined cohorts. We performed these analyses on the total data sets and for those subjects with urinary iodine concentrations <100 μg/L to determine whether the effects of perchlorate exposure on thyroid function tests were limited to individuals with low dietary iodine intake, as was seen in the NHANES data set (12).

We used multiple linear regression analyses to assess the associations between urine perchlorate and thyroid function tests, considered separately, adjusted for urine iodide concentration, TPO antibody titers, gestational age, and urine creatinine. These covariates were selected because of the likelihood that they might be associated with thyroid function. We suspected that women with TPO antibodies might have some underlying thyroid compromise and therefore be less able to maintain normal thyroid function in the setting of perchlorate exposure. Urinary iodide concentrations were included in the FT4 model because women with low dietary iodine intake might be more susceptible to thyroidal effects of perchlorate (although a spot urine iodine is an imperfect reflection of an individual's dietary iodine status). Creatinine was used to adjust for variable urine water excretion. Gestational age was included to account for expected physiological changes in thyroid hormone levels during the first trimester. For the multiple regression analyses, the variables TSH and perchlorate were distributed non-normally and were natural-log transformed so that they adequately followed a Gaussian distribution. Statistical tests were considered significant if the two-tailed p-value was <0.05.

Results

Patients and Thyroid Function Tests

All women were in the first trimester of pregnancy. 134 women (gestational age 9.1 +/- 2 weeks) and were recruited from the Los Angeles obstetric clinic and 107 women (gestational age 10.0 +/- 2 weeks) were recruited from the Cordoba obstetrical clinic. Thyroid function tests for the two cities are presented in Table 1. TPO antibodies were positive in 16% of the pregnant women residing in Cordoba and 17% in Los Angeles.

Urine Iodine and Perchlorate Values

The median urinary iodine concentrations were 144 µg/L in Los Angeles and 130 µg/L in Cordoba, values which were not significantly different between the two cities ($p = 0.4$) (Table 2). The median iodine value in the combined cohort was 136 µg/L. A subset of women had urinary iodine values less than 100 µg/L: 40 women from Los Angeles and 43 women from Cordoba. The median urinary perchlorate value was 7.8 µg/L (range 0.4-284) in Los Angeles and 13.5 µg/L (range 1.1-676) in Cordoba. The median perchlorate value in the combined groups was 10.6 µg/L. Urinary perchlorate was significantly higher in Cordoba ($p<0.003$).

Effects of Perchlorate on Thyroid Function Tests

Using Spearman rank correlation analyses, there was no association between urinary perchlorate concentrations and serum TSH, free T_4 index, and total T_3 values, including the subset of women with urinary iodine values less than 100 µg/L (Table 3).

In multivariate analyses using the combined Cordoba and Los Angeles data sets adjusting for urinary iodine concentrations, urine creatinine, gestational age, and TPO Ab status, urine perchlorate was not a significant predictor of thyroid function (Table 4). TPO antibody titer was positively associated with log serum TSH and inversely associated with serum FT4I. Gestational age was positively associated with serum T3. There was a slight but significant inverse association between urinary iodine concentration and serum FT4I. Results of multivariate analyses restricting the dataset to the TPO antibody negative women were similar (data not shown). Results of multivariate analyses using the individual data sets for Cordoba and Los Angeles were also similar (data not shown).

Statistical Power

Given the lack of association of urine perchlorate concentrations with first trimester serum TSH and FT4 values, we estimated our statistical power to detect associations. At an α of 0.05, we had 80% power to detect correlation coefficients of $\pm$ 0.27 for the Cordoba cohort, $\pm$ 0.24 for Los Angeles cohort, and $\pm$ 0.18 for the two cohorts combined.

Discussion

The present findings in first trimester pregnant women residing in Cordoba, Argentina and Los Angeles, California confirmed our recent report in pregnant women residing in Wales and Northern Italy that perchlorate exposure did not affect thyroid function, including in women with low iodine excretion (14). Although the present cohorts from the United States and Argentina are smaller than our European study, the

combined numbers from the two cities are sufficiently powered to detect changes in serum thyroid hormone and TSH concentrations induced by perchlorate. The environmental exposure to perchlorate in the present study was approximately two to three times greater than that previously reported from the United States (11) and Europe (14) using the same methodology. Although the explanation remains unclear, it is possible that perchlorate-rich Chilean fertilizers used in Argentina may have contributed to exposure from locally-grown foods in Argentina. In Los Angeles, the ground water and foodstuffs may contain more perchlorate than other regions in the United States since the geometric mean urine perchlorate in the NHANES study of 2,820 persons residing throughout the U.S. was 3.6 μg/L (11). It is also possible that the higher urine perchlorate concentration in the present study was due to the smaller sample size than in the 2 major studies cited above.

Exposure to quantities of perchlorate sufficient to decrease maternal thyroid function during the first trimester of pregnancy would be potentially detrimental to the fetus since transplacental passage of thyroid hormone is essential for early fetal brain development before fetal thyroid function ensues. It is also possible that fetal thyroid function beginning in the second trimester could be adversely affected by excess perchlorate which could directly inhibit fetal thyroid NIS and fetal thyroid iodine content and decrease thyroid function. Thus, it is reassuring that low level perchlorate exposure in first trimester pregnant women does not affect thyroid function in this and previous studies (14). However, infant and childhood development in relation to maternal thyroid function and exposure to perchlorate and other potential thyroid disruptors await further study.

It is difficult to reconcile the present negative findings and those from Europe with the adverse affects of environmental perchlorate on thyroid function reported previously in the study by Blount et al. (12). In the present and previous studies, TPO antibodies are associated with a significant increase in serum TSH and a significant decrease in FT_4I concentrations in first trimester pregnant women. In the 2001-2002 NHANES study, TPO antibodies and FT_4 were not measured and could be potential confounding factors. Ongoing NHANES surveys are addressing these factors.

Median iodine intake in the present study was lower than that recommended by WHO (i.e., optimal intake would be reflected by a median urinary iodine concentration of 150-249 µg I/L for pregnant women) and approximately one third of the values were less than 100 µg/L. The adverse effects of perchlorate on thyroid function in the NHANES study was primarily seen in women, but not men, with urine iodine values less than 100 µg/L. This was not observed in the large number of iodine deficient pregnant women in Europe and the small number of iodine deficient pregnant women in the present study.

In conclusion, no effect of environmental perchlorate on thyroid function was observed in first trimester pregnant women residing in Cordoba, Argentina and Los Angeles, California. However, adequate iodine intake may protect against the possible adverse effects of environmental perchlorate reported in the Blount et al. NHANES study.

Table 1: Thyroid Function Tests in Cordoba and Los Angeles

Test	Cordoba* (n = 107)	Los Angeles* (n = 134)	p-value for Cordoba vs. Los Angeles	Combined Cities* (n = 241)
TSH (μU/ml)	2.27 +/- 4.4	1.58 +/- 1.6	0.0004	1.89 +/- 3.3
T_4 (μg/dl)	11.9 +/- 2.3	10.7 +/- 2.0	0.0008	11.1 +/- 2.2
FT_4 Index	10.1 +/- 2.1	8.9 +/- 1.7	<0.0001	9.42 +/-1.9
T_3 (ng /dl)	174 +/- 38	163 +/- 37.3	0.03	168 +/- 37
FT_3 Index	153 +/- 40.2	135 +/- 30.0	0.003	143 +/- 35.8

*Values are mean +/- SD.

Table 2: Median Urine Iodine and Perchlorate Values in Cordoba and Los Angeles

Test	Cordoba Median (range)	Los Angeles Median (range)	Combined Cities Median (range)
Urine Iodine (µg/L)	130 (9 - 693) (n = 93)	144 (16-733) (n = 123)	136 (9-733) (n = 216)
Urine Perchlorate (µg/L)	13.5 (1 - 676) (n = 102)	7.8 (0.4 - 284) (n = 127)	10.6 (0.4-676) (n = 229)

*Missing data reflect either samples that did not arrive at the laboratory or small samples with insufficient volume to assay both iodine and perchlorate.

Table 3: Correlations between Urine Perchlorate Concentrations and Serum Thyroid Function Values

Cohort	Urine Perchlorate and Serum TSH		Urine Perchlorate and Serum FT4I		Urine Perchlorate and Serum T3	
	r	**p**	**r**	**p**	**r**	**p**
Cordoba						
Total (n = 102)	0.02	0.9	0.02	0.8	0.02	0.8
UIC <100 µg/L (n = 43)	-0.09	0.6	-0.06	0.7	-0.15	0.4
Los Angeles						
Total (n = 127)	-0.02	0.9	0.12	0.2	0.07	0.4
UIC <100µg/L (n = 44)	-0.06	0.7	0.21	0.2	0.09	0.5
Combined Cordoba and Los Angeles						
Total (n=229)	0.05	0.5	0.12	0.06	0.06	0.4
UIC <100 µg/L (n = 87)	-0.04	0.7	0.14	0.2	-0.03	0.8

Table 4a. Multivariable Regression Model Predicting the natural log of Serum TSH

Independent Variable	Coefficient	Standard Error	p-value
Intercept	0.3	0.4	0.5
Log Urine Perchlorate (µg/L)	-0.03	0.07	0.7
Creatinine (mg/L)	-0.00002	0.0001	0.9
TPO Titer (IU/ml)	0002	0.0004	0.0004
Urine Iodide (µg/L)	0.0008	0.0009	0.4
Gestational age (weeks)	-0.009	0.04	0.8

Table 4b. Multivariable Regression Model Predicting the Serum Free T4 Index

Independent Variable	Coefficient	Standard Error	p-value
Intercept	9.2	0.8	<0.0001
Log Urine Perchlorate (µg/L)	-0.0006	0.1	1.0
Creatinine (mg/L)	0.00008	0.0003	0.8
TPO Titer (IU/ml)	-0.002	0.0009	0.005
Urine Iodide (µg/L)	-0.004	0.002	0.04
Gestational age (weeks)	0.1	0.08	0.2

Table 4c. Multivariable Regression Model Predicting the Serum Total T3

Independent Variable	Coefficient	Standard Error	p-value
Intercept	105	15.2	<0.0001
Log Urine Perchlorate (µg/L)	-0.7	2.6	0.8
Creatinine (mg/L)	0.006	0.005	0.2
TPO Titer (IU/ml)	-0.01	0.02	0.5
Urine Iodide (µg/L)	-0.03	0.03	0.3
Gestational age (weeks)	7.1	1.4	<0.0001

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