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Urinary Perchlorate and Thyroid Hormone Levels in Adolescent and Adult Men and Women Living in the United States

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Abbreviations:

CDC: Centers for Disease Control and Prevention

CI: confidence interval

NAS: National Academy of Sciences

NHANES: National Health and Nutrition Examination Survey

NIS: sodium/iodide symporter

RfD: reference dose

U.S. EPA: U.S. Environmental Protection Agency

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Section Headings

Abstract

Introduction

Subjects and methods

Study design

Demographic variables

Laboratory methods

Statistical analysis

Results

Discussion

Conclusions

References

Tables

Abstract

Background: Perchlorate is commonly found in the environment and known to inhibit thyroid function at high doses. Assessing the potential effect of low-level exposure to perchlorate on thyroid function is an area of ongoing research.

Objectives: Evaluate the potential relationship between urinary levels of perchlorate and serum levels of thyroid stimulating hormone (TSH) and total thyroxine (T4) in 2299 men and women, aged 12 and older, participating in the National Health and Nutrition Examination Survey (NHANES) during 2001-2002.

Methods: Multiple regression models of T4 and TSH that included perchlorate and covariates known or likely to be associated with T4 or TSH levels: age, race/ethnicity, body mass index, estrogen use, menopausal status, pregnancy status, premenarche status, serum C-reactive protein, serum albumin, serum cotinine, hours of fasting, urinary thiocyanate, urinary nitrate, and selected medication groups.

Results: Perchlorate was not a significant predictor of T4 or TSH levels in men. For women overall, perchlorate was a significant predictor of both T4 and TSH. For women with urinary iodine < 100 µg/L, perchlorate was a significant negative predictor of T4 ($p < 0.0001$) and a positive predictor of TSH ($p = 0.001$). For women with urinary iodine ≥ 100 µg/L, perchlorate was a significant positive predictor of TSH ($p = 0.025$), but not T4 ($p = 0.550$).

Conclusions: These associations of perchlorate with T4 and TSH are coherent in direction and independent of other variables known to affect thyroid function, but are at perchlorate exposure levels unanticipated based on previous studies.

Introduction

Perchlorate is an inorganic anion used for a variety of products such as road flares, explosives, pyrotechnics and solid rocket propellant (Mendiratta et al. 1996). Perchlorate can also form naturally in the atmosphere leading to trace levels of perchlorate in precipitation (Dasgupta et al. 2005). Natural processes are considered to concentrate perchlorate in some locations such as regions of west Texas (Dasgupta et al. 2005) and northern Chile (Urbansky et al. 2001). A combination of human activities and natural sources has led to the widespread presence of perchlorate in the environment. As of November 2005, perchlorate was detected in drinking water samples from 4.1% of community water supplies in 26 different states with levels ranging from the method detection limit of 4 µg/L to a maximum at 420 µg/L (U.S. EPA 2005). Most of this drinking water contamination is likely due to contaminated source waters, although in rare instances perchlorate formation has been reported to occur in water distribution systems (Jackson et al. 2004). Additionally, perchlorate exposure from the diet is probable, because of the contamination of milk (Kirk et al. 2005), as well as vegetables (Sanchez et al. 2005), fruit (Sanchez et al. 2006a), grain (Sanchez et al. 2006b), and forage crops (Jackson et al. 2005). Perchlorate contamination has also been reported in dietary supplements and flavor enhancers (Snyder et al. 2006).

Trace levels of perchlorate in the environment leads to human exposure. Direct measurement of perchlorate in biological samples collected from people (NAS 2005) is considered an excellent assessment of their exposure. We recently assessed perchlorate exposure in a nationally representative sample of 2,820 U.S. residents, ages 6 years and

older, who participated in the National Health and Nutrition Examination Survey (NHANES) during 2001 and 2002 (Blount et al., In press).

Environmental perchlorate exposure is of potential health concern because much larger doses of perchlorate have been shown to competitively inhibit iodide uptake (Greer et al. 2002; Wyngaarden et al. 1953). Populations with low intake of iodine or increased demand for iodine may be more vulnerable to inhibition of iodide uptake. Sustained inhibition of iodide uptake can lead to hypothyroidism, although perchlorate-induced changes to thyroid function have not been previously demonstrated in any human population exposed to perchlorate, even at doses as high as 0.5 milligrams per kilogram body weight per day (NAS 2005). The thyroid plays a crucial role in energy homeostasis and neurological development. Hypothyroidism can lead to metabolic problems in adults and abnormal development during gestation and infancy (Braverman and Utiger 2000). Severe hypothyroidism due to iodine deficiency during pregnancy is a preventable cause of cretinism, a permanent cognitive impairment of the developing fetus (CDC 2005; Glinioer 2000). Mild hypothyroidism during pregnancy has been associated with subtle cognitive deficits in children (Haddow et al. 1999; Klein et al. 2001), leading the National Academy of Sciences to recommend that consideration be given to adding iodide to all prenatal vitamins (NAS 2005). Therefore, we examined relationships between urinary perchlorate, and serum thyroid hormones in men and women, 12 years and older, who participated in NHANES 2001 – 2002.

Subjects and Methods

Study design. NHANES is conducted by the National Center for Health Statistics of the Centers for Disease Control and Prevention (CDC). This survey is designed to assess the health and nutrition status of the civilian, non-institutionalized U.S. population.

NHANES uses a complex multistage probability sampling designed to be representative of the U.S. population based on age, sex, race/ethnicity and income. Data were collected using an extensive household interview addressing health conditions and health-related behaviors and a standardized physical examination including medical blood and urine tests, which were conducted in mobile examination centers. NHANES 2001–2002 was conducted in 30 locations throughout the United States. Overall, the survey interview response rate was 83.9% and the exam response rate was 79.6%. A full description of the NHANES survey is available at the NHANES website (CDC 2004). The study protocol was reviewed and approved by the CDC institutional review board; additionally, informed written consent was obtained from all subjects before they took part in the study.

Urinary perchlorate levels were measured by the Division of Laboratory Sciences, National Center for Environmental Health at CDC on a representative random one-third subsample consisting of 2,820 study participants (males and females), aged 6 years and older (Blount et al., In press). For ages 12 and older, 2517 persons were in the random subsample. Serum levels of thyroid stimulating hormone (TSH) and total thyroxine (T4) were only available for 2299 participants aged 12 years and older.

Demographic variables. Sociodemographic data was self-reported by study participants. Race/ethnicity was derived from self-reported questionnaire data, and categorized as: non-Hispanic white, non-Hispanic black, Mexican Americans, and Other. Each of these race/ethnicity categories was used in the regression modeling. Non-Hispanic whites were used as the referent group in regression analysis.

Laboratory methods. During the physical examinations, whole blood and spot urine specimens were collected from participants, aliquoted, and stored cold (2–4°C) or frozen until shipment. Whole blood was collected into a red top 15 ml Vacutainer tube, mixed, allowed to clot for 30 – 45 min, centrifuged, and ~1 mL serum stored frozen in a cryovial for future analysis for TSH and T4. Serum samples collected in 2001 were assayed for TSH and T4 by the Coulston Foundation (Alamogordo, New Mexico) using a microparticle enzyme immunoassay for the quantitative determination of TSH, and a Hitachi 704 chemistry analyzer for the quantitative determination of T4 (CDC 2003). Serum samples collected in 2002 were assayed for TSH and T4 by Collaborative Laboratory Services (Ottumwa, Iowa) using a chemiluminescent immunoassay (Access Immunoassay System, Beckman Instruments, Fullerton, CA) (CDC 2003). The National Center for Health Statistics, CDC evaluated the TSH and T4 data sets from the two laboratories and determined that the values are comparable across the 2 years.

Surplus urine samples from NHANES 2001-2002 were shipped on dry ice to the Division of Laboratory Sciences, National Center for Environmental Health, CDC and analyzed for perchlorate, thiocyanate and nitrate using ion chromatography tandem mass spectrometry (Blount et al., In press; Valentin-Blasini et al. 2005). These samples were

stored frozen (-70°C) for up to 4 years before perchlorate analysis. Experiments evaluating storage at -70°C for greater than 2 years indicate no changes in urinary levels of this analyte. Reported results for all assays met the Division's quality control and quality assurance performance criteria for accuracy and precision (similar to specifications outlined by Westgard et al. (1981)). Urine samples from the same study participants had previously been analyzed for iodine using inductively coupled plasma mass spectrometry (Caldwell et al. 2005).

Statistical analysis. Initial multiple regression analysis found perchlorate to be a significant predictor of both T4 and log TSH in women, but perchlorate did not predict either T4 or log TSH in men (data not shown). Therefore, subsequent analysis focused on women and the analysis of women is described in this section.

Of the 1318 women aged 12 and older, 92 had missing TSH and T4 values – leaving 1226. Of these 1226 women, 91 were excluded from analysis because they reported a history of thyroid disease or current use of thyroid medications – leaving 1135 women. Of these 1135 women, 3 had extreme values of T4 and/or TSH and were excluded. One of these women had a total T4 of 27 µg/dL and a TSH of 0.04 IU/L. This woman was clearly hyperthyroid and excluded from the analysis. Two other women had very high TSH levels of 43 and 68 and were excluded. Of the remaining 1132 women, 21 had missing perchlorate measurements, leaving a sample size of 1111 women.

The major design variables for NHANES are age, sex, race/ethnicity, and income related to the poverty level. The values of these variables for the initial 1318 women and the final 1111 women are: mean of age – 41.6 and 39.8 years; percent non-Hispanic

whites – 70.8% and 69.4%; percent non-Hispanic blacks – 11.8% and 12.5%; percent Mexican Americans – 7.0% and 7.0%; and percent below the poverty level – 13.9% and 14.9%.

Covariates for the multiple regression analyses were chosen which are known or likely to be associated with T4 or TSH. We selected a broad number of covariates to evaluate the independence of the perchlorate relationship. These covariates were: age, race/ethnicity, body mass index (BMI), serum albumin, serum cotinine (a marker of tobacco smoke exposure), estimated total caloric intake, pregnancy status, post-menopausal status, premenarche status, serum C-reactive protein, hours fasting before sample collection, urinary thiocyanate, urinary nitrate, and use of selected medications.

For these covariates, Table 1 provides means (or geometric means if lognormally distributed) for continuous variables, percent in category for categorical variables, and number of missing results for each covariate. Thyroid function has been previously reported to vary with the constitutional variables of age, race, sex, pregnancy, and menopause. Serum cotinine is a marker of tobacco smoke exposure and smoking is associated with altered thyroid function. Serum C-reactive protein was included as a marker for inflammatory conditions that have been associated with alterations in thyroid function. Both total caloric intake (based on a 24 hour dietary recall survey and a U.S. Department of Agriculture database (Food and Nutrition Database for Dietary Studies (USDA 2004)) and body mass index are related to thyroid function, but the interrelationship as to cause or effect is unclear.

Serum albumin was included as a possible surrogate for T4 serum protein binding. NHANES 2001-2002 included total T4 measurements but not free T4

measurements; total T4 varies with the concentrations of specific binding proteins. Concentrations of these proteins can change with physiologic state and health conditions. Free T4 varies less with such protein concentration changes than does total T4. Serum albumin accounts for 15-20% of T4 binding and thyroid binding protein and prealbumin (not measured in NHANES) account for the remaining percentage (Robbins 2000). Thyroid autoantibody measurements were not available for 2001-2002. For autoantibodies to affect the relationship between perchlorate and T4 or TSH, presence of autoantibodies would have to correlate with perchlorate levels. We have found no such correlation in the literature and we are unaware of a rationale for such an association.

Medications known to affect thyroid function were also considered. As noted above, women taking medication containing thyroid hormone (e.g. levothyroxine) or antithyroid drugs (e.g. methimazole or propylthiouracil) were excluded. Use of beta-blockers, estrogen formulations, steroids, and furosemide were each modeled using an indicator variable in the regressions. An 'other drug' category was also modeled by an indicator variable. This 'other drug' category consisted of a heterogeneous group of other medications that have possible effects on thyroid function, protein binding, or measurements, including: salicylates, dopaminergics, anticonvulsants and barbiturates, narcotic analgesics, androgenic agents, lithium, and several others (a total of 28 drug codes).

Log of urinary creatinine was included in the models to adjust for variable water excretion. A non-linear relationship was evaluated by adding the square of log of perchlorate to final models, but it was not significant. Models were also checked for significance of interaction terms involving main effects. Partial regression plots were

examined to identify any unduly influential data points. No unduly influential points were found. Indicator variable coefficients in the models (e.g., for non-Hispanic blacks) should be interpreted: 1 = group member and 0 = not a group member. Urine samples were collected in three sessions of the day from 8 am through 10 pm. Mean perchlorate levels were not statistically different across sessions ($p=0.49$).

Univariate statistics and distribution plots were examined for each dependent and independent variable to look for outliers and to assess the distribution shape. TSH, perchlorate, cotinine, body mass index, urinary thiocyanate, urinary nitrate, and C-reactive protein were \log_{10} transformed to normalize their distributions.

Regression models, including log of perchlorate as one of the predictor variables, were constructed separately for thyroxine and log of TSH. The initial phase of analysis used ordinary least squares regression (OLS) (SAS Proc Reg, SAS v. 9.0, SAS Institute, Cary, NC) and purposefully did not adjust for the NHANES complex survey design in order to obtain a broad group of potentially significant predictor variables. Forward stepwise and backward elimination procedures were used on both population-weighted and unweighted data. The entry p-value for forward elimination models was 0.10 and the retaining p-value for backward elimination was 0.10 in order to identify significant and borderline significant predictors. The forward stepwise and backward elimination approaches produced models that were generally in good agreement.

This OLS analysis produced a generous list of significant and borderline significant variables for regression analysis using SUDAAN (SUDAAN version 9.0.1, Research Triangle Institute, Research Triangle Park, NC), which provides an analysis that adjusts for the complex survey design. SUDAAN regression models were tested using a

manual backward elimination approach starting with the variables obtained from the OLS regression modeling. Selected variables that were excluded in the SUDAAN backward elimination process were added to the final model to assure they were not significant. The stability of the perchlorate coefficient was monitored during the SUDAAN backward elimination process.

The main SUDAAN regression analysis used population weights to represent women 12 years and older in the U.S. population for the years 2001 and 2002. In addition, we performed separate regression analyses with SUDAAN using unweighted data and verified that regression coefficients were in good agreement with those obtained using population weights. Reported regression model results in the tables use the population-weighted analysis.

Women were categorized based on a urinary iodine cut-point of 100 $\mu\text{g/L}$ and analyzed separately. The 100 $\mu\text{g/L}$ cut-point was used based on the World Health Organization definition of sufficient iodine intake in populations. WHO noted that the prevalence of goiter begins to increase in populations with median urinary iodine less than 100 $\mu\text{g/L}$ (WHO 1994). A urine iodine level of 100 $\mu\text{g/L}$ represents about the 36th percentile of urinary iodine concentrations in women living in the United States (Caldwell et al. 2005). Women with lower iodine intake could be more vulnerable to perchlorate's effects to impair iodine uptake. From this analysis, the significance of urinary perchlorate as a predictor of thyroid function in women was found to be largely determined by women with urinary iodine < 100 $\mu\text{g/L}$. Consequently, we report here results for women divided into groups based on urinary iodine levels.

Compared to use of the average of multiple spot urine measurements or use of 24 hour urine specimens, use of a single spot urine for perchlorate and iodine measurement has more imprecision in estimating true urine levels (Andersen et al, 2001). This imprecision is a source of random error (not bias) and therefore decreases statistical power to detect an association between perchlorate and either TSH or T4 compared to these other urine collection approaches.

Results

For all women 12 years and older, multiple regression analysis found urinary perchlorate to be a significant predictor of serum TSH and a significant predictor of serum T4 (data not presented). Since low iodine levels had potential to affect the relationship of perchlorate with T4 and TSH, women with urinary iodine $< 100 \mu\text{g/L}$ were analyzed separately from women with urinary iodine $\geq 100 \mu\text{g/L}$. Results of this analysis are presented in Tables 2 and 3 for T4 and in Tables 4 and 5 for TSH.

For women with urinary iodine $< 100 \mu\text{g/L}$, multiple regression analysis found perchlorate to be a significant predictor ($p < 0.0001$) of T4 with a coefficient for log perchlorate of -0.8917. The result of regression of T4 on perchlorate and urinary creatinine without other covariates yielded a coefficient of -0.8604 ($p < 0.0001$). Perchlorate was also a significant predictor ($p = 0.0010$) of log TSH with a coefficient of 0.1230. The result of regression of log TSH on perchlorate and urinary creatinine without other covariates found a coefficient of 0.1117 ($p = 0.0031$). The signs of these coefficients are coherent, with increased perchlorate associated with less production of

T4 and an increase in TSH to stimulate additional T4 production. For women with urinary iodine $\geq 100 \mu\text{g/L}$, perchlorate was not a significant predictor of T4 ($p = 0.5503$), but remained a significant predictor of log TSH ($p = 0.0249$). The regression analysis results in Tables 2 – 5 include variables that were borderline significant ($0.05 \leq p < 0.10$) to give ample opportunity for other variables to explain variance and better evaluate the independence of the perchlorate effect.

Regression results for men (not shown) indicated that perchlorate was not a significant predictor of either T4 or log TSH. This finding also held when examining men with urinary iodine levels $< 100 \mu\text{g/L}$.

From the regression coefficients for women with urinary iodine $< 100 \mu\text{g/L}$, we calculated the predicted effect size (i.e., the change in T4 and TSH) for different levels of perchlorate exposure. We chose perchlorate levels corresponding to the 5th, 10th, 25th, 50th, 75th, 90th, and 95th percentiles of urinary perchlorate in women 12 years and older. The minimum and maximum perchlorate values are observed results for this population sample, they are not estimates of the 0th and 100th percentile for the U.S. population. As such, they would be expected to change in another population sample. The effect size was calculated from the difference between the minimum level of perchlorate measured in women and the level of perchlorate corresponding to the specific percentile. For example, the 50th percentile of urinary perchlorate for women is $2.9 \mu\text{g/L}$ and the minimum level was $0.19 \mu\text{g/L}$. Increasing exposure from 0.19 to $2.9 \mu\text{g/L}$ would result in a predicted decrease in T4 of $1.06 \mu\text{g/dL}$.

For TSH, one more step is needed in the calculation. Since TSH was modeled as log TSH, the change in TSH from a given change in perchlorate depends on the starting

level of TSH. Our calculations use the approximate 50th and 90th percentiles of TSH as starting points to estimate the predicted perchlorate effect size for TSH. Results of these calculations for T4 and TSH are presented in Table 6. For comparison, the normal range for T4 is 5–12 µg/dL and for TSH is 0.3–4.5 IU/L.

To search for a threshold for the perchlorate relationship with T4 and TSH, piecewise regression models (Neter et al. 1985) were fit to the data. No inflection point was found for the perchlorate relationship with T4 or TSH. However, statistical power is limited to detect such a threshold, if present.

Discussion

Increased urinary perchlorate was associated with increased TSH and decreased T4 for women with urinary iodine levels < 100 µg/L, a group possibly more susceptible to competitive inhibition of thyroid iodine uptake by perchlorate. The statistically significant associations of urinary perchlorate with decreased serum T4 and increased serum TSH were consistent with competitive inhibition of iodide uptake.

For women with urine iodine ≥ 100 µg/L, perchlorate was also a statistically significant predictor for TSH but not for T4. Greater iodine intake may have diminished the effect of perchlorate on T4 in these women. The significant association with TSH, but not with T4, in this group may be due to the greater sensitivity of TSH to impairment of thyroid function; that is, normal T4 levels are maintained by increasing TSH to compensate for impaired thyroid function.

Predicted changes in serum TSH and T4 with increasing perchlorate exposure (Table 6) can span a notable portion of the normal medical range of TSH and T4 values. Compared to a urine level of 0.19 $\mu\text{g/L}$, perchlorate exposure at a urine level of 13 $\mu\text{g/L}$ (95th percentile) yields a predicted decrease in T4 of 1.64 $\mu\text{g/dL}$. The normal range for T4 is 5–12 $\mu\text{g/dL}$. A similar exposure would increase TSH by 2.12 IU/L, for a woman starting with a TSH level of 3.11 IU/L (90th percentile for TSH in women 12 years and older). Normal range for TSH is 0.3–4.5 IU/L. Effect size estimates that start with the 90th percentile of TSH have more uncertainty than estimates starting with the 50th percentile because the predicted TSH levels fall further from the central portions of the original data.

The mechanism of perchlorate's effect is competitive inhibition of iodide uptake by the thyroid (Clewell et al. 2004; Wolff 1998). Based on this mechanism, individuals with less iodide available to compete with perchlorate may be more vulnerable to impaired iodide uptake. Chronically impaired iodide uptake could lead to changes in serum thyroid hormones, consistent with the increased TSH and decreased T4 we find associated with increased perchlorate exposure in women with urinary iodine < 100 $\mu\text{g/L}$. WHO has identified median urinary iodine levels ≥ 100 $\mu\text{g/L}$ as indicating sufficient iodine intake for a population (WHO 1994). Based on concerns about adequate iodine intake, the National Research Council recently recommended that consideration be given to adding iodine to all prenatal vitamins (NAS 2005).

Perchlorate was not found to be a significant predictor of T4 or TSH in men. Previous studies report that women have a much higher risk of goiter than do men, especially in populations with marginal iodine intake (Laurberg et al. 2000). The

increased vulnerability of women may partially be caused by increased susceptibility to autoimmune thyroid disease in women, the increased demands on the thyroid during pregnancy, or the effect of estrogens on thyroid function. Estradiol has been shown to block TSH-induced sodium/iodide symporter (NIS) expression in the FRTL5 rat follicular cell line (Furlanetto et al. 1999). Impaired NIS expression could lead to reduced ability of the thyroid follicular cells to import iodide, and thus an increased vulnerability to NIS-inhibitors such as perchlorate. Also, estrogens increase thyroxine-binding globulin and thus increase the demand for thyroxine so that free thyroxine levels can remain constant.

Covariates in the regression models predicted T4 and TSH levels in a manner generally consistent with previous studies. We found that estrogen use was a significant, independent and positive predictor of T4 in both low and sufficient iodine models of women aged 12 and older, but was not a significant predictor in either of the TSH models. Similar to estrogen use, pregnancy was a significant or borderline significant predictor of T4 but not TSH. Both estrogen use and pregnancy raise estrogen levels, increase thyroid binding proteins, and increase serum T4 concentrations (Glinioer 1997). Menopause lowers estrogen levels and was a significant predictor of T4 in the regression for women with urinary iodine levels < 100 µg/L.

In NHANES III (1988-94), non-Hispanic blacks were shown to have lower TSH than other groups and Mexican Americans had higher thyroxine levels than non-Hispanic blacks and whites (Hollowell et al. 2002). The models for TSH and T4 were consistent with these previous findings concerning race/ethnicity. Non-Hispanic blacks have also been shown to have lower urinary perchlorate levels than non-Hispanic whites, although

the reason for this difference is not known (Blount et al., In press). Age was positively associated with TSH in women with urinary iodine levels $\geq 100 \mu\text{g/L}$, but not significant for women with urinary iodine levels $< 100 \mu\text{g/L}$. A positive association of age and TSH was seen in NHANES III and other studies (Canaris et al. 2000; Hollowell et al. 2002).

BMI was significant in the TSH model for women with urinary iodine levels $\geq 100 \mu\text{g/L}$ and total caloric intake was significant in T4 model for women with urinary iodine levels $< 100 \mu\text{g/L}$. Thyroid function clearly has an effect on BMI as seen clinically and documented in populations (Nyrenes et al. 2006). The reverse is also true, since BMI and total caloric intake can influence hypothalamic-pituitary-thyroidal axis, though usually at the extremes of body weight and caloric intake (Acheson et al. 1984; Burger et al. 1987; Danforth et al. 1979; Loucks et al. 1992; Loucks and Heath 1994). Total caloric intake in NHANES is a 24 hour recall of food intake. Depending on how well recent intake reflects long term intake, total caloric intake may parallel the effect of BMI, which was not seen in this study. Increased caloric intake is known to increase thyroid hormone disposition through deiodination pathways (Burger et al. 1987; Danforth et al. 1979), increasing the conversion of T4 to the active form, T3, and increasing conversion of T3 to inactive forms. The effect of changes in calories and carbohydrate composition of the diet on thyroid disposition may have different short and long term effects on T3 and T4 levels. Hours of fasting before sample collection was a borderline significant predictor in one regression model: T4 in women with sufficient iodine. Fasting for 60 hrs can reduce TSH in humans, but fasting for shorter periods has unknown effects on thyroid function.

Beta-blocker drugs are commonly used to treat hypertension and other cardiovascular conditions. Beta-blocker drugs inhibit the conversion of T4 to the more active form, T3, and increase serum TSH (Kayser et al. 1991). Use of these drugs was positively associated with TSH in the regression for women with urinary iodine < 100 µg/L. Serum C-reactive protein was positively associated with T4 in women in each of the iodine groups. C-reactive protein is an acute phase reactant protein increased in many inflammatory conditions in response to production of tissue-generated cytokines, particularly interleukin-6; and has been used as a marker for both specific and systemic low-level inflammation conditions. It is unclear if C-reactive protein is associated with thyroid function other than thyroiditis (Jublanc et al. 2004; Pearce et al. 2003; Tuzcu et al. 2005). However, the stimulus for C-reactive protein, interleukin-6, has a firm inverse relationship with serum T3 in non-thyroidal illnesses. Also, C-reactive protein and serum T4 binding proteins are synthesized by the liver; C-reactive protein may vary with an unrecognized health or physiologic condition that affects the synthesis of both proteins. The association of C-reactive protein and T4 in our study is unclear.

Other variables that are known to possibly affect thyroid function or measurements were not significant predictors in the regression models, including the categories of medications (other than estrogen use and beta-blockers), serum albumin, and serum cotinine. Generally, other medication categories were small and unlikely to have significant effects. Serum albumin did not appear in the final models. Factors such as estrogen use that increase protein binding of thyroid hormones may have accounted for variance in T4 due to protein binding that serum albumin may have otherwise explained. Serum cotinine is a marker of tobacco smoke exposure and smoking is associated with

altered thyroid function (Belin et al. 2004; Bertelsen and Hegedus 1994). However, tobacco smoke also contains other factors that can inhibit TSH secretion (Bartalena et al. 1995), and perhaps is an explanation for the absence of an association of serum cotinine with either TSH or T4.

Cyanide in tobacco smoke is metabolized to thiocyanate, a competitive inhibitor of iodide uptake (Tonacchera et al. 2004). Also, nitrate from dietary sources and from formation by intestinal bacteria can compete with iodide. In vitro studies indicate that perchlorate is a more potent inhibitor of human NIS, with potencies 15, 30, and 240 times greater than thiocyanate, iodide, and nitrate, respectively (Tonacchera et al. 2004). Thus, the ability of NIS to transport adequate amounts of iodide depends on the relative concentrations of these competing anions. Based on the relative concentrations of perchlorate, nitrate and thiocyanate likely to be found in human serum, several researchers have predicted that nitrate and thiocyanate are more likely than perchlorate to impair thyroid function (DeGroef et al. 2006; Gibbs 2006). Thiocyanate-induced NIS inhibition is a plausible explanation of the association of smoking with goiter in populations with low iodine intake (Knudsen et al. 2002), and is analogous to the association of perchlorate exposure with thyroid hormones levels observed in our study. However, in women with urinary iodine levels $< 100 \mu\text{g/L}$ urinary thiocyanate was negatively associated with serum TSH, a direction unexpected based on a mechanism of NIS inhibition. The explanation for this is unclear. Urinary nitrate was negatively associated with serum T4 in women with urinary iodine levels $\geq 100 \mu\text{g/L}$, a direction consistent with inhibition of NIS. Goitrogenic effects of nitrate intake in animal studies have been observed, but there are few studies in humans.

Recently the National Research Council (NRC) of the National Academy of Sciences (NAS) evaluated the potential health effects of perchlorate ingestion (NAS 2005). Based on studies of long-term treatment of hyperthyroidism and clinical studies of healthy adults, the NRC panel estimated that a perchlorate dose of more than 0.40 mg/kg per day would be required to cause hypothyroidism in adults, although lower doses may lead to hypothyroidism in sensitive subpopulations (NAS 2005).

Comparison of our results to previous studies requires consideration of 1) target population group studied, 2) estimated dose of perchlorate, 3) duration of exposure to perchlorate dose, and 4) sample size (statistical power). First, for men, our results found no relationship with perchlorate and T4 or TSH. This finding is in general agreement with predicted effects of this level of perchlorate exposure based on reported studies of exposure in men. Lawrence et al administered 10 mg perchlorate daily (~ 0.14 mg/kg) to iodine-sufficient adult males for 14 days and found a 10% decrease in radioactive iodine uptake (RAIU), but with no change in TSH or free T4 (Lawrence et al. 2000).

Greer et al administered perchlorate to 16 male and 21 female volunteers for 14 days, and found increasing RAIU inhibition for doses between 0.02 and 0.5 mg/kg/day, with no perchlorate-related change in TSH or free T4 (Greer et al. 2002). An unknown number of women in the Greer study may have had urinary iodine < 100 µg/L, but if the women were typical of the U.S. population (Caldwell et al. 2005), the predicted number of women with low urinary iodine would be 7 to 8. Braverman et al administered perchlorate to 13 iodine-sufficient male and female volunteers at daily doses of 0.5 mg and 3 mg for 6 months, and found no change in RAIU, TSH or free T4 (Braverman et al. 2006). Two other studies have also found that workers exposed to perchlorate

intermittently for long periods did not have significant changes to serum TSH or T4 levels (Braverman et al. 2005; Lamm et al. 1999). These study populations were either exclusively (Braverman et al 2005) or predominantly (Lamm et al 1999) male.

For women, only two perchlorate studies have focused on women or included a large percentage of women. A recent study of 184 pregnant Chilean women, with mean urinary perchlorate levels near the 99th percentile for women in NHANES 2001-2002, found no perchlorate relationship with thyroid function (Tellez et al. 2005). Of these 184 women, 181 had mean urinary iodine levels $\geq 100 \mu\text{g/L}$ and only 3 had mean levels $< 100 \mu\text{g/L}$. Therefore, the results of this study would compare to our results for women with urinary iodine levels $\geq 100 \mu\text{g/L}$. Urinary iodine levels in the Chilean study population (median 269 $\mu\text{g/L}$) were higher than urinary iodine levels found in the NHANES 2001 – 2002 population (median 168 $\mu\text{g/L}$, CI 159 – 178 $\mu\text{g/L}$). The Chilean women were also pregnant, which increases the variability in T4 and TSH. This increased variability would make an association between perchlorate and thyroid function harder to find. The second study with a large percentage of women was the Greer study discussed previously. These two studies are compared with the current study in Table 7.

The comparison in Table 7 indicates our study is the first to target and separately analyze results for women with lower levels of urinary iodine, a potentially susceptible population. A second special attribute of the current study is the much larger sample size of women, affording more statistical power to detect a potential effect. By averaging over many women, the current data likely represents a good approximation of a population steady-state exposure to perchlorate that women have had for a long period of time. If a mid- to long-term exposure is needed for perchlorate to affect thyroid function,

this data would have a better opportunity to detect that effect compared to study designs that use short-term exposures. The influence of duration of exposure merits further study.

Accurate assessment of exposure is critical to detect biochemical endpoints potentially related to exposure. Our laboratory recently developed an improved method for measuring urinary perchlorate which enhances individual perchlorate exposure assessment (Valentin-Blasini et al. 2005). The use of this new urinary perchlorate measurement strengthens the ability of this study to detect potential associations with T4 and TSH.

This study has the general limitations of a cross-sectional analysis. Therefore, the relationship between urinary perchlorate and thyroid function was examined with attention to the potential influences of chance, bias, or confounding. Perchlorate (as with any of the significant predictor variables) could be a surrogate for another unrecognized determinant of thyroid function. We also assumed in this analysis that urinary perchlorate correlates with levels in the thyroid stroma and tissue, a kinetically distinct compartment. This would be the case in a population with stable, chronic exposures; which is likely, but not certain in this population. A large sample size helps to average such potential kinetic differences. Lastly, a measurement of free T4 would be an improvement to the study.

Conclusions

Urinary perchlorate is associated with an increased TSH and decreased total T4 in women 12 and older, who have urine iodine levels $< 100 \mu\text{g/L}$, in the U.S. population during 2001-2002. For women with urine iodine levels $\geq 100 \mu\text{g/L}$, urine perchlorate is a significant predictor of TSH but not T4. These effects of perchlorate on T4 and TSH are coherent in direction and independent of other variables known to affect thyroid function, but are at perchlorate exposure levels unanticipated based on previous studies. Further research is recommended to affirm these findings.

References

- Acheson K, Jequier E, Burger A, Danforth E. 1984. Thyroid hormones and thermogenesis: the metabolic cost of food and exercise. *Metabolism* 33:262-265.
- Andersen S, Pedersen KM, Pedersen IB, Laurberg P. 2001. Variations in urinary iodine excretion and thyroid function. A 1-year study in healthy men. *Eur J Endocrinol* 144:461-465.
- Bartalena L, Bogazzi F, Tanda ML, Manetti L, Dell'Unto E, Martino E. 1995. Cigarette smoking and the thyroid. *Eur J Endocrinol* 133:507-512.
- Belin RM, Astor BC, Powe NR, Ladenson PW. 2004. Smoke exposure is associated with a lower prevalence of serum thyroid autoantibodies and thyrotropin concentration elevation and a higher prevalence of mild thyrotropin concentration suppression in the third National Health and Nutrition Examination Survey (NHANES III). *J Clin Endocrinol Metab* 89:6077-6086.
- Bertelsen JB, Hegedus L. 1994. Cigarette smoking and the thyroid. *Thyroid* 4:327-331.
- Blount BC, Valentin-Blasini L, Osterloh JD, Mauldin JP, Pirkle JL. In press. Perchlorate Exposure of the U.S. Population, 2001- 2002. *Journal of Exposure Science and Environmental Epidemiology*.

Braverman LE, He X, Pino S, Cross M, Magnani B, Lamm SH, et al. 2005. The effect of perchlorate, thiocyanate, and nitrate on thyroid function in workers exposed to perchlorate long-term. *J Clin Endocrinol Metab* 90:700-706.

Braverman LE, Pearce EN, He X, Pino S, Seeley M, Beck B, et al. 2006. Effects of Six Months of Daily Low-Dose Perchlorate Exposure on Thyroid Function in Healthy Volunteers. *J Clin Endocrinol Metab* 91:2721 – 2724; doi:10.1210/jc.2006-0184.

Braverman LE. and Utiger RD. 2000. Introduction to Hypothyroidism. In: Werner & Ingbar's *The Thyroid: A fundamental and clinical text.* (Braverman LE, Utiger RD, editors). 8th edition. Philadelphia, PA: Lippincott Williams & Wilkins, pp 719-720.

Burger AG, O'Connell M, Scheidegger K, Woo R, Danforth E. 1987. Monodeiodination of triiodothyronine and reverse triiodothyronine during low and high calorie diets. *J Clin Endocrinol Metab* 65:829-835.

Caldwell KL, Jones R, Hollowell JG. 2005. Urinary iodine concentration: United States National Health And Nutrition Examination Survey 2001-2002. *Thyroid* 15:692-699.

Canaris GJ, Manowitz NR, Mayor G, Ridgway EC. 2000. The Colorado thyroid disease prevalence study. *Arch Intern Med* 160:526-534.

CDC (Centers for Disease Control and Prevention). 2003. National Health and Nutrition Examination Survey Lab Methods 2001-2002.

Available:http://www.cdc.gov/nchs/data/nhanes/nhanes_01_02/l40t4_b_met_b_t4.pdf

[Accessed 20 March 2006].

CDC. 2004. National Health and Nutrition Examination Survey. Available:

<http://www.cdc.gov/nchs/nhanes.htm> [Accessed 20 March 2006].

Clewell RA, Merrill EA, Narayanan L, Gearhart JM, and Robinson PJ. 2004. Evidence for competitive inhibition of iodide uptake by perchlorate and translocation of perchlorate into the thyroid. *Int. J. Toxicol.* 23:17-23.

Danforth E Jr, Horton ES, O'Connell M, Sims EA, Burger AG, Ingbar SH, et al. 1979. Dietary-induced alterations in thyroid hormone metabolism during overnutrition. *J Clin Invest* 64:1336-1347.

Dasgupta PK, Martinelango PK, Jackson WA, Anderson TA, Tian K, Tock RW, et al. 2005. The origin of naturally occurring perchlorate: the role of atmospheric processes. *Environ Sci Technol* 39:1569-1575.

DeGroef B, Decallonne BR, van der Geyten S, Darras VM, and Bouillon R. 2006. Perchlorate versus other environmental sodium/iodide symporter inhibitors: potential thyroid-related health effects. *European Journal of Endocrinology.* 155:17-25.

Furlanetto TW, Nguyen LQ, Jameson JL. 1999. Estradiol increases proliferation and down-regulates the sodium/iodide symporter gene in FRTL-5 cells. *Endocrinology* 140:5705-5711.

Gibbs JP. 2006. A comparative toxicological assessment of perchlorate and thiocyanate based on competitive inhibition of iodide uptake as the common mode of action. *Human and Ecological Risk Assessment* 12:157-173.

Glinoe D. 1997. The regulation of thyroid function in pregnancy: pathways of endocrine adaptation from physiology to pathology. *Endocr Rev* 18:404-433.

Glinoe D. 2000. Thyroid disease during pregnancy. In: Werner & Ingbar's *The Thyroid: A fundamental and clinical text*. (Braverman LE, Utiger RD, eds). Philadelphia, PA:Lippincott Williams & Wilkins, 1013-1027.

Greer MA, Goodman G, Pleus RC, Greer SE. 2002. Health effects assessment for environmental perchlorate contamination: the dose response for inhibition of thyroidal radioiodine uptake in humans. *Environ Health Perspect* 110:927-937.

Haddow JE, Palomaki GE, Allan WC, Williams JR, Knight GJ, Gagnon J, et al. 1999. Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child. *N Engl J Med* 341:549-555.

Hollowell JG, Staehling NW, Flanders WD, Hannon WH, Gunter EW, Spencer CA, et al. 2002. Serum TSH, T(4), and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). *J Clin Endocrinol Metab* 87:489-499.

Jackson A, Arunagiri S, Tock R, Anderson TA, Rainwater K. 2004. Electrochemical generation of perchlorate in municipal drinking water systems. *Journal of the American Water Works Association* 96:103-108.

Jackson WA, Joseph P, Laxman P, Tan K, Smith PN, Yu L, et al. 2005. Perchlorate accumulation in forage and edible vegetation. *J Agric Food Chem* 53:369-373.

Jublanc C, Bruckert E, Giral P, Chapman MJ, Leenhardt L, Carreau V, et al. 2004. Relationship of circulating C-reactive protein levels to thyroid status and cardiovascular risk in hyperlipidemic euthyroid subjects: low free thyroxine is associated with elevated hsCRP. *Atherosclerosis* 172:7-11.

Kayser L, Perrild H, Feldt-Rasmussen U, Hegedus L, Skovsted L, Hansen JE. 1991. The thyroid function and size in healthy man during 3 weeks treatment with beta-adrenoceptor-antagonists. *Horm Metab Res* 23:35-37.

Kirk AB, Martinelango PK, Tian K, Dutta A, Smith EE, Dasgupta PK. 2005. Perchlorate and iodide in dairy and breast milk. *Environ Sci Technol* 39:2011-2017.

Klein RZ, Sargent JD, Larsen PR, Waisbren SE, Haddow JE, Mitchell ML. 2001. Relation of severity of maternal hypothyroidism to cognitive development of offspring. *J Med Screen* 8:18-20.

Knudsen N, Bulow I, Laurberg P, Ovesen L, Perrild H, Jorgensen T. 2002. Association of tobacco smoking with goiter in a low-iodine-intake area. *Arch Intern Med* 162:439-443.

Lamm SH, Braverman LE, Li FX, Richman K, Pino S, Howearth G. 1999. Thyroid health status of ammonium perchlorate workers: a cross-sectional occupational health study. *J Occup Environ Med* 41:248-260.

Laurberg P, Nohr SB, Pedersen KM, Hreidarsson AB, Andersen S, Bulow-Pedersen I, et al. 2000. Thyroid disorders in mild iodine deficiency. *Thyroid*. 10:951-963.

Lawrence JE, Lamm SH, Pino S, Richman K, Braverman LE. 2000. The effect of short-term low-dose perchlorate on various aspects of thyroid function. *Thyroid* 10:659-663.

Loucks AB, Heath EM. 1994. Induction of low-T3 syndrome in exercising women occurs at a threshold of energy availability. *Am J Physiol* 266:R817-R823.

Loucks AB, Laughlin GA, Mortola JF, Girton L, Nelson JC, Yen SS. 1992. Hypothalamic-pituitary-thyroidal function in eumenorrheic and amenorrheic athletes. *J Clin Endocrinol Metab* 75:514-518.

Mendiratta SK, Dotson RL, Brooker RT. 1996. Perchloric acid and perchlorates. In: Kirk-Othmer Encyclopedia of Chemical Technology, 4th ed., vol. 18 (Kroschwitz JJ, Howe-Grant M, eds). New York:John Wiley & Sons, Inc, 157–170.

NAS (National Academy of Sciences) 2005. Health Implications of Perchlorate Ingestion. Washington, D.C.: National Research Council, National Academy Press.

Neter J, Wasserman W, Kutner M. 1985. Applied Linear Statistical Models, 2nd ed. Homewood, Illinois. Richard D. Irwin, Inc, 346-8.

Nyrnes A, Jorde R, Sundsfjord J. 2006. Serum TSH is positively associated with BMI. *Int J Obes (Lond)* 30:100-105.

Pearce EN, Bogazzi F, Martino E, Brogioni S, Pardini E, Pellegrini G, et al. 2003. The prevalence of elevated serum C-reactive protein levels in inflammatory and noninflammatory thyroid disease. *Thyroid* 13:643-648.

Robbins J. 2000. Thyroid hormone transport proteins and the physiology of hormone binding. In: Werner & Ingbar's *The Thyroid: A fundamental and clinical text*. (Braverman LE, Utiger RD, eds). Philadelphia, PA: Lippincott Williams & Wilkins, pp. 105-120.

Sanchez CA, Krieger RI, Khandaker N, Moore RC, Holts KC, Neidel LL. 2005. Accumulation and perchlorate exposure potential of lettuce produced in the Lower Colorado River region. *J Agric Food Chem* 53:5479-5486.

Sanchez CA, Krieger RI, Khandaker N, Valentin-Blasini L, Blount BC. 2006a. Potential Perchlorate Exposure from Citrus sp. Irrigated with Contaminated Water. *Analytica Chimica Acta* 567:33-38.

Sanchez CA, Krieger RI, Valentin-Blasini L, Blount BC, Khandaker N. 2006b. Perchlorate Accumulation and Potential Exposure from Durum Wheat Irrigated with Colorado River Water. *Journal of ASTM International*. doi:10.1520/JAI100397.

Snyder SA, Pleus RC, Vanderford BJ, Holady JC. 2006. Perchlorate and chlorate in dietary supplements and flavor enhancing ingredients. *Analytica Chimica Acta* 567:26–32. doi:10.1016/j.aca.2006.03.029.

Tellez RT, Chacon PM, Abarca CR, Blount BC, Landingham CB, Crump KS, et al. 2005. Long-term environmental exposure to perchlorate through drinking water and thyroid function during pregnancy and the neonatal period. *Thyroid* 15:963-975.

Tonacchera M, Pinchera A, Dimida A, Ferrarini E, Agretti P, Vitti P, et al. 2004. Relative potencies and additivity of perchlorate, thiocyanate, nitrate, and iodide on the inhibition

of radioactive iodide uptake by the human sodium iodide symporter. *Thyroid* 14:1012-1019.

Tuzcu A, Bahceci M, Gokalp D, Tuzun Y, Gunes K. 2005. Subclinical hypothyroidism may be associated with elevated high-sensitive c-reactive protein (low grade inflammation) and fasting hyperinsulinemia. *Endocr J* 52:89-94.

Urbansky ET, Brown SK, Magnuson ML, Kelty CA. 2001. Perchlorate levels in samples of sodium nitrate fertilizer derived from Chilean caliche. *Environ Pollut* 112:299-302.

USDA (U.S. Department of Agriculture). 2004. Food and Nutrient Database for Dietary Studies, 1.0. Beltsville, MD: Agricultural Research Service, Food Surveys Research Group. Available: <http://www.ars.usda.gov/Services/docs.htm?docid=7673> [Accessed 20 March 2006].

U.S. EPA. 2005. Unregulated Contaminant Monitoring Regulation (UCMR) data from public water systems. Available: <http://www.epa.gov/safewater/ucmr/data.html> [Accessed 20 March 2006].

Valentin-Blasini L, Mauldin JP, Maple D, Blount BC. 2005. Analysis of perchlorate in human urine using ion chromatography and electrospray tandem mass spectrometry. *Anal Chem* 77:2475-2481.

Westgard JO, Barry PL, Hunt MR, Groth T. 1981. A multi-rule Shewhart chart for quality control in clinical chemistry. *Clin Chem* 27:493-501.

WHO (World Health Organization). 1994. Indicators for assessing iodine deficiency disorders and their control through salt iodization. WHO/NUT/94.6. Geneva:World Health Organization (WHO)/International Council for the Control of Iodine Deficiency Disorders.

Wolff J. 1998. Perchlorate and the thyroid gland. *Pharmacolog. Rev.* 50:89-105.

Wyngaarden JB, Stanbury JB, Rapp B. 1953. The effects of iodide, perchlorate, thiocyanate and nitrate administration upon the iodide concentrating mechanism of the rat thyroid. *Endocrinology* 52:568-574.

Table 1. Means and percent in category for covariates used in the multiple regression, women ages 12 and older, NHANES 2001-2002.^a

variable	N	N missing	Arithmetic mean (95% CI) ^b	Geometric mean (95% CI)	Percent in category (95% CI)
age (years)	1111	0	39.8 (38.1 - 41.6)		
fasting (hrs)	1111	0	10.4 (9.85 - 10.9)		
serum albumin (g/dL)	1111	0	4.20 (4.17 - 4.23)		
serum T4 (µg/dL)	1111	0	8.27 (7.97 - 8.58)		
total kilocalories (kcal / 1000)	1072	39	1.93 (1.87 - 1.99)		
body mass index	1075	36		25.8 (25.2 - 26.5)	
serum cotinine (µg/L)	1104	7		0.33 (0.23 - 0.48)	
serum C-reactive protein (mg/dL)	1111	0		0.16 (0.14 - 0.18)	
serum TSH (IU/L)	1111	0		1.36 (1.31 - 1.42)	
urine creatinine (mg/dL)	1109	2		81.4 (76.7 - 86.5)	
urine iodine (µg/L)	1111	0		126 (115 - 138)	
urine nitrate (µg/L x 1000)	1106	5		38.0 (35.9 - 40.3)	
urine perchlorate (µg/L)	1111	0		2.84 (2.54 - 3.18)	
urine thiocyanate (µg/L x 1000)	1104	7		1.20 (1.08 - 1.33)	
<i>Race</i>					
Non-Hispanic White	1111	0			69.4 (62.9 - 75.4)
Non-Hispanic Black	1111	0			12.5 (7.49 - 19.1)
Mexican American	1111	0			7.02 (5.14 - 9.34)
Other race	1111	0			11.1 (7.04 - 16.3)
<i>Medication usage</i>					
Furosemide	1111	0			1.99 (1.25 - 3.01)
Glucocorticoids and androgens	1111	0			2.23 (1.24 - 3.67)
Beta Blocker	1111	0			4.48 (3.34 - 5.87)
Estrogen	1111	0			17.1 (13.2 - 21.7)
Other drug	1111	0			1.04 (0.52 - 1.88)
Menopausal or Post menopausal	1028	83			35.9 (30.1 - 41.9)
Pregnant	1111	0			3.84 (2.74 - 5.21)
Pre-menarchal	1019	92			1.06 (0.48 - 2.02)

^aexcludes women with missing TSH, T4 or perchlorate, women with history of thyroid disease or taking thyroid drugs, and three women with outlier values of T4 or TSH (see text)

^b95% confidence interval

Table 2. Regression of serum thyroxine on perchlorate and covariates for women 12 years and older with urine iodine < 100 µg/L, NHANES 2001-2002.

Dependent variable: serum thyroxine (N = 348, R ² =0.240)			
Independent variables	Coefficient	Standard error	p-value
Intercept	8.6508	0.5428	< 0.0001
log(urinary perchlorate)	-0.8917	0.1811	< 0.0001
log(urinary creatinine)	0.6897	0.3338	0.0391
estrogen use	1.5117	0.4421	0.0007
log(C-reactive protein)	0.8249	0.1774	< 0.0001
Mexican American ^a	0.6296	0.3684	0.0878
menopause	-0.5908	0.2578	0.0221
pregnant (by test)	0.7389	0.3662	0.0439
total kilocalorie intake (divided by 1000)	-0.3334	0.1173	0.0046
premenarche	0.6401	0.2722	0.0189

^aReferent group for race is non-Hispanic whites

Table 3. Regression of serum thyroxine on perchlorate and covariates for women 12 years and older with urine iodine ≥ 100 $\mu\text{g/L}$, NHANES 2001-2002.

Dependent variable: serum thyroxine (N = 724, R ² = 0.149)			
Independent variables	Coefficient	Standard error	p-value
Intercept	10.6652	1.2345	< 0.0001
log(urinary perchlorate)	0.2203	0.3687	0.5503
log(urinary creatinine)	1.3138	0.7183	0.0677
estrogen use	0.8278	0.2722	0.0024
log(C-reactive protein)	0.5783	0.1247	< 0.0001
Mexican-American ^a	0.5763	0.2522	0.0225
pregnant (by test)	1.6175	0.3334	< 0.0001
log(urinary nitrate)	-1.1215	0.4994	0.0249
hours of fasting	0.0290	0.0156	0.0630

^aReferent group for race is non-Hispanic whites

Table 4. Regression of serum TSH on perchlorate and covariates for women 12 years and older with urine iodine < 100 µg/L, NHANES 2001-2002.

Dependent variable: Log of serum TSH (N = 356, R ² =0.061)			
Independent variables	Coefficient	Standard error	p-value
Intercept	0.2654	0.1183	0.0403
log(urinary perchlorate)	0.1230	0.0373	0.0010
log(urinary creatinine)	-0.0954	0.0761	0.2103
betablocker use	0.1881	0.0595	0.0016
estrogen use	-0.0918	0.0404	0.0233
premenarcho	0.1288	0.0262	< 0.0001

Table 5. Regression of serum TSH on perchlorate and covariates for women 12 years and older with urine iodine ≥ 100 $\mu\text{g/L}$, NHANES 2001-2002.

Dependent variable: Log of serum TSH (N = 697, R ² = 0.145)			
Independent variables	Coefficient	Standard error	p-value
Intercept	-0.6948	0.3415	0.0600
log(urinary perchlorate)	0.1137	0.0506	0.0249
log(urinary creatinine)	-0.1198	0.0910	0.1884
age in years	0.0025	0.0006	< 0.0001
log(body mass index)	0.4812	0.1346	0.0004
non-Hispanic black ^a	-0.1125	0.0335	0.0008
log(urinary nitrate)	0.1087	0.0591	0.0660
log(urinary thiocyanate)	-0.0816	0.0352	0.0206

^aReferent group for race is non-Hispanic whites

Table 6. Predicted change in serum thyroxine^a and serum TSH^b levels from changes in urinary perchlorate levels, women aged 12 and older, with urine iodine < 100 µg/L, NHANES 2001-2002.

Change in urine perchlorate (0.19 µg/L is minimum level measured)	Change in thyroxine (µg/dL)	Change in TSH (IU/L) (depends on initial TSH level)	
		initial TSH of 1.40 IU/L	initial TSH of 3.11 IU/L
		(50th TSH percentile)	(90th TSH percentile)
0.19 to 0.65 µg/L (5 th percentile)	0.48	0.23	0.51
0.19 to 0.92 µg/L (10 th percentile)	0.61	0.30	0.67
0.19 to 1.6 µg/L (25 th percentile)	0.83	0.42	0.93
0.19 to 2.9 µg/L (50 th percentile)	1.06	0.56	1.24
0.19 to 5.2 µg/L (75 th percentile)	1.28	0.70	1.56
0.19 to 9.0 µg/L (90 th percentile)	1.49	0.85	1.89
0.19 to 13 µg/L (95 th percentile)	1.64	0.95	2.12
0.19 to 100 µg/L (maximum)	2.43	1.63	3.61

^aNormal range for thyroxine: 5-12 µg/dL

^bNormal range for TSH: 0.3-4.5 IU/L

Table 7. Comparison of perchlorate studies targeting women or including a high percentage of women.

	Greer et al. 2002	Tellez et al. 2005	Current study
Number of women studied	21 females of 37 in study	184	1111
Number of women with urine iodine < 100 µg/L	Unknown (estimate 7-8)	3 ^a	348 – T4 analysis 356 – TSH analysis
Women with urine iodine < 100 µg/L analyzed separately	no	no	yes
Perchlorate dose and duration of exposure	Up to 0.5 mg/kg/day for 14 days	Long term environmental exposure	Long term environmental exposure
Comments		All women pregnant, increasing variability of T4 and TSH	

^aaverage of 1 - 3 spot urine samples