

Long-Term Environmental Exposure to Perchlorate Through Drinking Water and Thyroid Function During Pregnancy and the Neonatal Period

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We have conducted a longitudinal epidemiologic study among pregnant women from three cities in northern Chile: Taltal with 114 $\mu\text{g/L}$, Chañaral with 6 $\mu\text{g/L}$, and Antofagasta with 0.5 $\mu\text{g/L}$ perchlorate in the public drinking water. We tested the hypothesis that long-term exposure to perchlorate at these levels may cause a situation analogous to iodine deficiency, thus causing increases in thyrotropin (TSH) and thyroglobulin (Tg) levels and decreased levels of free thyroxine (FT_4), in either the mother during the early stages of gestation or the neonate at birth, or in the fetus cause growth retardation. We found no increases in Tg or TSH and no decreases in FT_4 among either the women during early pregnancy (16.1 \pm 4.1 weeks), late pregnancy (32.4 \pm 3.0 weeks), or the neonates at birth related to perchlorate in drinking water. Neonatal birth weight, length, and head circumference were not different among the three cities and were consistent with current U.S. norms. Therefore, perchlorate in drinking water at 114 $\mu\text{g/L}$ did not cause changes in neonatal thyroid function or fetal growth retardation. Median urinary iodine among the entire cohort was 269 $\mu\text{g/L}$, intermediate between that of pregnant women in the United States at National Health and Nutrition Examination Survey (NHANES) I and at NHANES III and consistent with current World Health Organization (WHO) recommendations. Median breast milk iodine was not decreased in the cities with detectable perchlorate. Analysis of maternal urinary perchlorate excretion indicates an additional dietary source of perchlorate.

Introduction

IN RECENT YEARS, perchlorate has been found in drinking water in the United States (1). Concern about the possibility of environmental perchlorate inducing a relative iodine deficiency during pregnancy, especially among women with lower iodine intake, and thereby resulting in adverse neurodevelopmental effects in the fetus has led to proposed drinking water standards as low as 1 $\mu\text{g/L}$ (2).

Recent studies in Europe and the United States have determined that maternal thyroid underfunction during pregnancy, even when mild and considered subclinical, and especially when occurring during early gestation, may be associated with an impairment of normal brain development and intelligence in offspring (3–8). Whether decreased ma-

ternal free thyroxine (FT_4) or increased maternal thyrotropin (TSH) is the most important marker for potential adverse neurodevelopmental outcome remains unclear.

Depending on the degree of iodine deficiency during pregnancy, maternal responses include increased thyroid volume and goiter prevalence, increased TSH, thyroglobulin (Tg), and decreased FT_4 (9–22). In addition to the same general pattern of hormonal responses, fetal responses may also include decreased birth weight, length, and head circumference (18,23,24).

We previously conducted a study among first-grade schoolchildren in northern Chile where perchlorate levels in drinking water range from nondetectable by ion chromatography (IC) to 100–120 $\mu\text{g/L}$ (25). No decrease in FT_4 , increase in TSH, or goiter attributable to perchlorate levels

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The Centers for Disease Control and Prevention's (CDC) role in this study dealt exclusively with perchlorate exposure assessment. The findings and conclusions in this report are those of the authors and do not necessarily represent the views of CDC.

in drinking water was detected. Analysis of neonatal screening records from the same region did not find elevated TSH levels associated with perchlorate levels in water over a 3-year period. Iodine excretion among the schoolchildren was approximately 3 times higher than among children in the United States in 1988–1994 (National Health and Nutrition Examination Survey [NHANES] III) and 75% higher than that of children age 1–11 in the United States in 1971–1974 (NHANES I).

The International Council for the Control of Iodine Deficiency Disorders has reported on the iodine status in Chile intermittently over the past decade (26–30). Iodized salt was first mandated by law in Chile in 1959. This law was repealed in 1982, but a new law was passed in 1990 mandating that salt contain 100 ppm potassium iodate. In 1991 a survey among schoolchildren indicated mild to moderate iodine deficiency in various regions in Chile. In 1995 a median urinary iodine excretion of 750 $\mu\text{g}/\text{L}$ was reported. In 1997 levels for iodized salt were lowered to 40–100 ppm as potassium iodate. Median urinary iodine was reported as 565 $\mu\text{g}/\text{L}$ in 1998 and 540 $\mu\text{g}/\text{L}$ in 1999, second highest in the world after Japan. Average urinary iodide of 640 $\mu\text{g}/\text{g}$ creatinine was reported in pregnant women in Santiago in 1998 (31). Average urinary iodine of 717 $\mu\text{g}/\text{L}$ was reported among schoolchildren in northern Chile in 1999 (25). In 2000, the iodide content of salt was reduced to 20–60 ppm. In terms of other common public health parameters (birth rate, life expectancy, and infant death rate), Chile ranks a close third behind Canada and the United States, respectively, in the Western hemisphere.

This longitudinal epidemiologic study was designed to address the possible impact of perchlorate in drinking water at concentrations of 100–120 $\mu\text{g}/\text{L}$ on maternal thyroid function during pregnancy, neonatal thyroid function and developmental status at birth, and breast milk iodide and perchlorate levels during lactation. It was conducted in the same three coastal cities in northern Chile in which schoolchildren were previously studied (25). These cities, situated along a 200-mile span of the west coast of Chile, centered at approximately 25° south latitude, have very similar climates, and the population appears racially homogeneous. This region is in the Atacama Desert, which is the driest inhabited area in world. Saltpeter, which is known to contain perchlorate, has been mined in this region for over 200 years. The use of local water for irrigation is not an issue because there is no local agriculture. Municipal drinking water sources, which are different for each city, have been previously described (25).

Materials and Methods

All research activities and consent forms were approved in advance by the review board of the Southeast Metropolitan Health Service in Santiago and by the Coquimbo-Atacama Health Service Institutional Review Board (IRB). Additionally, the research protocol was reviewed and approved by the Centers for Disease Control and Prevention IRB. Informed consent was obtained upon enrollment of the women in the study, both for themselves and for their newborns. The same protocol was applied in each of the three study cities; Antofagasta, Taltal, and Chañaral. The study was conducted between November 2002 and April 2004.

Pregnant women were enrolled in a consecutive manner in Antofagasta, Taltal, and Chañaral when first presenting for prenatal care. Because we were most concerned about maternal thyroid changes during early pregnancy, we excluded women who first presented for prenatal care after 24 weeks' gestation. Additional exclusion criteria included women who had resided in their respective cities less than 6 months, or who were taking thyroid medications or iodine-containing medications during the 3 months before entering the study. The target number of subjects per city was 60. A schematic of the study design is shown as Figure 1.

At the first prenatal study visit, each subject provided socioeconomic and cultural information elicited from a questionnaire, and a sample of home tap water. During the postpartum study visit, a breast milk sample was obtained when possible. During both prenatal study visits and the postpartum study visit, maternal serum and urine samples were obtained, frozen, and subsequently shipped by air to Santiago as was the newborns' cord blood serum. All serum and urine samples were analyzed at the Catholic University Clinical Laboratories in Santiago. TSH, FT₄, and triiodothyronine (T₃) were analyzed using chemiluminescence (ADVIA Centaur, Bayer HealthCare, Berkshire, United Kingdom). Tg was analyzed using radioimmunoassay and antibodies to Tg were analyzed using radioimmune separation (32). Antibodies to thyroid peroxidase (TPO) were analyzed using hemagglutination (Thymune-M, Murex, Dartford, England). Urine iodine determinations were made using oxidation with ammonium persulfate (33).

Serum and urine samples were analyzed as they were received by the laboratory. FT₄ was observed to be significantly lower in sequential samples from two sample batches collected in February 2004 than samples from other batches. Retained frozen serum samples corresponding to those tested in February were retested at the Catholic University Laboratory in June 2004 and the June results substituted into the final data set. No batch effect was evident with TSH analyses.

As an external laboratory quality control, 194 remaining frozen samples of cord serum, and maternal serum from the first and second prenatal visits (with sufficient quantity), were re-analyzed at the Boston Medical Center Laboratory for TSH, T₃, T₄, FT₄, and T₃ uptake as a single batch in July 2004. The Boston laboratory also uses the Bayer ADVIA Centaur Automated Chemiluminescence System for these analyses.

During both prenatal study visits and the postpartum visit, a physical examination of the maternal thyroid was conducted by an endocrinologist (Dr. Téllez in Taltal, Drs. Michaud and Téllez in Antofagasta, and Drs. Reyes and Téllez in Chañaral). The criteria recommended by WHO/UNICEF/CIL TCCY (34) were used to classify goiters.

Home tap water samples, breast milk samples, serum samples, and urine samples were shipped via air from Santiago to the United States. Thirty-one tap water samples from Antofagasta, 58 tap water samples from Taltal, and 51 from Chañaral were analyzed for perchlorate at the U.S. Air Force Research Laboratory (AFRL) using ion chromatography (U.S. EPA Method 314.0). Thirty-four tap water samples from Antofagasta and 4 from Taltal were analyzed by the Kerr-McGee Chemical LLC laboratory in Oklahoma City using the

TABLE 1. WATER ANALYSES FOR PERCHLORATE AND OTHER POTENTIAL GOITROGENS

	<i>Antofagasta</i>			<i>Chañaral</i>			<i>Taltal</i>		
	n	Mean	SD	n	Mean	SD	n	Mean	SD
Perchlorate, $\mu\text{g}/\text{L}^{\text{a}}$	66	N.D.	—	53	5.82	0.63	62	113.9	13.3
Iodide, $\mu\text{g}/\text{L}^{\text{b}}$	5	156	35	5	87	18	5	114	14
Arsenic, $\mu\text{g}/\text{L}^{\text{c}}$	5	11	6	5	N.D.	—	5	15	6
Lithium, $\mu\text{g}/\text{L}^{\text{d}}$	5	848	205	5	370	72	5	75	10
Nitrate nitrogen mg/L ^e	6	0.61	0.47	6	0.64	0.4	6	2.06	0.84
Perchlorate, $\mu\text{g}/\text{L}^{\text{f}}$	6	0.46	0.29	—	—	—	—	—	—

^aPerchlorate analyses using IC: not different from measurements previously reported (21).

^bIodide: urinary iodide excretion was not different among the three cities.

^cArsenic: current U.S. standard is 10 $\mu\text{g}/\text{L}$.

^dLithium: average diet contains 2 mg/d. Therapeutic dose is , 100 mg/d.

^eNitrate nitrogen: U.S. standard is 10 mg/L.

^fAnalyses at CDC using IC-MS/MS, range 0.26–1.0 $\mu\text{g}/\text{L}$ (35).

SD, standard deviation; IC, ion chromatography; MS, mass spectrometry.

mean concentration of 0.46 and range of 0.26–1.0 $\mu\text{g}/\text{L}$. Although there are some differences among the three water sources in arsenic, lithium, nitrate, and iodine, these levels are not sufficiently high to cause concern about confounding.

Maternal demographics are listed in Table 2. The women enrolled in Chañaral were slightly older, had more previous pregnancies, and had lived in their respective city longer than women enrolled in either Taltal or Antofagasta. Women from Chañaral and Taltal were heavier than those from Antofagasta. There was no difference in cigarette smoking or self-reported tap water consumption among women from the three cities. The subjects from the three cities appeared to be racially homogeneous, so analysis by race is not included. Contingency table analysis of socioeconomic data found no significant difference among women from the three cities in terms of education (categorized as high school graduate or more versus less than high school graduate), economic status (type of house, categorized as owner, renter or other), or marital status (categorized as married or not married). For various reasons (e.g., subject moved away, did not attend subsequent study visits, were not pregnant, early mis-

carriage, or gave birth in other sites where we could not obtain staff collaboration), a total of 8 women from Antofagasta, 10 women from Taltal and 6 women from Chañaral were lost to follow-up.

Figure 2 compares maternal urinary iodine levels (all three cities, first and second prenatal study visits combined) in the study cohort compared with pregnant women included in two recent National Health and Nutrition Examination Surveys (NHANES). As shown in this figure, iodine excretion among the study cohort is consistent with the current World Health Organization (WHO) recommendations and intermediate between levels in the United States in the early 1970s (NHANES I) and early 1990s (NHANES III).

Table 3 contains maternal measurements from the three study visits. The women from Chañaral had a significantly higher goiter prevalence at both prenatal visits compared with women from the other two cities and this difference could not be explained by age, parity or antithyroid antibodies. The women in Taltal had increased goiter prevalence at the second prenatal visit relative to the first, and at the post partum visit relative to the second prenatal visit. No difference in goiter prevalence among the three cities was ob-

TABLE 2. MATERNAL DEMOGRAPHICS

	<i>Antofagasta</i>			<i>Chañaral</i>			<i>Taltal</i>			P value
	n	Mean	SD	n	Mean	SD	n	Mean	SD	
Mother's Age	65	23.1	6.2	53	28.2	6.3	66	25.0	6.0	<0.0001 ^a
Years in City	63	16.1	8.5	50	20.6	11.2	66	16.8	9.5	0.02 ^a
Previous Pregnancies	65	0.83	1.18	52	1.92	1.56	66	1.50	1.45	<0.0001 ^a
Height (cm)	56	156.4	6.1	46	156.8	5.2	63	157.4	5.8	0.72 ^a
Weight (kg)	46	61.4	13.7	47	66.8	13.2	60	66.8	14.6	0.04 ^a
Smoking, cigs/week	65	1.62	5.77	53	2.74	8.95	66	0.52	2.72	0.21 ^a
Housing—% Owners	65	43.1		52	51.9		66	52.2		0.60 ^b
Education—% HS+	65	46.2		52	44.2		66	52.2		0.65 ^b
Marital—% Married	65	18.5		52	25.0		66	26.9		0.49 ^b
Tap water, L/d	61	1.04	0.63	47	1.03	0.43	66	0.92	0.41	0.38 ^a

^aKruskal-Wallis *p* value.

^bContingency table *p* value.

SD, standard deviation.

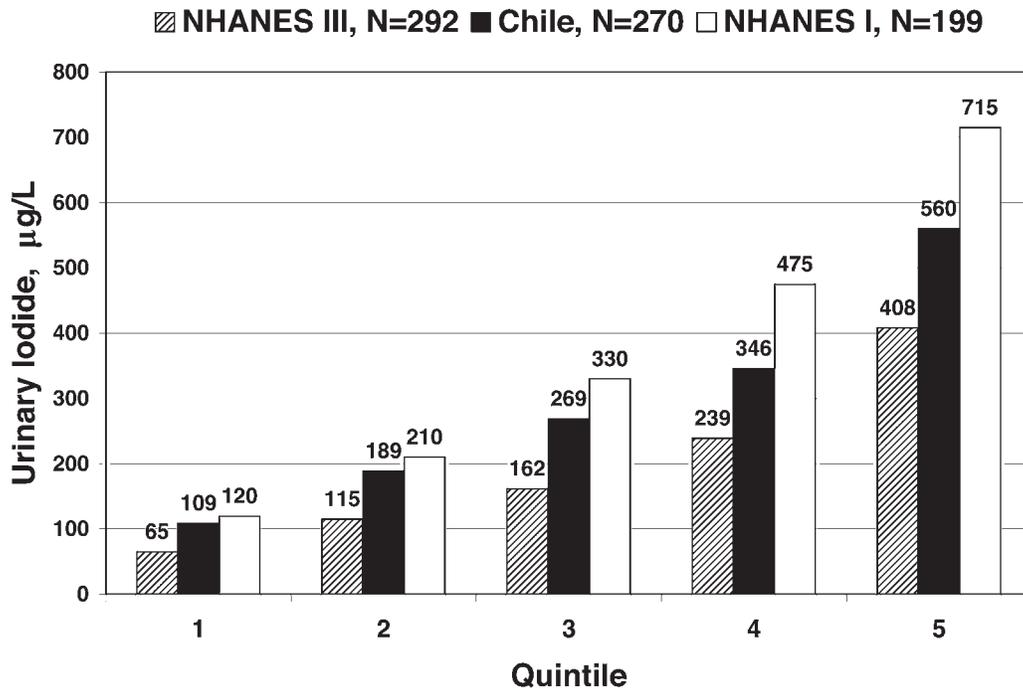


FIG. 2. Comparison of urinary iodine distribution by quintiles in the current study (combined first and second prenatal visits from all three cities) with pregnant women from (NHANES) I and NHANES III surveys.

served at the postpartum visit. With the exception of one subject in Chañaral and one in Antofagasta who had grade 2 (visible) goiters at both prenatal visits, all goiters classified in this study were grade 1.

Urinary iodine excretion was lower in Taltal than the other two cities. The women from Chañaral had a significantly higher T_3 level than women from the other two cities at both prenatal visits, however no differences were observed at the postpartum visit. FT_4 was significantly lower at the second prenatal visit compared to the first visit in all three cities. At the second prenatal visit the Kruskal-Wallis p value is highly significant for a difference in FT_4 levels among the three cities, however, with regression analysis that controlled for differences in maternal age among the three cities (Table 2), the differences among cities were no longer significant. Figure 3 shows maternal FT_4 levels at the first and second prenatal visits compared with weeks of gestation at those visits. TSH remained unchanged throughout gestation and there were no differences among the three cities. Maternal TSH cumulative distributions for the first and second prenatal visits combined were nearly identical for the subjects from the three cities as shown in Figure 4. The results of the 194 frozen serum samples analyzed at the Boston Medical Center for T_3 , FT_4 , and TSH (not shown) were highly consistent with the results from the Catholic University Laboratory for the same specimens. R^2 values from linear regression of the Catholic University data on the corresponding Boston Medical Center data without intercept were 0.98, 0.96, and 0.83 for FT_4 , T_3 , and TSH, respectively.

One woman from Chañaral was taking T_4 throughout gestation and was enrolled without recognition of this exclusion factor. Three subjects from Chañaral, four from Taltal, and one from Antofagasta had a serum TSH greater than 4.5

$\mu\text{UI}/\text{mL}$ and according to protocol, were maintained on T_4 after the first prenatal visit. One additional subject from each city had a serum TSH greater than 4.5 $\mu\text{UI}/\text{mL}$ at the second prenatal visit and was subsequently maintained on T_4 for the duration of pregnancy. Differences among the cities for subjects placed on T_4 were not significant. In all, 12% of the entire cohort (16 from Antofagasta, 13 from Taltal, and 8 from Chañaral) had a TSH greater than the laboratory non-pregnant reference range of 4.2 $\mu\text{UI}/\text{mL}$ at either the first or second prenatal visit. The data presented in Table 3 represent the entire data set including the women taking T_4 . A similar analysis (not shown) excluding subjects who were taking T_4 at the time of the prenatal and postpartum visits did not change any of the significant findings. One woman from Antofagasta without antithyroid antibodies developed hypothyroidism by the second prenatal visit (TSH = 61.8). Excluding this one subject the mean TSH among 47 women from Antofagasta was $2.46 \pm 1.44 \mu\text{UI}/\text{mL}$. One additional woman from Antofagasta who had a high titer of anti-TPO antibodies became hypothyroid by the time of the postpartum visit. Excluding those two outliers, the mean TSH among the 40 other women from Antofagasta was $2.39 \pm 2.8 \mu\text{UI}/\text{mL}$ at the postpartum visit.

Serum selenium levels on a subset of second prenatal visit maternal samples were slightly different among the three cities, but were well within the reference interval, suggesting that selenium deficiency was unlikely among this study population. Serum thiocyanate levels among nonsmoking mothers at the postpartum visit were not significantly different among the three cities and were all in the lower range of what has been generally reported, indicating that dietary thiocyanate was not likely to be a confounder in this study.

TABLE 3. MATERNAL PARAMETERS

	Antofagasta			Chañaral			Taltal			City effects significance	
	n	Mean	SD	n	Mean	SD	n	Mean	SD	Kruskal-Wallis p value	Regression ^a p value
First Prenatal											
Weeks' gestation	46	16.0	4.4	45	17.3	4.4	64	15.3	4.1	0.05	
Weight (kg)	46	62.1	13.5	45	67.6	13.1	60	66.8	14.6	0.04	
% with goiter	46	8.7%		45	24.4%		64	9.4%		0.04 ^b	
T ₃ (ng/dL)	64	183	35.7	52	207	38.5	65	187	36.1	0.001 ^c	0.0004
Free T ₄ (ng/dL)	64	0.97	0.15	52	0.95	0.13	65	0.99	0.13	0.19	0.18
TSH (μU/mL)	64	2.63	1.54	52	2.81	1.78	65	2.61	1.45	0.91	0.75
Thyroglobulin (ng/mL)	58	4.32	3.63	45	3.67	3.49	58	3.64	3.31	0.30	
% Anti-TPO	64	6.3%		52	15.4%		64	9.4%		0.26	
% Anti-Tg	67	9.4%		49	8.2%		64	9.4%		0.97	
Urine Iodine, μg/dL	63	32.1	17.9	40	30.3	18.0	62	32.5	18.4	0.73	
Iodine/g creatinine	62	407	191	39	363	140	62	323	157	0.02	
Urine perchlorate μg/L	61	24.5	23.9	53	66.7	84.9	59	132.9	103.9	<0.0001	
Perchlorate/g creatinine	61	28.4	22	53	80.2	129.6	59	135.5	95	<0.0001	
Serum perchlorate μg/L ^d	3	<4		7	<4		14	10.9	2.1	0.0001	
Second Prenatal											
Weeks' gestation	39	32.2	4.1	33	30.8	4.3	46	33.2	2.7	0.02	
Weight (kg)	38	69.2	14.4	31	74.3	13.0	46	75.3	12.2	0.02	
% with goiter	39	7.7%		33	36.4%		46	15.2%		0.01 ^b	
T ₃ (ng/dL)	48	196	46.3	40	206	40.7	38	173	39.9	0.003 ^c	0.006
Free T ₄ (ng/dL)	48	0.86	0.13	40	0.82	0.09	38	0.83	0.12	0.015	0.61
TSH (μU/mL)	48	3.69	8.69	40	2.55	2.12	38	2.08	0.86	0.63	0.68
Thyroglobulin (ng/mL)	47	2.97	2.05	38	2.99	2.39	35	3.70	2.78	0.23	
% Anti-TPO	49	6.1%		40	7.5%		37	8.1%		0.93	
% Anti-Tg	48	4.2%		38	0.0%		37	5.4%		0.38	
Urine iodine, μg/dL	47	24.2	16.4	27	40.4	19.4	37	21.7	10.9	<0.0001	
Iodine/g creatinine	47	368	192	27	422	123	37	358	120	0.08	
Serum Se, μg/L	5	94.2	12.0	5	133.6	30.7	4	134.0	18.9	0.048	
Urine perchlorate μg/L	35	16	14.3	36	73.2	178.1	27	128.9	127	<0.0001	
Perchlorate/g creatinine	35	23.5	18.2	36	71.9	148.9	27	192.1	138.6	<0.0001	
Serum perchlorate μg/L ^d	5	<4		5	<4		6	13.2	1.7	0.0009	
Postpartum											
Weeks' gestation	45	16.2	10.2	45	13.3	8.7	49	9.2	3.8	0.01	
Weight (kg)	38	60.3	10.2	43	68.0	12.3	48	68.1	13.2	0.01	
% with goiter	45	11.1%		45	24.4%		49	22.5%		0.21	
T ₃ (ng/dL)	42	115	40.3	26	114	50.2	46	107	28.1	0.74	0.48
Free T ₄ (ng/dL)	42	0.93	0.24	26	1.00	0.41	46	0.95	0.20	0.95	0.97
TSH (μU/mL)	42	8.25	27.02	26	2.34	1.86	46	1.95	1.38	0.79	0.13
Thyroglobulin (ng/mL)	41	3.80	5.31	20	6.50	8.05	44	5.78	10.03	0.16	
% Anti-TPO	41	7.3%		25	16.0%		36	83%		0.49	
% Anti-Tg	42	2.4%		20	0.0%		46	4.3%		0.60	
Urine Iodine, μg/dL	35	32.6	20.3	25	32.3	15.2	40	20.9	11.6	0.004	
Iodine/creatinine ratio	34	382	488	25	344	95	39	244	114	0.003	
Urine perchlorate μg/L	6	22.3	23.8	4	17.5	10	16	49.1	35.2	0.016	
Perchlorate/g creatinine	6	49.7	88.8	4	23	18	16	54	36.9	0.19	
Serum perchlorate μg/L ^d	4	<4		0			0				
Breast milk perchlorate μg/L	14	81.6	277.1	16	18.3	17.7	25	95.6	54.6	<0.0001	
Breast milk iodine, μg/L	14	45.4	37.4	16	32.5	15.0	25	38.4	15.4	0.41	
Serum SCN, μmol/L	9	20.0	10.9	21	17.4	16.1	10	12.6	6.4	0.072	

^aRegression with city, parity, antibodies, mother's age and weeks (gestation, postpartum or gestation age), transformations of the dependent variables were used to assure normality of the residuals.

^bGoiter prevalence was not different between Antofagasta and Taltal at the first (p=0.97) or second (p=0.1) prenatal visits.

^cT₃ was not different between Antofagasta and Taltal at the first (p=0.48) or second (p=0.08) prenatal visit.

^dThere was no attempt to measure serum perchlorate in all samples

SD, standard deviation; T₃, triiodothyronine; T₄, thyroxine; TSH, thyrotropin; TPO, thyroid peroxidase; Tg, thyroglobulin.

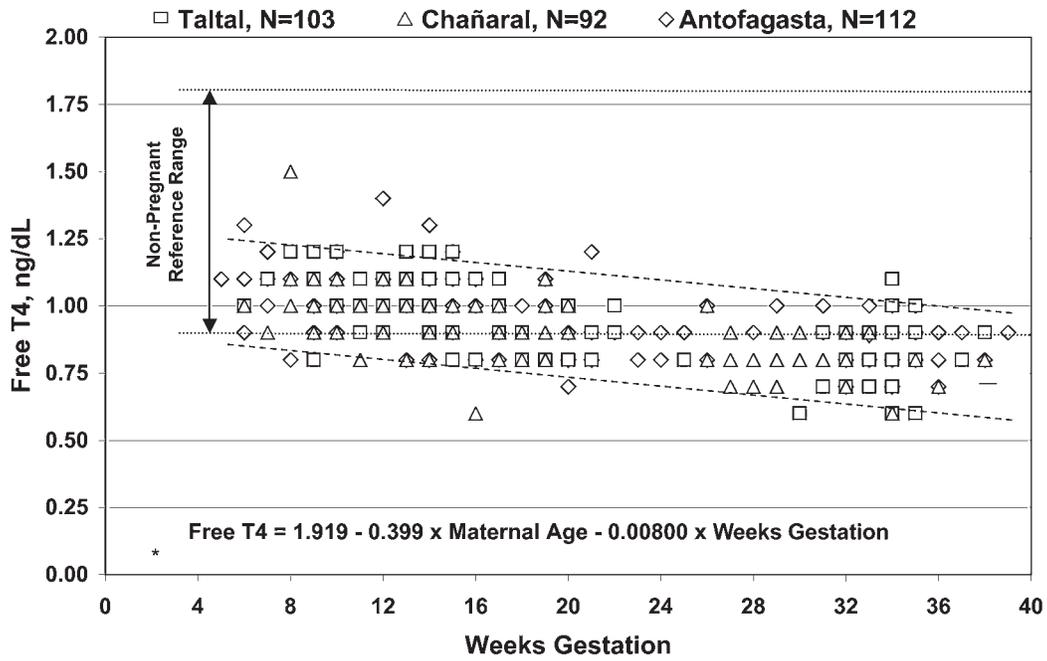


FIG. 3. Maternal free thyroxine (FT₄) levels during gestation (first and second prenatal visits combined) from each city. Initial regression using repeated measures included a city effect ($p = 0.1543$), maternal age ($p = 0.004$), and weeks' gestation ($p < 0.0001$). Above regression was performed excluding a city effect. Mixed model repeated measures regression of FT₄ with maternal age and weeks gestation, $n = 307$, variance = 0.01444. The dashed lines are the 10th and 90th percentiles on the overall regression.

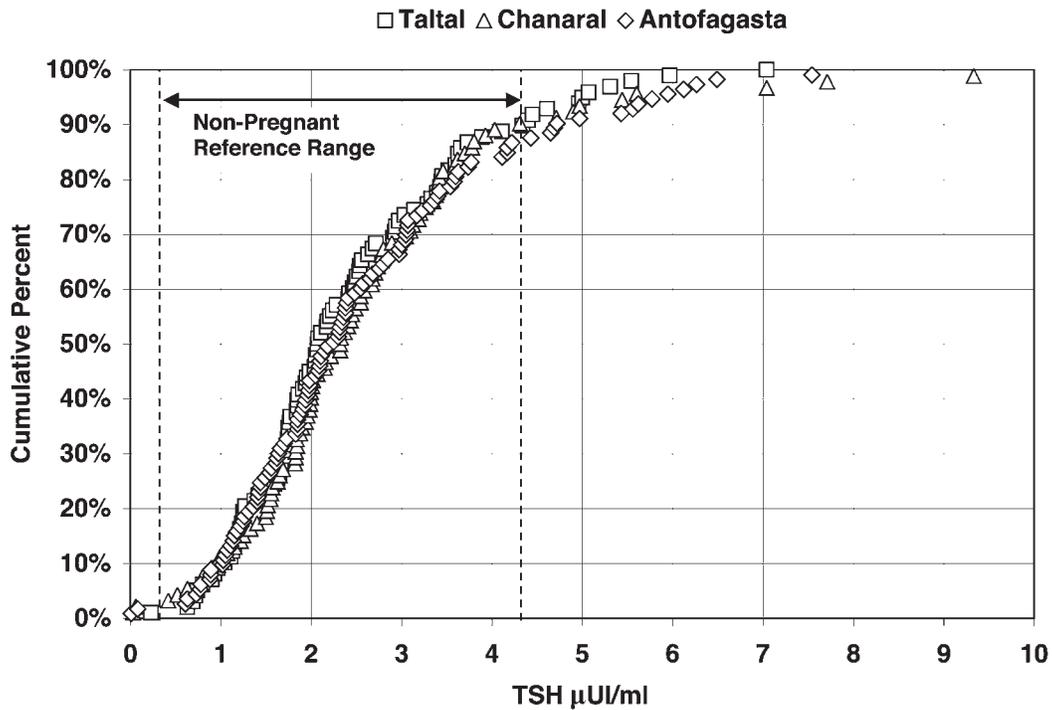


FIG. 4. Cumulative distribution of maternal thyrotropin (TSH) levels during gestation (first and second prenatal visits combined) from each city. Note: Two second prenatal data points with TSH > 10, one from Antofagasta and one from Chañaral not shown on graph.

TABLE 4. NEONATAL PARAMETERS

	Antofagasta			Chañaral			Taltal			City effect significance	
	n	Mean	SD	n	Mean	SD	n	Mean	SD	Kruskal-Wallis p value	Regression ^a
Gestational age (weeks)	55	38.9	3.9	49	38.2	4.1	55	38.4	2.6	0.01	
% Male	55	49.1%		48	54.2%		55	72.7%		0.03	
% Vaginal birth	56	60.7%		49	69.4%		57	40.4%		0.01	
% Alive	56	96.4%		49	95.9%		57	94.7%		0.90	
Length, cm	55	49.9	2.1	48	49.7	3.1	54	49.6	3.3	0.98	0.46
Weight, g	55	3459	488	48	3464	705	55	3356	593	0.30	0.98
Head circumference, cm	42	35.2	2.8	45	35.1	1.4	50	34.7	1.5	0.32	0.51
T ₃ (ng/dL)	35	79	13.4	42	73	17.9	28	82	20.6	0.03	0.003 ^b
Free T ₄ (ng/dL)	35	1.07	0.16	42	1.04	0.13	28	1.03	0.14	0.73	0.08
TSH (μ UI/ml)	33	6.20	2.96	40	6.69	4.13	28	6.31	2.91	0.99	0.69
Thyroglobulin (ng/mL)	30	16.79	9.93	36	14.03	10.98	28	18.11	12.82	0.29	0.01 ^c
Serum perchlorate, μ g/L ^d	4	<4		1	<4		14	19.9	5.0	0.005	

^aCity significance in regression with city, parity, antibodies, mother's age, gender and gestation age. Transformations of dependent variables were used to assure normality of the residuals.

^bT₃ was not different between Antofagasta and Taltal (p=0.76).

^cThyroglobulin was not different between Antofagasta and Taltal (p=0.95).

^dThere was no attempt to measure serum perchlorate in all samples.

SD, standard deviation; T₃, triiodothyronine; T₄, thyroxine; TSH, thyrotropin.

Although the median breast milk perchlorate concentrations at the postpartum visit (less than 4, 19, and 104 μ g/L for Antofagasta, Chañaral, and Taltal, respectively) were similar to drinking water concentrations in the respective cities, the breast milk perchlorate levels were highly variable. The levels ranged from less than 4 μ g/L in each city to a maximum of 1042, 61, and 204 in Antofagasta, Chañaral, and Taltal, respectively. The woman with the highest (1042 μ g/L, confirmed on repeat analysis) breast milk concentration in Antofagasta had less than 4 μ g/L perchlorate in her home tap water. Analysis of a urine samples collected on the same day as this milk sample also contained elevated levels of perchlorate (230 μ g perchlorate per gram creatinine), confirming recent significant exposure. On a second postpartum visit, her breast milk concentration was 74 μ g/L. We were unable to determine the source of the elevated breast milk perchlorate in this individual. No significant correlations could be established between breast milk perchlorate and either urine perchlorate or breast milk iodine concentrations.

Neonatal characteristics for all of the infants are summarized in Table 4. A similar analysis (not shown) excluding infants born to mothers who were taking T₄ at the time of delivery did not change any of the findings. There were no differences in indicators of fetal development (birth weight, length, head circumference) among the three cities. Birth weight distributions among the neonates from the three cities were similar to the distribution reported for U.S. neonates in NHANES III (39) as shown in Table 5. There were no differences in FT₄ or TSH in cord sera attributable to city of origin. T₃ and thyroglobulin were statistically lower among neonates from Chañaral in contrast to the statistically higher maternal T₃ levels. T₃ and Tg were not significantly different between Antofagasta and Taltal. The percentage of male births (72.7%) in Taltal was significantly higher than expected, however, we reported a 50.7% prevalence of males among 484 births occurring between 1996 and 1998 in Taltal (25).

The percentage of cesarean births was significantly higher

TABLE 5. NEONATAL BIRTH WEIGHT DISTRIBUTION (GRAMS)

	n	Percentile						
		5%	10%	25%	50%	75%	90%	95%
Antofagasta	53	2710	2924	3160	3390	3700	4068	4306
Chanaral	47	2283	2768	3190	3570	3885	4260	4388
Taltal	56	2645	2870	3080	3350	3740	4055	4090
NHANES III ^a	—	2440	2680	3030	3360	3700	4000	4195

^aMean for males and females

in Taltal (57.1%) relative to Antofagasta (39.3%) and Chañaral (30.6%). In contrast to Antofagasta and Chañaral, where the majority of women chose to deliver in their home cities, 45% of the women from Taltal chose to deliver in Antofagasta, which is 125 miles to the north. Of the women from Taltal who delivered in Antofagasta, a significantly higher percentage ($p = 0.013$) delivered via Caesarian section (76.0%) compared to those that delivered via cesarean section in Taltal (41.9%). There was no indication of fetal distress as the reason for higher cesarean-section rates for those women from Taltal who delivered in Antofagasta. Cord TSH levels were slightly but not significantly lower for infants delivered by cesarean relative to those delivered vaginally in each of the three cities, however, the difference nearly reached significance in the three cities combined ($p = 0.06$).

Analyses of 20 maternal prenatal serum samples from Taltal (combined first and second prenatal visits) for perchlorate (Table 3) averaged $12.2 \pm 2.4 \mu\text{g/L}$, while analyses of 14 cord serum samples from Taltal averaged $19.9 \pm 5.0 \mu\text{g/L}$ (Table 4). Perchlorate was nondetectable in a total of 12 maternal samples each from Chañaral and Antofagasta. Four cord serum samples from Antofagasta and one from Chañaral were also nondetectable for perchlorate. These data have been used by the AFRL to further the development of a PBPK model for perchlorate (40).

Cumulative urine perchlorate excretion ($\mu\text{g/g creatinine}$) distributions based on the 297 urine perchlorate measurements performed at the CDC are presented in Figure 5. Overall the distributions are significantly different from one another, and are generally consistent with the differences in perchlorate concentrations in the respective municipal water systems. Somewhat surprisingly, there is significant overlap of perchlorate excretion among the women from the three

cities. Additionally, the median perchlorate excretions in Antofagasta and Chañaral significantly exceed what would be reasonably expected if the respective municipal water supplies were the only source of perchlorate. Serum thyroid hormones were available corresponding to 281 of the urine perchlorate values. Regression analysis for the entire dataset indicates no significant correlation between perchlorate excretion and T_3 , FT_4 , TSH, or Tg. Urine perchlorate excretion was not different for those subjects with goiter observed at the first or second prenatal visit ($77 \mu\text{g/g creatinine}$, $n = 33$) than for those subjects without goiter ($84 \mu\text{g/g creatinine}$, $n = 238$, $p = 0.76$).

Forty-two of the women who provided breast milk samples during the postpartum visit were nonsmokers while two from Chañaral and one from Antofagasta were smokers. There was no decrease in breast milk iodine associated with the perchlorate in municipal water in Taltal (Table 3). Perchlorate was found in 3 of the 14 breast milk samples from Antofagasta, with the highest being $1042 \mu\text{g/L}$. Perchlorate was not detectable in three of the 25 samples from Taltal or in 6 of the 16 samples from Chañaral. Median breast milk iodine to urine iodine ratios are presented in Table 6 for 39 nonsmoking subjects who provided both a urine and a breast milk sample at the postpartum visit. Median breast milk iodine to urine iodine ratios were higher in Taltal relative to the other two cities and this difference reached marginal statistical significance.

Discussion

This is the first epidemiologic study of potential thyroid-related health effects among pregnant women with environmental perchlorate exposure. Perchlorate levels in the

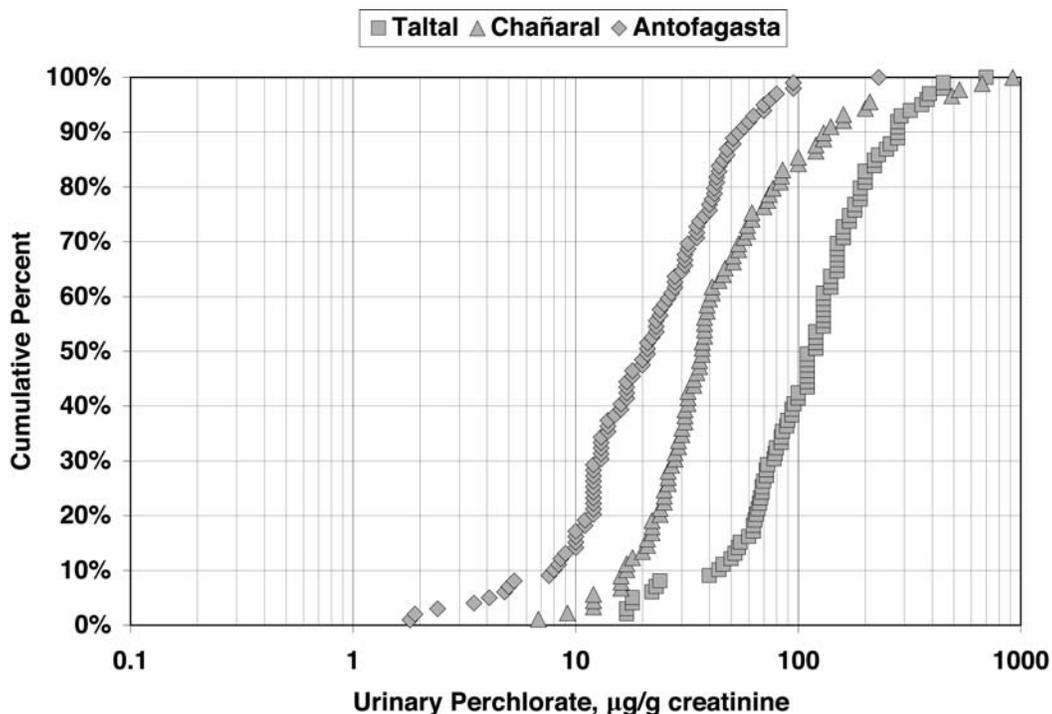


FIG. 5. Cumulative distribution of urine perchlorate excretion by city.

TABLE 6. BREAST MILK/URINE IODINE RATIOS (NON-SMOKERS ONLY)

	<i>Antofagasta</i>			<i>Chañaral</i>			<i>Taltal</i>			Kruskal-Wallis p value
	n	Mean	Interquartile Range	n	Mean	Interquartile Range	n	Mean	Interquartile range	
Breast milk iodine/ urine iodine	9	1.04	1.05	9	1.10	0.35	21	1.88	2.42	0.03
Breast milk iodine/urine Iodine/grams creatinine	9	1.18	1.28	9	0.81	0.55	20	1.64	1.14	0.06

pregnant women's home tap water were essentially the same as reported in public drinking water samples from the same cities in 2000 (25). The urinary perchlorate excretion distributions indicate a difference in perchlorate dose among the three cities that is generally consistent with the perchlorate levels in the municipal tap water.

In Table 2, the self-reported mean tap water consumption estimates are approximately 1 L/d, approximately half the 2 L/d default assumption used in risk assessments for environmental contaminants. An additional mean fluid consumption from bottled water and sodas, etc., of 670 mL/d was reported by this cohort. The mean creatinine excretion rates among pregnant women has been reported to be 1.08 g/d (41). Table 7 compares estimates of the median daily perchlorate excretion based on this creatinine excretion rate to estimates of the consumption of perchlorate through tap water. In each city, median perchlorate excretion exceeds the intake attributable to tap water by 21.7–33.8 $\mu\text{g}/\text{d}$. While this analysis is for median values, it can be seen in Figure 5 that the actual urine perchlorate concentrations are highly variable. This is consistent with a significant and highly variable dietary source of perchlorate that is similar in all three cities. It is likely that the highly variable breast milk perchlorate concentrations found at the postpartum visit can also be attributed to a variable dietary source of perchlorate. We did not, however, identify any specific dietary source of perchlorate.

In Taltal, perchlorate doses were sufficient to result in detectable perchlorate in all of the cord and maternal serum samples tested using IC, which provides an additional measure of internal dose. Thus perchlorate exposure and dose have both been well defined in this population.

Chile has previously had documented excess iodine excretion levels that likely explain, at least in part, the high goi-

ter prevalence that we previously described as well as the high prevalence of relatives of school children with thyroid conditions (25). Since 2000, iodine excretion levels have dropped considerably in Chile. As shown in Figure 2, iodine excretion is intermediate between levels in the United States in the early 1970s (NHANES I) and early 1990s (NHANES III) and considered acceptable under current WHO criteria, which recommends that the ideal dietary allowance of iodine is 200 μg of iodine per day for pregnant women. We did not find evidence to suggest that nitrate, thiocyanate, selenium, arsenic, or lithium levels were potential confounders in this study. The goiter prevalence observed in this study is higher than current U.S. experience, possibly because of the high iodine levels in the recent past. Goiter was not significant in repeated measures modeling (first and second prenatal visits) of the dependent variables (FT_4 , T_3 , and TSH) with independent variables including maternal age, parity, antibodies, and weeks' gestation (results not shown).

We do not have an explanation of higher frequency of goiter in Chañaral. To limit the effect of possible differences in their physical examinations of the thyroid, the examiners distributed themselves in the three cities (Dr. Michaud in Antofagasta, Dr. Reyes in Chañaral, and Dr. Téllez in Taltal). The examiners tested their degree of concordance in the physical examination of thyroid gland. Dr. Téllez accompanied both Dr. Reyes and Dr. Michaud to their respective cities several times, and independently, they examined each woman and annotated the results on separate sheets. When there were differences in their examinations, they examined the mother again to get consensus. The agreed upon result was recorded in the study database. Dr. Téllez and Dr. Michaud both examined a total of 37 mothers from Antofagasta and the chance-corrected agreement (κ statistic, 42) between them was 0.84. Dr. Téllez and Dr. Reyes examined a

TABLE 7. ANALYSIS OF DIETARY AND DRINKING WATER SOURCES OF PERCHLORATE

	<i>Antofagasta</i>	<i>Chañaral</i>	<i>Taltal</i>
Median estimated tap water consumption, L/d	0.84	1.06	0.82
Mean tap water perchlorate, $\mu\text{g}/\text{L}$	0.5	5.8	114
Calculated perchlorate consumed daily from tap water, $\mu\text{g}/\text{d}$	0.42	6.1	93.5
Median measured perchlorate in urine, $\mu\text{g}/\text{g}$ creatinine	20.5	37	110
Estimated creatinine excretion, g/day (41)	1.08	1.08	1.08
Calculated median perchlorate excretion, $\mu\text{g}/\text{d}$	22.1	40.0	118.8
Perchlorate "gap", $\mu\text{g}/\text{d}$ (urine excretion minus water consumption)	21.7	33.8	25.3

Note: individual urine perchlorate levels varied considerably. The difference between median excretion and median water consumption is assumed to represent dietary intake.

total of 80 mothers (some of them were examined both during pregnancy and postpartum) with $\kappa = 0.63$. Most of the thyroid examinations in Chañaral were done by both Dr. Reyes and Dr. Téllez (80/123). Altogether 41.5% of thyroid examinations in Antofagasta were done by both Dr. Michaud and Dr. Téllez, but their concordance was high. Therefore, the higher goiter prevalence in Chañaral cannot be explained by different examiners.

The vast majority of pregnant women present themselves for prenatal care before 24 weeks of pregnancy. In the smaller cities of Taltal and Chañaral, we incorporated almost all pregnant women who presented for prenatal care, except, for a few who did not consent to participate. For this reason, the probability that the study inadvertently excluded more complicated pregnancies, or women with thyroid disease is low. We cannot exclude that some women (very few) self-selected for diversion to other medical care facilities. Overall, we feel that the women who participated in this study are a representative sample.

Numerous studies have reported increases in maternal Tg (15–17,19–21) and/or increases in TSH (4,10,11,13,16,17,19, 20) during gestation or at delivery in regions with iodine deficiency. These observations lead to the conclusion that the combination of pregnancy and relative iodine deficiency causes stress on the maternal thyroid. A decrease in TSH in early gestation in response to the increase in human chorionic gonadotropin (hCG) followed by an increase in later gestation has been reported (22). We did not observe this in the current study, perhaps because the first prenatal visit occurred on average at approximately the 16th week of gestation. We did not observe an increase in either TSH or Tg during gestation in Taltal, indicating that the 114 $\mu\text{g/L}$ perchlorate in the municipal drinking water there does not induce a significant relative iodine deficiency.

We observed an average of 14% decrease in maternal FT₄ throughout gestation in all three cities between the first and second prenatal visits, and the mean FT₄ level at the second prenatal visit was slightly below the nonpregnant reference range. Regression analysis indicated that there is a significant effect of maternal age on FT₄ levels but no difference between cities. Thus, perchlorate at the concentrations seen in Taltal did not diminish maternal FT₄ during gestation. Decreases in FT₄ in the range of 12%–38% between the first and third trimesters have been reported by others (10–13,17, 19–21) with the largest decreases observed in regions with iodine deficiency.

Maternal and cord serum FT₄, TSH, and Tg levels have been reported in both iodine-sufficient and iodine-deficient populations. We can perhaps make some meaningful comparisons between the current study and others by comparing the ratio of cord and maternal serum values, although estimates from the current study are based on the second prenatal visit and not at delivery. The cord mean FT₄ to maternal mean FT₄ ratio in the present overall cohort is 1.25, consistent with a range of 1.03 to 1.49 (12,15,21,43) reported elsewhere. The ratio of median cord TSH to median maternal TSH is 2.87, slightly lower than the available range of 3.09 to 7.05 (11,15,18,21,43,44). The ratio of mean cord serum Tg to mean maternal serum Tg is 5.08, slightly higher than 2.2–3.5 (12,15,21) observed in other recent studies. Thus, combined results from the three cities in this study are reasonably consistent with other published studies. Moreover,

the cord serum concentrations of FT₄, TSH, and Tg in Taltal do not suggest that perchlorate in the municipal drinking water stressed the neonatal thyroid.

It has been speculated (45) that perchlorate from environmental sources might cause a decrease in breast milk iodine concentrations. This appears to be the case with thiocyanate because breast milk iodine to urine iodine concentration ratios among women who smoke cigarettes (0.57) (44) and among women in a region that relies heavily on cassava as a food staple (0.57) (46) are lower than those reported in other populations, which range from 0.8 to 2.0 (18,44,47,48). Breast milk iodine concentrations were not decreased, and the breast milk iodine to urinary iodine ratio was not decreased (Table 6), by perchlorate at 114 $\mu\text{g/L}$ in Taltal, which suggests that the effect of perchlorate at these levels is negligible compared with that of thiocyanate from cigarette smoking or cassava consumption in other populations.

Based on urinary iodine excretion, the highest and median perchlorate doses in Taltal were 0.017 and 0.0018 mg/kg per day, respectively. Research in dairy cows (49) has shown a threshold for inhibition of iodine transfer to milk by perchlorate at 0.1 mg/kg per day and maximal effect at 2.0 mg/kg per day. Thus, total perchlorate doses seen in the current study are orders of magnitude lower than doses likely to significantly reduce breast milk iodine.

We found a statistically significant increase in maternal T₃ and goiter prevalence in Chañaral during gestation that was not observed at the post partum visit. It is not likely that these differences are the result of the low perchlorate concentrations in municipal drinking water in Chañaral, considering that the concentration in Taltal was approximately 20-fold higher and no similar changes were observed there.

The mechanism of action of perchlorate is well known. At some serum perchlorate concentration, iodine uptake will be sufficiently inhibited to cause decreases in serum FT₄ and breast milk iodine, and increases in serum Tg and TSH. In this study, we did not see any of these changes in either the mother during gestation or the neonate at birth. This indicates that the perchlorate dose in Taltal was not sufficiently high to stress the maternal or neonatal thyroid.

The mean cord perchlorate concentration in Taltal was 19.9 $\mu\text{g/L}$ (0.199 $\mu\text{mol/L}$), which can be estimated to cause approximately 13% inhibition of iodine uptake in the neonate (50). A recent report by the National Research Council (51) indicates that iodine uptake inhibition is not an adverse event, and that greater than 75% inhibition would be necessary before adverse effects on thyroid hormones would be likely. It can be estimated that a 75% inhibition of iodine uptake would require a serum perchlorate concentration of approximately 4 $\mu\text{mol/L}$ (50). Thus, the cord serum perchlorate levels in Taltal are 20 times lower than the lowest levels that could be anticipated to cause adverse thyroid hormonal effects.

The potency of perchlorate in iodide uptake inhibition at the human sodium iodide symporter (NIS) has recently been determined to be 15 times that of thiocyanate on a serum molar concentration basis (50). The mean maternal serum perchlorate concentration in Taltal of 12.2 $\mu\text{g/L}$ (0.122 $\mu\text{mol/L}$), was therefore equivalent to 1.83 $\mu\text{mol/L}$ thiocyanate in iodine uptake inhibition. Typical serum thiocyanate concentrations are 10–70 and 80–120 $\mu\text{mol/L}$ among nonsmokers and smokers respectively (50). Thus, it is ap-

parent that inhibition of iodine uptake at the NIS resulting from the internal perchlorate doses in Taltal is negligible compared with the inhibition of iodine uptake attributable to dietary thiocyanate. This is consistent with the lack of observed effect of perchlorate on breast milk iodine or on serum FT₄, TSH, or Tg in the current study.

In our previous study in this region among schoolchildren, the urinary iodine excretion among the children was found to be excessive (25). It has been postulated that the high dietary iodide intake makes this geographic area unsuitable for studies of the thyroid effects of perchlorate exposure. The contention is that the iodide intake is so high that substantial competition with perchlorate cannot be detected. The National Academy of Sciences recently addressed this concern and concluded (51) "on the basis of the iodide-inhibition analyses, the additional comparisons, and a review of information on urinary iodide excretion, the committee concluded that the data from Chile could be considered in the evaluation of the U.S. experience with perchlorate in drinking water." Because total iodine nutrition among the current cohort of pregnant women is similar to that of U.S. pregnant women, it is clearly not an issue in the current study.

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