Published in final edited form as:

Environ Res. 2016 August ; 149: 179–188. doi:10.1016/j.envres.2016.05.017.

Thyroid antagonists and thyroid indicators in U.S. pregnant women in the Vanguard Study of the National Children's Study

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Abstract

The sodium iodide-symporter (NIS) mediates uptake of iodide into thyroid follicular cells. This key step in thyroid hormone synthesis is inhibited by perchlorate, thiocyanate (SCN) and nitrate (NO3) anions. When these exposures occur during pregnancy the resulting decreases in thyroid hormones may adversely affect neurodevelopment of the human fetus. Our objectives were to describe and examine the relationship of these anions to the serum thyroid indicators, thyroid stimulating hormone (TSH) and free thyroxine (FT4), in third trimester women from the initial Vanguard Study of the National Children's Study (NCS); and to compare urine perchlorate results with those in pregnant women from the National Health and Nutritional Examination Survey (NHANES).

Urinary perchlorate, SCN, NO3, and iodine, serum TSH, FT4, and cotinine were measured and a food frequency questionnaire (FFQ) was administered to pregnant women enrolled in the initial Vanguard Study. We used multiple regression models of FT4 and TSH that included perchlorate equivalent concentration (PEC, which estimates combined inhibitory effects of the anions perchlorate, SCN, and NO3 on the NIS). We used multiple regression to model predictors of each urinary anion, using FFQ results, drinking water source, season of year, smoking status, and demographic characteristics. Descriptive statistics were calculated for pregnant women in NHANES 2001–2012.

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Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention, the National Institutes of Health, or the U.S. Department of Health and Human Services. Use of trade names is for identification only and does not imply endorsement by the Centers for Disease Control and Prevention, the Public Health Service, or the U.S. Department of Health and Human Services.

Conflict of Interest: None of the authors has any actual or potential conflicts of interest to disclose.

The geometric mean (GM) for urinary perchlorate was 4.04 μg/L, for TSH 1.46 mIU/L, and the arithmetic mean for FT4 1.11 ng/dL in 359 NCS women. In 330 women with completed FFQs, consumption of leafy greens, winter season, and Hispanic ethnicity were significant predictors of higher urinary perchlorate, which differed significantly by study site and primary drinking water source, and bottled water was associated with higher urinary perchlorate compared to filtered tap water. Leafy greens consumption was associated with higher urinary NO3 and higher urinary SCN. There was no association between urinary perchlorate or PEC and TSH or FT4, even for women with urinary iodine < 100 μg/L. GM urinary perchlorate concentrations in the full sample (n=494) of third trimester NCS women (4.03 μg/L) were similar to pregnant women in NHANES (3.58 μg/L).

Keywords

National Children's Study; Perchlorate; Biomonitoring; NHANES; Pregnancy

1.0 Introduction

The perchlorate anion is an oxidizing agent, and its ammonium salt has been used in rocket and missile propellant systems, fireworks, matches, and for several other industrial uses (Trumpolt et al, 2005). Perchlorate is formed in the atmosphere and can accumulate in the soils of arid regions (Dasgupta et al, 2005). Human exposure sources include contaminated drinking water and consumption of foods containing perchlorate; mainly, milk and high surface area plants such as leafy green vegetables (Murray et al, 2008; Sanchez et al, 2009). Human health concerns are related to the ability of perchlorate to competitively inhibit iodide uptake by the sodium-iodide symporter (NIS) of the thyroid gland (Dohan and Carrasco, 2003; Tonacchera et al, 2004). So effective is it for blocking iodide uptake that high dose potassium perchlorate (up to 1000 mg per day) historically was used to treat hyperthyroidism (Crooks and Wayne, 1960). Current concerns about potential anti-thyroid effects of environmental perchlorate exposure focus largely on pregnant women. During fetal development, adequate thyroid hormone is essential for neurological development, and until 20 weeks gestation, the fetus is dependent on maternal thyroid hormone (Pearce, 2012). At the same time, adequate maternal iodine intake is essential for thyroid hormone production. Iodine intake is considered to be adequate in a population of pregnant women with a urinary median iodine concentration of 150–249 μg/L (WHO, 2008). Perchlorate absorbed into the body easily passes through the placenta, and fetal exposure parallels maternal exposure (Blount et al 2009).

The U.S. Environmental Protection Agency (EPA) has begun to develop a drinking water standard for perchlorate (U.S. EPA, 2011), but the majority of the U.S. population exposure to perchlorate derives from dietary sources. Huber et al (2011) used National Health and Nutrition Examination Survey (NHANES) data and estimated that approximately 80% of urinary perchlorate was derived from dietary sources. Others also have shown diet as the primary perchlorate exposure source for U.S. residents, based on tap water perchlorate concentrations (Mendez et al, 2010), dietary analysis (Murray, et al 2008), and analysis of

tap water and exposure data from NHANES (Blount et al, 2010; Lau et al, 2013; Yang et al, 2012).

Perchlorate is widespread in the U.S. population, with virtually 100% of participants in the NHANES having detectable urinary concentrations (Blount et al, 2007; CDC, 2015). The ubiquitous exposure to perchlorate, in combination with decreasing iodine intake, especially in women of child-bearing ages (Caldwell et al, 2013), has given impetus to examine the relationship among thyroid function, perchlorate exposure, and urinary iodine. Results have been inconsistent. U.S. women with marginal iodine intake (urinary iodine < 100 μg/L) and higher urinary perchlorate had lower thyroxine and higher thyroid stimulating hormone (TSH) values compared to women with urinary iodine > 100 μg/L (Blount et al, 2006). Prenatal perchlorate exposure increased the risk for elevated TSH in the newborn (Steinmaus et al, 2010) and was associated with lower IQ in childhood (Taylor et al, 2014). However, no association was found between urinary perchlorate and TSH or free thyroxine (FT4) in California women who had experienced perchlorate contamination of drinking water (Gold et al, 2013). Most studies in pregnant women have not found an association between measures of thyroid function (e.g. TSH, FT4, or thyroglobulin) and urinary perchlorate concentrations measured in the first or second trimesters, regardless of urinary iodine status (Pearce et al, 2010, 2011, and 2012; Tellez et al, 2005). However, others have found that urinary perchlorate was a significant predictor for increased TSH and decreased FT4 (Charatcharoenwitthaya et al, 2014; Steinmaus et al, 2015).

The anions thiocyanate (SCN) and nitrate (NO3) also inhibit the NIS and are virtually ubiquitous exposures because of their presence in green leafy vegetables and other foods (Clements 1960; Hord et al, 2009). Cruciferous vegetables, including cabbage, kale, broccoli, and cauliflower, are rich in SCN (Clements 1960). Cyanide in tobacco smoke also contributes significantly to urinary SCN in smokers (Buratti et al, 1997). Higher concentrations of these anions may act together: increased urine SCN (from smoking) and perchlorate interacted to reduce serum thyroxine in women with lower urine iodine concentrations (< 100 μg/L) (Steinmaus et al, 2013). The perchlorate equivalent concentration (PEC) has been proposed as a tool to estimate combined inhibitory effects of perchlorate, SCN, and NO3 on the NIS, (Bruce et al, 2013; Tonacchera et al, 2004) and to examine the combined effects of these anions on thyroid function. However, the PEC was weakly predictive of thyroxine and no other measures of thyroid function, and PEC was not predictive of thyroid indicators in women with inadequate iodine intake (Bruce et al, 2013).

Two sources of variability may contribute to the inconsistent results of studies that examined effects of perchlorate or PEC on thyroid hormones in pregnancy. First pertains to the physiologic changes in FT4 and TSH, particularly in early pregnancy (first-to-early second trimester). FT4 concentrations typically increase by as much as 50% in the first trimester, and TSH decreases because of increasing human chorionic gonadotropin (hCG) produced by the placenta (Stagnaro-Green et al, 2011). Second is that widely used immunologic assays for FT4 can be unreliable and subject to biases related to protein bound T4 and related proteins (Sapin et al, 2003). We sought to reduce these sources of variability by limiting our sample to third trimester women and by measuring serum FT4 in a method that uses equilibrium dialysis to separate free from protein-bound T4.

In this analysis, we evaluated perchlorate and a combined effect of the NIS inhibitors (as PEC) on FT4 and TSH, and also examined determinants of urinary perchlorate, SCN, and NO3 in third trimester women enrolled in the Vanguard Study of the NCS. We also present urinary perchlorate results from similar-aged pregnant women in NHANES 2001–2012 for comparison.

2. Methods

2.1. Study populations

The NCS Vanguard Study was a feasibility study to test the proposed recruitment, enrollment, and study visit assessment methodologies for a planned large-scale epidemiological cohort study of children and their parents. As described by Baker et al. (2014), 1399 women were enrolled in the NCS initial Vanguard Study from 2009—2010 from seven locations: Queens County, New York; Duplin County, North Carolina; Salt Lake County, Utah; Orange County, California; Montgomery County, Pennsylvania; Waukesha County, Wisconsin; and a composite location of four adjacent counties in South Dakota and Minnesota. During pregnancy, women had up to two visits that included an extensive interview, a physical examination, and collection of blood and urine specimens and environmental samples. This study reports a sample of third trimester pregnant women enrolled in the Vanguard Study and measurements made as part of a pilot study conducted with the Centers for Disease Control and Prevention (Mortensen and Hirschfeld, 2012).

NHANES has been conducted annually since 1999, releases data in two year cycles, and provides an ongoing assessment of the health, nutrition, health-related behaviors, and environmental chemical exposures in the U.S. population (CDC, 2013). Using a stratified, multistage, probability cluster design, NHANES obtains a representative sample of the non-institutionalized U.S. population. Additional information is available at: http://www.cdc.gov/nchs/nhanes/about_nhanes.htm. Each year, approximately 5000 residents randomly selected in 15 counties across the U.S. are asked to participate through an advance letter, providing information that the household has been selected as part of the NHANES sample. A field interviewer conducts screening and enrollment, and completes the household interview at the home. Subsequent interviews, physical examination, and biological specimen collections are conducted at the Mobile Examination Center (MEC). The average participation rate for data collected at the MEC is approximately 80% (NCHS, 2013). Informed consent was obtained from all NHANES participants prior to collecting, data or specimens, and the analysis presented here used only de-identified data that were publicly available.

We used NHANES 2001–2012 urine perchlorate results for pregnant women ages 16–44 years to obtain a large number of pregnant women for comparison with the Vanguard Study sample results. Over these NHANES survey cycles, urinary perchlorate results for the U.S. population and for females appeared to be stable, with little variance in the geometric means and selected percentiles (CDC, 2015).

2.2 Vanguard Study Data collection

The prenatal study visit interview inquired about demographic characteristics, health conditions, medicines, environmental influences, and health-related behaviors. Age in years at the time of the study visit, race/ethnicity (non-Hispanic black, non-Hispanic white, Hispanic, other), income (<$50,000, >=$50,000), education (not high school graduate, high school graduate and/or some college, college graduate or higher), and self-reported thyroid disorder diagnosis were candidate predictors for the regression modeling. The interview also queried participants about their primary source of drinking water with response categories of tap, filtered tap, bottled, and other source. For two-thirds of participants, this question was asked at a study visit prior to the one in which they provided a urine sample.

Following the visit, participants completed a paper Food Frequency Questionnaire (FFQ), which was based on the NCI Diet History Questionnaire (Subar et al, 2001). The FFQ queried respondents about foods consumed over the past three months and the size of their usual portion.

Written informed consent was obtained from all participants and the study protocol was approved by the NICHD Institutional Review Board (IRB) and the IRBs at each Vanguard Study institution. The involvement of the CDC laboratory was determined not to constitute engagement in human subjects research.

2.3 NHANES pregnant women

Public-release NHANES files from 2001–2012 were used to obtain records for pregnant women ages 16–44 years who were identified by a positive pregnancy test result. Trimester of pregnancy was determined by response to the question “What month of pregnancy are you in?” Participants self-reported race/ethnicity (categorized as All Hispanic, non-Hispanic white, non-Hispanic black, and other), age at last birthday, and household annual income (categorized as < $55,000 or >$55,000). Education attainment was reported by category: not high school graduate, high school graduate and/or some college, college graduate or higher. The season of participation was categorized as winter/spring (11/1-4/30) or summer/fall (5/1-10/31). Pregnant women ages 16–44 years with urine perchlorate, SCN, and NO3 results comprised the analysis group.

2.4. Serum measurements

Blood was collected from Vanguard Study participants in serum separator tubes (SST tubes, Becton Dickinson; Franklin Lakes, NJ, USA) for thyroid measures and into no-additive serum red top tubes (Becton Dickinson; Franklin Lakes, NJ, USA) for serum cotinine. Samples were centrifuged locally at ambient temperature within 2 hours of collection and shipped on ice packs to the NCS repository. At the NCS repository, red top tubes were re-spun. Serum from the red top and SST tubes were aliquotted into pre-screened metal-free cryovials. Aliquots were stored using vapor phase liquid nitrogen (−196°C) until shipment on dry ice to the analytical laboratory. Quest Diagnostics Nichols Institute (San Juan Capistrano, CA, USA) analyzed serum samples for FT4 and TSH. The free direct dialysis method separates free T4 from protein-bound T4, and then FT4 is measured directly from the protein-free dialysate. TSH was measured using an immunochemiluminometric assay

(ICMA) method. Analytical sensitivities were 0.2 ng/dL (FT4) and 0.01 mIU/L (TSH). The laboratory's reference intervals for third trimester of pregnancy were 0.8–1.7 ng/dL for FT4 and 0.43–2.91 mIU/L for TSH. Serum cotinine was analyzed by the Environmental Health Laboratory at CDC using the method described by Bernert et al. (1997 and 2000). The limit of detection for serum cotinine was 0.015 ng/mL.

2.5. Urine measurements

NCS spot urine samples were collected into pre-screened, metal-free containers, frozen locally and shipped on dry ice to the NCS Repository (Fisher Bioservices, Rockville, MD) where urine was aliquotted into pre-screened metal-free cryovials and at stored at −80°C until shipment on dry ice to the CDC laboratory. NHANES spot urine samples were collected and aliquotted in the Mobile Examination Centers, and samples were shipped on dry ice to CDC's National Center for Environmental Health, where samples were stored at or below −20°C until analyzed. All urine measurements were performed by the Environmental Health Laboratory at CDC.

Perchlorate, thiocyanate and nitrate were analyzed by isotope dilution and ion chromatography/tandem mass spectroscopy (IC-MS/MS) using a slightly modified version of the method of Valentin-Blasini et al. (2007) and available at http://www.cdc.gov/nchs/data/nhanes/nhanes_09_10/PERNT_F_met.pdf. 0.250 mL of urine was diluted to 1.0 mL with aqueous internal standard solution containing stable isotope labeled perchlorate ($Cl^{18}O_4^-$), thiocyanate ($SC^{15}N^-$), and nitrate ($^{15}NO_3^-$). Samples were vortex-mixed and queued for injection. Each analytical batch consisted of a blank, calibration standards, and four quality control (QC) samples (two QC low and two QC high). Analyte quantification was based on the peak area ratio of the analyte to stable isotope-labeled internal standard. Limits of detection (LOD) for measurements made from 2001–2012 were as follows: perchlorate 0.05 μg/L; NO3 0.7 mg/L; and SCN 0.02 mg/L. Iodine was measured by the method of Caldwell et al (2003 and 2005) that used inductively coupled plasma dynamic reaction cell mass spectrometry. Sample preparation used 0.5 mL of urine, diluted 1:10 with 1% (v/v) tetramethyl ammonium hydroxide, 0.2% Triton™ X-100 (Mallinckrodt Baker, Inc., Phillipsburg NJ), 25 μg/L tellurium, 5 μg/L bismuth, 5% (v/v) ethanol, 1000 μg/L gold, and 0.5 g/L EDTA. Iodine quantification was based on the peak as a ratio of analyte to internal standard tellurium, and the LOD was 1.4 μg/L. Reported results for iodine, perchlorate and other anions met the accuracy and precision guidelines of the quality assurance/quality control program of the Division of Laboratory Sciences, National Center for Environmental Health, CDC (Caudill et al, 2008).

For NHANES 2001–2008, urine creatinine was measured based on the Jaffé rate reaction and performed on a Beckman CX3 Chemistry Analyzer (Beckman Instruments Inc., Brea, CA, USA). Details are available at http://www.cdc.gov/nchs/data/nhanes/nhanes_01_02/l16_b_met_creatinine.pdf. Starting in 2009, urine creatinine was measured on a Roche/Hitachi Modular P Chemistry Analyzer, which employs an enzymatic method that is less susceptible to interferences from non-creatinine chromogens compared to older methods. Details are available at http://www.cdc.gov/NCHS/data/nhanes/nhanes_09_10/ALB_CR_F_met_creatinine.pdf.

3.0 Data Analysis and Processing

3.1 NCS Dietary Data Processing

The FFQ analysis program, Diet*Calc 1.4.3, (NCI, 2013) transforms the FFQ responses to estimates of energy and nutrients (values taken from USDA's Food and Nutrient Database for Dietary Studies, FNDDS) and also food group equivalent values (values taken from the Center for Nutrition Policy and Promotion's MyPyramid Equivalent Database). Diet*Calc provides estimated consumption for dairy, total fruits, total vegetables, total fish, and total energy intake, among many other foods and nutrients. Values for dark green leafy vegetables, shellfish, and finfish were obtained from the USDA Food Commodity Intake Database (FCID) (U.S. EPA, 2010) and appended to Diet*Calc so we could estimate these intakes. The FCID converts foods in the USDA FNDDS database into corresponding retail commodities. The continuous food consumption variables were categorized into three groups: low consumers (<25th percentile), medium consumers (25th to 75th percentile), and higher consumers (>75th percentile) for presentation. For use in regression modeling to predict urinary perchlorate, thiocyanate, and nitrate, the continuous values were energy-adjusted, resulting in estimates of the grams or cup equivalents per 1,000 kcal consumed for each participant.

3.2 Statistical Analysis

Data were analyzed using Statistical Analysis System (SAS, version 9.3; SAS Institute, Inc., Cary NC). For both NCS and NHANES women, we calculated geometric means (GMs) and selected percentiles stratified by demographic factors, and other variables of interest. Unless otherwise specified, all reported concentrations are geometric means (GMs). The derived variable perchlorate equivalency concentration (PEC) was calculated based on work by Tonacchera et al (2004) and Bruce, et al (2013). The PEC calculation uses relative potencies of perchlorate, SCN, and NO3 inhibition of iodine uptake as determined *in vitro.* The PEC estimates the NIS inhibitory effects of the mixture of these anions, although these estimates may not be physiologically relevant *in vivo* because of the *in vitro* derivation that does not factor in gene expression or regulation and NIS transport to the basolateral surface of the human thyrocyte.

We examined factors associated with FT4 and TSH using multiple linear regression analysis. Serum TSH was log 10-transformed because the data were positively skewed. The variables included as predictors of FT4 and TSH were age, race/ethnicity (non-Hispanic black, non-Hispanic white, Hispanic, Other), education (not high school graduate, high school graduate/some college, college graduate or more, unknown), income (<$50,000, > $50,000, unknown), smoking status (smoker >10 ng/mL; second hand smoke exposed 0.015 to <10 ng/mL; non-smoke exposed <0.015 ng/mL [< LOD]), study site, urinary iodine, thyroid disease status (not hypothyroid, hypothyroid and untreated, and hypothyroid and unknown if treated), and PEC. There were 23 women who reported current treatment for hypothyroidism and were excluded from this study.

To predict the urinary anions, we used stepwise regression with entry into the model set at p=0.20 and remain in model set at p=0.10. The candidate predictors for these models were

age, race/ethnicity, education, income, smoking status, study site, urinary creatinine, dietary variables (energy-adjusted consumption of leafy green vegetables, other vegetables, fruit, fish, and dairy), season of the year at time of study visit (winter = 12/20 to 3/19; spring = 3/20 to 6/19; summer = 6/20 to 9/19; fall = 9/20 to 12/19), primary drinking water source (bottled, filtered tap, unfiltered tap, or other), and cooking water source (bottled, filtered tap, unfiltered tap, or other). Final models included the resulting variables from the stepwise regression and, due to expected differences in the urinary anion concentrations by age, race/ethnicity, and location, these variables were included in the final models even if they did not meet the selection criteria. The urinary anions were log10-transformed because these data were positively skewed.

We also assessed correlations between log10 transformed urinary anions and iodine by calculating Pearson's correlation coefficients.

4.0 Results

4.1 Study Populations and Urinary Anion Distributions

4.1.1 NCS Women—There were 494 NCS pregnant women with urinary perchlorate, SCN, and NO3 results, after excluding the 23 who reported current treatment for hypothyroidism. Of these, 359 (73.1%) women also had FT4 and TSH results and 330 (66.3%) had urine and serum results and completed FFQ information. The demographic characteristics of these groups were similar: predominantly non-Hispanic white, college or higher educational attainment, and approximately half with annual household income of $50,000 per year or greater (data for the full NCS sample shown in Table 1). These characteristics reflect the diverse geographic distribution as well as the rural and urban settings of the recruitment populations. The distribution of urine sample collections was approximately equal across spring, summer, fall, and winter (Supplemental Table 1). Urinary perchlorate, NO3, and SCN concentrations were similar in the NCS subsamples described above, but results are not shown.

Distributions of urinary perchlorate, SCN, and NO3 concentrations in the NCS women shown in Supplemental Table 1 were stratified by the potential covariates used in the regression modeling. Although the mean (95% CI) perchlorate concentration was somewhat higher in women from Utah (4.93 μg/L [4.21, 5.78]) and in samples collected in winter (5.21μg/L [4.39, 6.17]), there was little difference among the categories compared to the overall mean of 4.03 μg/L (3.72, 4.36). Although urinary perchlorate was higher in women who primarily used bottled water, the sources for drinking water were not mutually exclusive. Urine SCN concentrations were slightly higher in non-Hispanic black women (1.17 mg/L [0.71, 1.93]) and those reporting annual incomes <$50,000 (1.03 mg/L [0.90, 1.17]) compared to the overall mean (0.88 mg/L [0.81, 0.95]). Women with serum cotinine 10 ng/mL or higher had the highest SCN concentrations (4.57 mg/L [3.25, 6.43]), consistent with tobacco smoke as a source of cyanide exposure and increased urinary SCN. The overall mean urine NO3 (45.6 mg/L [42.9, 48.4]) was similar among the categories, with the exception of women with serum cotinine 10 ng/mL or higher (59.1 mg/L [47.4, 73.7]) and those from Utah (56.2 mg/L [49.0, 64.5]). These urine anion concentrations appeared similar to values observed in all females in NHANES cycles from 2001–2012 (CDC, 2015).

In the 359 women with serum TSH and FT4 results, the overall mean (95% CI) TSH was 1.46 mIU/L (1.37, 1.56) and the overall arithmetic mean FT4 was 1.11 ng/dL (1.08, 1.13) (Table 2). All FT4 and 95% of TSH values were within the laboratory reference range for third trimester women (FT4 0.8–1.7 ng/dL and TSH 0.43–2.91 mIU/L). TSH and FT4 were higher in younger women and in non-Hispanic white and Hispanic women but the numbers in other racial/ethnic groups were relatively small. These differences were not statistically significant at $p<0.05$.

Urine iodine results in this sample have already been described (Caldwell et al, 2013). The median concentration of 167 μg/L provided evidence of adequate iodine intake, although there was variation across study sites, ranging from 107–217 μg/L.

4.1.2. NHANES Pregnant Women—In NHANES 2001–2012, there were 533 pregnant women with urinary perchlorate, NO3, and SCN results. On average, there were fewer than 100 pregnant women in each NHANES 2-year cycle, too few to be representative of the U.S. population of pregnant women. Thyroid measurements were not available for all these NHANES survey periods so an analysis similar to that done in the NCS sample was not possible. Compared with the NCS sample, a higher percentage of NHANES women were ages 30–44 years, were Hispanic, and reported a lower education attainment; a majority (61.7 %) who reported household incomes had <$55,000 per year (Table 1). Unlike the NCS sample, the NHANES women were distributed across all the trimesters.

Urinary perchlorate results were similar in the NCS and pregnant NHANES women (Table 1). The overall means (95% CI) were 4.04 μg/L (3.74, 4.37) in NCS and 3.58 μg/L (2.98, 4.18) in NHANES women, and the interquartile ranges (IQRs) were similar for both groups. The NCS winter/spring mean (4.61 μg/L [4.15, 5.12]) was higher than the NHANES mean (3.44 μg/L [2.73, 4.16]). Urinary perchlorate concentrations appeared to be similar across trimesters in the NHANES women: 3.99 (2.35, 5.62), 2.96 (2.26, 3.67), and 3.27 (2.59, 3.95) μg/L in the first, second, and third trimesters, respectively.

4.2 Correlations Between Urinary Anions and Iodine in NCS Women

Log-transformed perchlorate, NO3, and iodine were significantly correlated with each other, with correlation coefficients (r) ranging from 0.45 to 0.51. Log-transformed SCN was less strongly correlated with the other analytes; r=0.14 with log10 perchlorate, r=0.35 with log10 nitrate, and r=0.33 with log10 iodine.

4.3 Predictors of Urinary Perchlorate, SCN, and NO3 in NCS Women

4.3.1 Dietary Intakes and Urinary Anions—For the NCS pregnant women who also had complete dietary information available, Supplemental Table 2 shows mean and selected percentiles for each urinary anion, stratified by food categories and reported dietary intakes. No relationship was apparent between mean perchlorate concentrations and any dietary intake. Urinary SCN and NO3 concentrations increased with total vegetable intakes and decreased with total fruit intakes. Urinary SCN concentrations increased with leafy green vegetable intake; whereas, urine NO3 mean concentrations decreased slightly with total dairy intake. No other relationships between intakes and anion concentrations were apparent.

4.3.2 Regression Analyses—In models to examine predictors for each log-transformed anion, the food intakes were energy adjusted; that is, the amount consumed was divided by total calories, then multiplied by 1000 (to provide intake per 1000 kCal). Significant predictors (at $p<0.05$) of log10 transformed urinary perchlorate were race/ethnicity, study site, season, drinking water source, leafy green vegetable intake, and log10 urinary creatinine. Adjusted GM urinary perchlorate was highest in Hispanic women (5.07 μg/L [3.90, 6.60]) compared to other racial/ethnic categories, in women from Wisconsin (4.88 μg/L [3.59, 6.64]) compared to the other study sites, in women who were sampled in winter (4.10 μg/L [3.20, 5.26]), and in women who reported drinking bottled water (4.17 μg/L [3.41,5.10]). Leafy green vegetable intake was associated with increased urinary perchlorate concentrations, with each additional g/1,000 kCal resulting in an increase of 0.005 in the log10 urinary perchlorate concentration (Table 3). Significant predictors of log10 transformed urinary SCN were age, race/ethnicity, log10 urinary creatinine, smoking status, study site, and intakes of total fruits and leafy green vegetables. Adjusted GM SCN concentrations were higher in non-Hispanic blacks and whites (1.23 mg/L [0.83, 1.84] and 1.27 mg/L [1.09, 1.47], respectively) compared to Hispanic and other racial/ethnic categories and highest in women with serum cotinine >10 ng/mL (3.51 mg/L [2.44, 5.06]). Women from Montgomery, PA had the lowest adjusted GM SCN concentrations (0.70 mg/L [0.52, 0.95]) and women from Orange, CA and Salt Lake, UT had the highest (1.32 mg/L [0.98, 1.76] and 1.28 mg/L [1.01, 1.63]), Leafy green vegetable intake was associated with increasing SCN concentrations, and age and fruit intake were inversely related to urinary SCN (Table 3). Significant predictors of log10 transformed urinary NO3 were study site, leafy green vegetable intake, and log10 urinary creatinine. Women from Waukesha, WI and Salt Lake, UT had the highest adjusted GM SCN concentrations (52.3 mg/L [44.3, 61.9] and 51.8 [44.9, 59.7], respectively) and women from Duplin, NC had the lowest (37.8 [33.3, 42.9]. Leafy green vegetable intake was associated with increasing urinary NO3 concentrations.

Neither PEC nor iodine were significant predictors of thyroid function (Table 4). Replacing PEC in the models with perchlorate or the creatinine-adjusted PEC or perchlorate resulted in similar findings (data not shown). No predictor was significant at $p<0.05$ of either FT4 or log10 transformed TSH. These results were unchanged when we examined only women with urinary iodine <100 μg/L (data not shown).

5.0 Discussion

Neither urinary perchlorate nor PEC were predictive of TSH or FT4 in the NCS women, regardless of urinary iodine concentration. Our results are similar to studies of first trimester women that found no association between perchlorate exposure or PEC and TSH or FT4, even when urine iodine was <100 μg/L (Pearce et al, 2010, 2011, and 2012; Tellez et al, 2005). In contrast, urinary perchlorate was predictive of increased TSH and reduced FT4 in first trimester Thai women (Charatcharoenwitthaya et al, 2014) and in pregnant women exposed via contaminated drinking water (Steinmaus, et al, 2015). Studies using NHANES data also have had inconsistent findings: urinary perchlorate or PEC were not predictive of TSH or T4, even in women with urinary iodine < 100μg/L (Bruce et al., 2013); whereas high urinary perchlorate alone (Blount et al, 2006; Mendez et al, 2012) or in combination with

high SCN was associated with significantly lower T4 and/or TSH in individuals with urinary iodine < 100μg/L (Steinmaus et al, 2013). NHANES data also provided evidence that smoking, with subsequent higher urinary SCN, may interact with perchlorate to affect thyroid function, especially in women with urinary iodine < 100 μg/L (Steinmaus et al, 2007).

Differences in thyroid hormone analytical methods and pregnancy-related hormonal changes, in addition to study design and sample differences, may contribute to differences in various study results. Immunologic assays for total thyroxine (T4) and FT4 were subject to biases from pregnancy-related serum protein changes and may not be sufficiently precise (Steele et al, 2005). The method used in the present study, which employs equilibrium dialysis, separates free from protein bound thyroxine so that the free concentration can be measured directly (Thienpont et al, 2013). In fact, the variability and lack of comparability of FT4 and TSH results obtained from different analytical methods is a recognized clinical interpretation challenge (Thienpont et al, 2015). During pregnancy, thyroid hormones increase by about 50% and TSH decreases, particularly in the first trimester (Stagnaro-Green et al, 2011). Thus, changing hormone concentrations during pregnancy in addition to variability in thyroid hormone measurements may obscure effects of low level environmental exposures.

We expected urinary perchlorate concentrations to increase with consumption of leafy green vegetables and dairy, but when intakes were adjusted for race/ethnicity, study location, and season, only leafy green vegetable consumption was a significant predictor. These results are consistent with an analysis of perchlorate sources in the U.S. diet (Murray et al., 2008). That the adjusted GM urinary perchlorate was highest in Hispanic compared to other racial/ethnic categories is likely the result of dietary differences, but the small subsample size limited further analysis. The highest adjusted urinary perchlorate concentrations were observed in winter, possibly because fresh vegetables available and consumed at that time of year came from arid regions where perchlorate from natural and anthropogenic sources result in higher food crop levels (English et al, 2011; Sanchez et al, 2008 and 2009). Leafy green vegetable intake was predictive of urine SCN, consistent with the observation that various foods, including root and cruciferous vegetables (e.g., kale, broccoli, cauliflower) contain SCN or cyanide that is subsequently metabolized to SCN (Clements, 1960). In addition, all three anions and iodine were highly correlated, most likely because they are concentrated by similar mechanisms in the food supply and share dietary sources, so it is not surprising that leafy green vegetable intake was associated with higher concentrations of all three anions. Defined as serum cotinine 10 ng/mL or higher, smoking was highly predictive of urine SCN, but there were few smokers in the NCS sample. Somewhat unexpectedly, bottled water was predictive of higher urinary perchlorate, but the interview question asked primary source and did not include sufficient detail to permit any apportionment across drinking water sources. Ours may be a chance finding, or possibly, women residing where naturally-occurring perchlorate is present in the water may be aware and so avoid tap water for most but not all of their consumption.

Urinary perchlorate concentrations in this large sample of third trimester NCS women were similar to concentrations in similar-aged pregnant women who participated in NHANES

2001–2012, and both groups had concentrations similar to those of all females in the U.S. population (CDC, 2015). Means (95% CI) were similar in the three groups: NCS, 4.03 μg/L (3.72, 4.36); pregnant women in NHANES, 3.58 μg/L (2.98, 4.18); and all females (6+ years) in NHANES 2001–2012, ranging from 2.65 to 3.42 μg/L (CDC, 2015). Urinary SCN and NO3 means in the NCS women also were similar to those of all U.S. females in NHANES: NO3, 45.6 mg/L (42.9, 48.4) vs. a range of 37.1 to 41.4 mg/L; SCN, 0.88 mg/L (0.81, 0.95) vs. a range of 0.93 to 1.26 mg/L, respectively. Pregnancy did not appear to alter exposure to perchlorate or the other anions. The few NCS women who were smokers (N=30 with serum cotinine >10 ng/mL [Supplemental Table 1]) had mean urinary SCN that was similar to U.S. adult women smokers: 4.57 mg/L (3.25, 6.43) vs. 4.19 mg/L (3.81, 4.61), respectively (CDC, 2015).

Study design and methodology differences may contribute to different findings in the studies of perchlorate exposure and thyroid function in pregnant women. It is also possible that environmental perchlorate exposures in the U.S., even when SCN and NO3 are considered, may be near or below a threshold that produces measurable thyroid effects in healthy pregnant women. (Given the critical role of adequate thyroid hormone for conception and pregnancy, we consider these study populations of pregnant women to be in good overall health, regarding thyroid status, at least.) Even those studies showing negative effects on TSH and FT4 are difficult to interpret clinically, because of physiologic changes in thyroid indicators during pregnancy and the different and relatively wide span of reference range values (Medici et al, 2015; Stagnaro-Green et al, 2011). For example, the clinical laboratory that analyzed our samples reported reference values for TSH of 0.26–2.66 mIU/L (first trimester), 0.55–2.73 mIU/L (second trimester), and 0.43–2.91 mIU/L (third trimester). Similar reference values for FT4 were 0.9–2.0 ng/dL (first trimester), 0.8–1.5 ng/dL (second trimester), and 0.8–1.7 ng/dL (third trimester) (Quest Diagnostics, 2015 http://www.questdiagnostics.com/home.html). This can make it difficult to infer clinical significance of statistically significant but small changes in thyroid indicators.

Strengths of the present study include the large sample of women at a similar stage of pregnancy and quality of the laboratory methods for measuring thyroid function. We were able to use third trimester-specific TSH and FT4 reference intervals and avoid the early pregnancy-related variability in these measures (Stagnaro-Green et al, 2011). The direct dialysis method used to measure FT4 in the NCS women has greater specificity than immunoassay methods (Sapin et al, 2003; Thienpont et al, 2013). Although ours was a convenience sample, the NCS women were geographically, economically, and racial/ethnically diverse, and the relatively large sample size extends the literature on perchlorate and other anion exposures in pregnancy.

Noteworthy limitations include the FFQ and single “spot” measures of anion exposure and thyroid indicators. The NCS used a FFQ questionnaire that was based on the National Cancer Institute’s Dietary History Questionnaire and asked about intakes during the previous 3 months, so recall bias and inaccurate reporting were possible but would have been random in these generally healthy women. The urinary anions measured are rapidly eliminated after entering the body, so the measurements indicate recent exposure. However, because diet was

the major exposure source, assuming that contributory food intakes are relatively consistent and recurrent is reasonable, the urinary anion concentrations may not fluctuate greatly.

6.0 Conclusions

Neither urine perchlorate nor PEC was a predictor of TSH or FT4, even in NCS women with urinary iodine <100 μg/L. Environmental perchlorate exposure may be below a threshold for clinically-detectable thyroid effects in pregnancy. We restricted our sample to third-trimester women in whom FT4 was measured using a highly accurate analytical method. Inattention to trimester-specific ranges may affect study results involving pregnant women across different trimesters, when thyroid hormone and TSH concentrations can change dramatically. Dietary sources were the major sources of perchlorate, NO3, and SCN in non-smokers. Urinary perchlorate concentrations in the NCS third trimester women were similar to those in pregnant women in NHANES 2001–2012.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This analysis was conducted as part of the National Children's Study, supported by the Eunice Kennedy Shriver National Institute of Child Health and Human Development, and funded, through its appropriation, by the Office of the Director of the National Institutes of Health under Contract nos. HHSN275200503414C, HHSN275200503411C, HHSN275200603416C, HHSN275200503415C, HHSN275200503413C, HHSN275200503410C, and HHSN275200503396C. Funding for the biospecimen analyses was provided by the National Center for Environmental Health, Centers for Disease Control and Prevention. The manuscript was developed by a Writing Team identified by the National Children's Study Publications Committee for the purpose of timely sharing of centrally collected NCS data. We acknowledge the contributions of the following Vanguard Centers and Principal Investigators: Children's Hospital of Philadelphia, Jennifer Culhane; Mt. Sinai Medical School, Phil Landrigan; South Dakota State University, Bonny Specker; University of California at Irvine, James Swanson and Dean Baker; University of North Carolina at Chapel Hill, Barbara Entwisle and Nancy Dole; University of Utah School of Medicine, Ed Clark; University of Wisconsin, Maureen Durkin. Dr. Michael Varner provided invaluable editorial review and insightful suggestions that sharpened our focus on thyroid measurement methods and issues. We are grateful to the NHANES staff for the quality and completeness of their efforts conducting this complex survey and specimen collection. We appreciate the dedication and diligence of the NCEH laboratory staff that performed the pilot study measurements.

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Highlights

1. Limited biomonitoring data are available in pregnant women, and we report results of perchlorate, thiocyanate, and nitrate measurements in 494 third trimester women.
2. We found no effect of perchlorate and related anions on the thyroid indicators, TSH and free T4, regardless of urinary iodine concentration, in a large sample of third trimester women.
3. Leafy green vegetables were a common dietary source for perchlorate, nitrate, and thiocyanate.

Table 1

Demographics and Urinary Perchlorate Concentrations (μg/L) in Pregnant Women Ages 16–44 Years in the NCS and NHANES, 2001–2012

	Geometric Means and Selected Percentiles with Interquartile Range (IQR)									
	NCS Women					NHANES Women				
		Geometric Mean		Percentiles			Geometric Mean		Percentiles	
Category	N	(95% CI)	IQR	50th	95th	N	(95% CI)	IQR	50th	95th
Total	494	4.03 (3.72,4.36)	4.35	4.0	17.5	533	3.58 (2.98, 4.18)	4.22	3.3	20.3
Women with Thyroid Results	359	4.04 (3.68,4.44)	3.88	4.0	18.1		--	--	--	--
Age, years										
16 to 29	258	4.21 (3.79,4.68)	4.25	4.0	17.7	368	3.52 (2.94, 4.10)	4.45	3.4	15.4
30 to 44	236	3.84 (3.41,4.32)	4.40	3.8	17.5	165	3.65 (2.69, 4.62)	3.55	3.3	29.1
Race/Ethnicity										
Hispanic	99	4.54 (3.77,5.46)	5.30	4.6	23.0	170	4.14 (3.1, 5.19)	4.88	4.5	20.2
Non-Hispanic white	318	4.05 (3.66,4.47)	4.14	4.0	17.5	209	3.81 (2.84, 4.78)	3.94	3.3	14.2
Non-Hispanic black	30	3.15 (2.48,4.01)	2.39	3.1	10.4	92	3.14 (2.27, 4.02)	4.09	3.2	14.7
Other	47	3.54 (2.79,4.50)	5.22	3.7	10.8	62	2.9 (0.95, 4.84)	4.54	2.7	29.8
Education										
Not high school graduate	77	3.88 (3.14,4.79)	4.01	3.8	18.7	174	4.02 (2.96, 5.08)	5.54	4.9	14.9
High school or some college	213	4.06 (3.65,4.50)	3.85	4.1	13.1	254	3.44 (2.49, 4.38)	4.39	3.3	26.7
College graduate or more	200	4.04 (3.52,4.64)	4.95	3.8	24.4	105	3.51 (2.60, 4.42)	3.00	2.9	14.0
Unknown	1_4									
Annual Income										
< $50,000	224	4.07 (3.62,4.57)	4.06	4.0	17.7		--	--	--	--
≥ $50,000	224	4.06 (3.60,4.59)	4.53	4.0	17.5		--	--	--	--
Unknown	46	3.67 (2.91,4.63)	3.87	3.8	13.1	1_{26}				
< $55,000		--	--	--	--	313	3.49 (2.68, 4.31)	4.47	3.3	25.0
≥ $55,000		--	--	--	--	194	3.58 (2.69, 4.47)	3.90	3.3	20.1

Geometric Means and Selected Percentiles with Interquartile Range (IQR)

	NCS Women					NHANES Women				
		Geometric Mean		Percentiles			Geometric Mean		Percentiles	
Category	**N**	**(95% CI)**	**IQR**	**50th**	**95th**	**N**	**(95% CI)**	**IQR**	**50th**	**95th**
Season										
Winter/Spring (Nov. 1–April 30)	257	4.55 (4.09,5.07)	4.46	4.2	18.2	278	3.44 (2.73, 4.16)	3.89	3.3	27.9
Summer/Fall (May 1–Oct.31)	237	3.52 (3.15,3.95)	3.86	3.3.6	13.1	255	3.69 (2.75, 4.64)	4.24	3.9	20.0
[2]**Trimester**										
First		--	--	--	--	100	3.99 (2.35, 5.62)	4.28	4.8	28.4
Second		--	--	--	--	187	2.96 (2.26, 3.67)	3.62	2.9	16.4
Third	494	4.03 (3.72,4.36)	4.35	4.0	17.5	172	3.27 (2.59, 3.95)	3.08	3.2	12.0

[1]Too few results to calculate.

[2]Pregnancy trimester could not be calculated for 74 women in NHANES.

Table 2

Serum Free T4 and TSH Results in NCS Third Trimester Pregnant Women

		Serum Free T4 (Thyroxine), ng/dL				Serum Thyroid Stimulating Hormone, mIU/L			
	N	Arithmetic Mean (95% CI)	IQR	50th	95th	Geometric Mean (95% CI)	IQR	50th	95th
Women with perchlorate & thyroid results	359	1.11 (1.08,1.13)	0.30	1.10	1.50	1.46 (1.37,1.56)	1.03	1.50	3.82
Age (years)									
16 to 29	189	1.11 (1.07,1.14)	0.30	1.10	1.40	1.46 (1.33,1.61)	0.97	1.47	3.97
30 to 44	170	1.10 (1.06,1.14)	0.30	1.10	1.60	1.46 (1.34,1.59)	1.09	1.58	3.35
Race/Ethnicity									
Hispanic	72	1.09 (1.03,1.15)	0.30	1.10	1.60	1.50 (1.31,1.72)	1.09	1.60	3.77
Non-Hispanic white	231	1.10 (1.07,1.13)	0.30	1.10	1.40	1.49 (1.37,1.63)	1.07	1.53	3.83
Non-Hispanic black	22	1.07 (0.97,1.16)	0.40	1.10	1.40	1.36 (1.07,1.73)	1.00	1.51	2.20
Other	34	1.18 (1.09,1.26)	0.30	1.20	1.50	1.26 (1.05,1.51)	0.64	1.23	2.99

Third trimester reference ranges: FT4 0.8–1.7 ng/dL; TSH 0.43–2.91 mIU/L

Table 3

Regression Results for Urinary Perchlorate, Thiocyanate, and Nitrate in Third Trimester NCS Women

	Perchlorate (μg/L)			Thiocyanate (mg/L)			Nitrate (mg/L)		
	β [b]	p-value	Geo. Mean [c] (95% CI)	β [b]	p-value	Geo. Mean [c] (95% CI)	β [b]	p-value	Geo. Mean [c] (95% CI)
All NCS Women (N=329[a])			4.12 (3.73,4.55)			0.88 (0.79,0.97)			45.8 (42.7,49.1)
Race/Ethnicity		0.003			0.04			0.46	
Non-Hispanic Black			2.26 (1.43,3.59)			1.23 (0.83,1.84)			40.74 (31.54,52.62)
Hispanic			5.07 (3.90,6.60)			0.92 (0.72,1.16)			50.52 (44.11,57.85)
Non-Hispanic White			3.34 (2.74,4.06)			1.27 (1.09,1.47)			46.21 (42.05,50.78)
Other			3.15 (2.19,4.52)			0.91 (0.67,1.23)			48.34 (39.79,58.73)
Education		0.06			---			0.20	
Not high school graduate			2.70 (1.97,3.72)		---	---			49.04 (41.23,58.33)
High school graduate or some college			3.32 (2.66,4.13)		---	---			43.21 (38.72,48.22)
College graduate or more			4.06 (3.19,5.17)		---	---			46.86 (41.70,52.65)
Study Site		0.002			0.01			0.02	
BYPL, SD, MN			4.12 (3.13,5.43)			1.14 (0.90,1.44)			45.73 (39.55,52.87)
Duplin, NC			2.38 (1.84,3.08)			1.06 (0.86,1.29)			37.80 (33.33,42.86)
Montgomery, PA			3.42 (2.40,4.88)			0.70 (0.52,0.95)			45.79 (37.87,55.37)
Orange, CA			3.20 (2.28,4.50)			1.32 (0.98,1.76)			46.63 (38.88,55.93)
Queens, NY			2.15 (1.37,3.37)			1.05 (0.72,1.55)			45.65 (35.67,58.41)
Salt Lake, UT			3.91 (2.97,5.14)			1.28 (1.01,1.63)			51.76 (44.85,59.73)
Waukesha, WI			4.88 (3.59,6.64)			1.06 (0.80,1.40)			52.34 (44.30,61.85)
Smoking Status (defined by serum cotinine)		---			<.0001			---	
≥10 ng/ml		---	---			3.51 (2.44,5.06)		---	---
0.015 to <10 ng/ml		---	---			0.73 (0.60,0.89)		---	---
<0.015 ng/ml)		---	---			0.71 (0.60,0.84)		---	---
Missing		---	---			0.72 (0.58,0.89)		---	---
Season		0.01			---			---	
Fall			2.74 (2.12,3.53)		---	---		---	---
Spring			3.31 (2.59,4.23)		---	---		---	---
Summer			3.25 (2.53,4.17)		---	---		---	---

	Perchlorate (µg/L)			Thiocyanate (mg/L)			Nitrate (mg/L)		
	β [b]	p-value	Geo. Mean [c] (95% CI)	β [b]	p-value	Geo. Mean [c] (95% CI)	β [b]	p-value	Geo. Mean [c] (95% CI)
Winter			4.10 (3.20,5.26)		---	---		---	---
Drinking Water Source		0.03			---			---	
Bottled			4.17 (3.41,5.10)		---	---		---	---
Filtered tap			3.02 (2.46,3.70)		---	---		---	---
Other			2.67 (1.59,4.48)		---	---		---	---
Unfiltered tap			3.60 (2.89,4.50)		---	---		---	---
Age (years)	−0.003	0.37		−0.007	0.030		−0.0003	0.90	
Leafy Green Vegetable intake (g/1000kCal)	0.005	0.003		0.005	0.001		0.003	0.005	
Fruit intake (cups/1000kCal)	---	---		−0.055	0.002		---	---	
Vegetables intake (cups/1000kCal)	---	---		---	---		---	---	
Dairy intake (cups/1000kCal)	---	---		---	---		---	---	
Urine creatinine (log-transfromed)	0.651	<.0001		0.533	<.0001		0.711	<.0001	

[a] One individual with mising data from drinking water source was excluded from the analysis.

[b] β coefficient presented for continuous variables. A one unit change in the predictor variable results in a change the size and direction of the estimate in the log 10-transformed urinary analyte.

[c] Least squares means from regression modeling for categorical variables

Table 4

Regression Results for Serum Free T4 and Thyroid Stimulating Hormone in Third Trimester NCS Women

	Serum Free T4, ng/dL			Serum log10(TSH, mIU/mL)		
	β [a]	p-value	Arith. Mean [b] (95% CI)	β [a]	p-value	Geo. Mean [b] (95% CI)
All NCS Women (N=359)			1.11 (1.08,1.13)			1.46 (1.37,1.56)
Age (years)	−0.005	0.06		−0.002	0.47	
Race/Ethnicity		0.05			0.47	
Non-Hispanic Black			1.02 (0.87,1.17)			1.63 (1.11,2.41)
Hispanic			1.02 (0.89,1.14)			1.72 (1.24,2.39)
Other			1.18 (1.05,1.31)			1.53 (1.09,2.14)
White			1.09 (0.98,1.19)			1.87 (1.42,2.46)
Education		0.05			0.70	
Not high school graduate			1.25 (1.09,1.41)			1.97 (1.31,2.98)
High school graduate or some college			1.19 (1.04,1.34)			1.92 (1.31,2.80)
College graduate or more			1.22 (1.07,1.38)			1.77 (1.18,2.65)
Unknown			*c*			*c*
Annual Income		0.97			0.09	
<$50,000			1.08 (0.96,1.19)			1.49 (1.12,2.00)
Unknown			1.08 (0.95,1.21)			2.01 (1.43,2.82)
>=$50,000			1.07 (0.95,1.19)			1.59 (1.16,2.19)
Smoking Status (defined by serum cotinine)		0.74			0.83	
≥10 ng/ml			1.09 (0.95,1.23)			1.52 (1.06,2.18)
0.015 to <10 ng/ml			1.05 (0.94,1.16)			1.69 (1.27,2.24)
<0.015 ng/ml)			1.08 (0.98,1.19)			1.69 (1.30,2.20)
Missing			1.07 (0.90,1.25)			1.86 (1.18,2.93)
Hypothyroidism [d]		0.07			0.23	
Yes, not treated			0.89 (0.67,1.12)			1.87 (1.05,3.36)
No			0.97 (0.85,1.10)			1.30 (0.94,1.80)
Unknown			1.36 (1.08,1.63)			1.96 (0.97,3.96)
Study Site		0.46			0.11	
BYPL, SD, MN			1.05 (0.92,1.18)			1.55 (1.12,2.16)

	Serum Free T4, ng/dL			Serum log10(TSH, mIU/mL)		
	β [a]	p-value	Arith. Mean [b] (95% CI)	β [a]	p-value	Geo. Mean [b] (95% CI)
Duplin, NC			1.07 (0.96,1.19)			1.67 (1.24,2.25)
Montgomery, PA			1.10 (0.96,1.24)			1.76 (1.24,2.51)
Orange, CA			1.14 (1.01,1.26)			1.42 (1.02,1.98)
Queens, NY			1.02 (0.88,1.16)			1.79 (1.26,2.55)
Salt Lake, UT			1.05 (0.93,1.17)			2.03 (1.50,2.76)
Waukesha, WI			1.10 (0.96,1.24)			1.63 (1.13,2.36)
Urinary iodine [e]	−0.00005	0.50		0.00006	0.48	
Urinary PEC [e]	0.00007	0.10		−0.00008	0.10	

[a] β coefficient presented for continuous variables. A one unit change in the predictor variable results in a change the size and direction of the estimate in FT4 and the log 10-transformed TSH.

[b] Least squares means from regression modeling for categorical variables

[c] n<10, data redacted

[d] 23 women who reported treatment for hypothyroidism were excluded from the analysis

[e] Using creatinine corrected urinary iodine and PEC yielded similar results.