January 2013

Urinary Uranium And Thyroid Hormone And Antibody Levels In U.s. Adults, 2007-2010

Min Hyung Hyung Suh
Yale University, marissa.suh@gmail.com

Urinary Uranium and Thyroid Hormone and Antibody Levels in U.S. Adults, 2007-2010

MPH Thesis
April 30, 2014

Marissa Suh
MPH Candidate, 2014
Yale School of Public Health
Chronic Disease Epidemiology

First reader:
Adrienne S. Ettinger, Sc.D., M.P.H.
Yale School of Public Health

Second reader:
Theodore R. Holford, PhD
Yale School of Public Health

ABSTRACT

Thyroid hormones play a crucial role in the functions of the nervous, reproductive, and cardiovascular systems in humans and have been shown to be sensitive to potential disruption of normal function by environmental contaminants. Several mechanisms have been studied in the role of environmental chemical exposure in alteration of thyroid hormones, including interference in iodine transport, thyroid hormone-binding proteins, deiodinases, and receptor binding. Uranium is a naturally occurring heavy metal that is found in the form of minerals in rocks, soil, surface water, groundwater, air, plants, and animals. The general population is primarily exposed to uranium through ingestion of uranium-contaminated food or water. We sought to examine the potential association between urinary uranium levels and serum thyroid hormones and antibodies in the U.S. population, using data from the National Health and Nutrition Examination Survey (2007-2010).

We used multiple linear regression and multiple logistic regression models to investigate the potential association between environmental uranium exposure and thyroid function. Environmental uranium exposure was measured by urinary uranium concentration. Thyroid function was measured by serum concentrations of thyroid stimulating hormone (TSH), total and free thyroxine (TT_4 and FT_4, respectively), total and free triiodothyronine (TT_3 and FT_3), thyroglobulin (Tg), thyroglobulin antibody (TgAb), and thyroperoxidase antibody (TPOAb). We observed a negative linear relationship between urinary uranium and serum TT_4 levels and a positive linear relationship between urinary uranium and serum thyroglobulin antibodies. Furthermore, subjects with elevated serum TSH had higher odds and those with elevated serum TT_3 had lower odds of having been exposed to environmental uranium compared to those with lower serum TSH and TT_3, respectively. More research is needed to identify specific mechanisms by which uranium affects thyroid function and to examine the association in populations with higher-than-normal exposures to uranium.

INTRODUCTION

Thyroid hormones play a crucial role in the functions of the nervous, reproductive, and cardiovascular systems in humans [1,2,3]. They are produced and regulated by three main components: the thyroid gland, the hypothalamus, and the pituitary gland. When the hypothalamus detects that there are low levels of thyroid hormone circulating in the blood, it produces thyrotroponin-releasing hormone (TRH), which stimulates the pituitary gland to increase its production of thyroid stimulating hormone (TSH). The main role of TSH is to stimulate the thyroid gland to release thyroid hormones. This cycle operates in a negative feedback loop in which excess thyroid hormone circulating in the blood inhibits TRH and TSH production. Large glycoproteins called thyroglobulin are produced in the thyroid gland and iodinized to produce thyroid hormones. [4]

The two main types of thyroid hormone are thyroxine (T_4) and triiodothyronine (T_3). Iodine is an important component of T_4 and T_4, and thus plays a critical role in the functioning of the thyroid gland. Approximately 90% of circulating thyroid hormones are in the form of T_4 and about 10% in the form of T_3. Both types are produced in the thyroid gland and released by Tg upon stimulation by TSH, although T_4 is produced in much greater quantities compared to T_3. Some of the circulating T_4 converts to T_3 at target cells. More than 95% of thyroid hormones circulating in the blood are bound to three main carrier proteins: thyroid-binding globulin (TBG), transthyretin, and albumin. The small amount of unbound, free T_4 and T_3 (often called FT_4 and FT_3) is believed to be metabolically active within the tissues. [4]

Abnormal thyroid hormone levels in the blood can manifest as subclinical or overt hypothyroidism or hyperthyroidism. Subclinical hypothyroidism occurs when serum TSH levels are high but serum FT_4 and FT_3 concentrations are normal, and subclinical hyperthyroidism

occurs when serum TSH levels are low but FT_4 and FT_3 concentrations are normal [5]. Overt hypothyroidism is defined as low serum FT_4 concentration with high serum TSH concentration. Overt hyperthyroidism can be defined as having very low serum TSH concentration with serum FT_4 and total and FT_3 levels above the normal reference range. [6] **Table 1** shows the reference ranges for various thyroid hormones and antibodies. Currently, the serum TSH test is most widely used by clinicians to diagnose various types of thyroid dysfunction because of its high sensitivity [4].

One study reports that the prevalence of subclinical hypothyroidism in the US adult population is approximately 4-8.5% among those without known thyroid disease. Approximately 2-5% of subclinical hypothyroidism cases progress to overt disease. The prevalence of subclinical hyperthyroidism is approximately 2%, and approximately 1-2% of those with serum TSH below 0.1 μIU/mL progress to overt hyperthyroidism. [6]

Thyroglobulin (Tg), thyroglobulin antibodies (TgAb), and thyroperoxidase antibodies (TPOAb) also play important roles in the diagnosis of thyroid disease. Serum thyroglobulin measurement is an important clinical tool for patients with differentiated thyroid cancer (DTC) [4]. The presence of thyroglobulin antibodies in patients with DTC has been shown to interfere with serum Tg measurement [7]. TPO is an enzyme that is involved in the synthesis of thyroxine and triiodothyronine. The presence of TPO antibodies in the blood was found to predict a higher risk of developing overt hypothyroidism [6]. The presence of TgAb in the absence of TPOAb is not significantly associated with thyroid disease [8].

Several mechanisms have been studied in the role of environmental chemical exposure in alteration of thyroid hormones, including interference in iodine transport, thyroid hormone-binding proteins, deiodinases, and receptor binding [5]. The majority of studies investigating

thyroid hormones have focused on polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers, and bisphenol A (BPA). [9,10]

Uranium is a naturally occurring heavy metal that is found in the form of minerals in rocks, soil, surface water, groundwater, air, plants, and animals. The U.S. Environmental Protection Agency set the maximum contaminant level (MCL) for uranium in drinking water at 30 μg/L. The MCL is enforced for public water systems, but not for private wells. [11]

Health effects of uranium exposure vary depending on the chemical form of uranium, dose, and route of exposure. The general population is primarily exposed to uranium through ingestion of uranium-contaminated food or water. Persons living or working near abandoned uranium mines or hazardous waste sites can also be exposed to uranium by inhalation or dermal contact. Numerous studies have found that uranium mining is associated with increased deaths from lung cancer [12]. Several animal studies have shown that exposure to uranium through ingestion was associated with adverse effects on reproductive function and decreased fertility [13,14,15]. A study on rats found that uranium exposure through drinking water was associated with degeneration of thyroid epithelium and disturbance in thyroid function [16].

Few human studies have examined the role of environmental heavy metals in thyroid function, and even fewer have specifically explored the uranium-thyroid association. One study that examined the relationship between heavy metals in blood and urine and thyroid function in NHANES 2007-2008 participants found that uranium concentrations in the urine was associated with decreased levels of total and free thyroxine in the blood [17]. However, this study investigated 15 other heavy metals as exposures and the authors reported that including multiple metals in their models may have led to unstable results due to collinearity among the exposures.

Furthermore, this study only investigated total and free T_4, total and free T_3, and TSH as primary outcomes.

This study aims to investigate the potential association between urinary uranium concentration and thyroid hormone and antibody levels in the U.S. adult population using data from the National Health and Nutrition Examination Survey (2007-2010).

METHODS:

Data Source

The National Health and Nutrition Examination Survey (NHANES) is a complex multistage stratified cluster survey that is designed to collect demographic, dietary, examination, laboratory, and questionnaire data from a nationally representative sample of the U.S. civilian population [18]. In 2007-2008, a sample of 10,149 subjects was included in the survey. In this cycle, measurements of thyroid function and of urinary uranium were taken for a subsample of 6,200 participants 12 years and older and of 2,627 participants 6 years and older, respectively. In 2009-2010, 10,537 participants were included in the survey. In this cycle, measurements of thyroid function and of urinary uranium were collected for a subsample of 2,262 subjects 12 years and older and of 2,848 subjects 6 years and older, respectively.

After combining data from the two cycles into an analytic dataset and excluding participants younger than 20 years of age, urinary uranium levels and serum thyroid hormone and antibody levels were measured in 3,537 adults. After further excluding subjects who reported a current thyroid problem (n=241), those who were pregnant based on a urine test (n=41), and had missing values for covariates (n=321) the analytic sample for this analysis consisted of 2,934 subjects. **Figure 1** shows how the analytic sample was determined from the two NHANES cycles (2007-2008 and 2009-2010).

Taking sampling weights into consideration, this analytic sample represented 165,593,286 non-pregnant adults in the U.S. general population who do not currently have a thyroid problem.

Urinary Uranium

In this analysis, urinary uranium (ug/L) was the primary exposure of interest. In NHANES 2007-2010, urine specimens were analyzed in the Division of Environmental Health Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention. The inductively coupled plasma-mass spectrometry (ICP-MS) method was used to measure uranium in the urine. Levels of uranium in urine reflect both recent and accumulated exposure [19,20].

Thyroid Hormone and Antibody Levels

Thyroid hormone and antibody levels were the primary outcomes of interest. Eight outcome variables were used in the analysis: thyroid stimulating hormone (TSH, uIU/mL), total thyroxine (TT_4, ug/dL), free thyroxine (FT_4, ng/dL), total triiodothyronine (TT_3, ng/dL), free triiodothyronine (FT_3, pg/mL), thyroglobulin (Tg, ng/mL), thyroglobulin antibodies (TgAb, IU/mL), and thyroid peroxidase antibodies (TPOAb, IU/mL). In 2007-2010, serum thyroid hormone and antibody levels were analyzed at the University of Washington in Seattle, WA. TSH level was measured using Access HYPERsensitive hTSH assay. TT_4, TT_3, and FT_3 levels were determined using a competitive binding immunoenzymatic assay. FT_4 level was measured using a two-step enzyme immunoassay. Tg level was measured using a simultaneous one-step assay. TgAb and TPOAb levels were measured using sequential two-step immunoenzymatic assays. [21,22]

Statistical Analysis

We plotted the survey weight against urinary uranium to look for outliers, and none with extremely large survey weights were identified. Therefore, we did not exclude any exposure outliers in the analysis. Additionally, we plotted the survey weight against each of the outcome

variables to look for outliers, and none with extremely large survey weights were identified. Therefore, we did not exclude any outcome outliers in the analysis. The following variables were natural log-transformed for continuous models because the data were not normally distributed: uranium, TSH, TT_4, FT_4, TT_3, and FT_3.

This analysis explored eight covariates and potential confounders: urinary creatinine, age, sex, race/ethnicity, poverty-to-income ratio (PIR), body mass index (BMI), serum cotinine, and urinary iodine. All were selected based on existing literature on the topic of environmental heavy metals and/or thyroid function [1,2,3,23]. Urinary creatinine was included in the models as a covariate because urinary uranium concentration is highly dependent on the level of urine dilution. Urine dilution may vary drastically from person to person and time to time depending on fluid consumption, physical activity level, and health [24]. PIR was included in the models to adjust for socioeconomic status. BMI was included because of its association with thyroid function. Serum cotinine was used as a proxy for smoking status, which is associated with thyroid disorders. Research has shown that active smokers almost always have serum cotinine concentrations that are higher than10 ng/mL while nonsmokers typically have serum cotinine concentrations that are less than 1 ng/mL [25]. Urinary iodine was used as a proxy for iodine status and was included in the models because iodine is essential in the body's production of thyroid hormones.

Urinary creatinine was treated as a continuous variable. Age and urinary iodine were treated as continuous variables while sex and race/ethnicity (non-Hispanic white, non-Hispanic black, Mexican Americans or other Hispanic, and other race including multi-racial) were treated as categorical variables in all of the models. We explored both continuous and categorical

treatment for the following variables: PIR (<1, 1-1.98, 1.99-3.78, ≥3.79), BMI (<25, 25-30, ≥30 kg/m^2), and serum cotinine (<3 and ≥3 ng/mL).

We used both multiple linear regression and multiple logistic regression models in order to investigate the association between environmental uranium exposure and thyroid function. In the linear regression model, each of the outcome variables were treated as continuous variables. In the logistic regression model, TSH, Tg, TgAb, and TPOAb were categorized based on the reference ranges shown in **Table 1**. While clinical cut-points were also available for TT_4, FT_4, TT_3, and FT_3, these outcomes were categorized as below or above the median value for the analytic sample. This is because when TT_4, FT_4, TT_3, and FT_3 were categorized as low, normal, or high based on referenced ranges, the low cell counts in the low and high categories led to unstable odds ratio estimates.

With respect to the four independent variables with which we explored both continuous and categorical treatment, we chose to treat urinary uranium, PIR, BMI, and serum cotinine differently for the linear and logistic regression models. Our decision to treat each of these variables as either continuous or categorical was based on comparison of Akaike information criterion (AIC) values for the models, holding all other variables constant. For example, in deciding whether to treat urinary uranium as continuous or as quintiles in the multiple linear regression model for TSH, we compared the AIC values for these two models containing the following independent variables:

Model 1: Uranium (continuous), creatinine, age, sex, race/ethnicity, PIR, BMI, cotinine, iodine

vs.

Model 2: Uranium (quintiles), creatinine, age, sex, race/ethnicity, PIR, BMI, cotinine, iodine.

The model with the smaller AIC was chosen in order to select the best treatment of each variable.

In the linear regression model, the AIC values did not appear meaningfully different for uranium, PIR, BMI, and cotinine for each of the outcomes. Therefore, we chose to treat each of these independent variables as continuous variables. In the logistic regression model, however, the AIC values appeared meaningfully different for the four variables, so we chose to treat uranium as categorical (quintile 1 as reference), PIR and BMI as continuous, and cotinine as categorical (<3 ng/mL as reference).

For both linear and logistic regression, four combinations of covariates were investigated: urinary creatinine-only adjusted model, core model (adjusting for urinary creatinine, age, gender, and race/ethnicity), parsimonious model (adjusting for urinary creatinine, age, sex, and race/ethnicity, and various other covariates), and full model (adjusting for urinary creatinine, age, sex, race/ethnicity, PIR, BMI, serum cotinine, and urinary iodine). In the parsimonious models, covariates included varied for each outcome because covariates were selected using backward elimination. Covariates that were not statistically significant ($p>0.1$) were eliminated one at a time from the full model until all covariates remaining in the model were statistically significant. Independent variables selected for each outcome variable in both the linear and logistic models, as well as how each variable was treated are summarized in **Table 2**.

In the logistic regression model where the uranium variable was categorized into quintiles, we tested for linear trend for the odds ratios. We also performed logistic regression with uranium as a continuous variable where the unit change was the interquartile range of uranium in the analytic sample (0.01 ug/L).

All analyses were performed using SAS version 9.3. PROC SURVEYREG and PROC SURVEYLOGISTIC were used to generate regression parameters and odds ratios, respectively, in order to account for the sampling weights and complex survey methods of NHANES.

RESULTS

Descriptive characteristics for this study's analytic sample are shown in **Table 3** (N=2,934). This analytic sample represented 165,593,286 adults in the U.S. general population with no reported thyroid disease or pregnancy. The mean age of the U.S. general population represented in this sample was 46. Approximately 53% of the subjects were male and 47% were female. Nearly half of the participants were non-Hispanic white, 20% non-Hispanic black, 28% Mexican American or other Hispanic, and 5% other race. Approximately 30% of the participants had a BMI <25 kg/m^2 and 21% had income that was below the poverty threshold. Estimating that having serum cotinine concentration greater than or equal to 3 ng/mL indicates exposure to heavy cigarette smoke (including active smokers and those heavily exposed through second hand smoking), approximately 29% of the participants were heavily exposed to cigarette smoke. The mean urinary iodine concentration was 218.17 ug/L and the mean for urinary creatinine was 119.56 mg/dL.

The weighted mean for serum thyroid hormone and antibody concentrations were: TSH, 1.89 uIU/mL; TT_4, 7.83ug/dL; FT_4, 0.78 ng/dL; TT_3, 114.96 ng/dL; FT_3, 3.20 pg/mL; Tg, 15.35 ng/mL; TgAb, 9.23 IU/mL; TPOAb, 18.26 IU/mL. The weighted mean for urinary uranium was 0.013 ug/L. The mean creatinine-corrected urinary uranium concentration was 0.01 ug/g creatinine.

Statistically significant associations between ln-transformed urinary uranium and ln-transformed serum TT_4 and TPOAb were observed in the linear regression models (**Table 4**). A statistically significant negative association between uranium and TT_4 as observed in the core (β=-0.016, p=0.031), parsimonious (β=-0.015, p=0.037; adjusted for age, sex, race/ethnicity, urinary creatinine, BMI, and urinary iodine), and full (β=-0.016, p=0.030) models. A statistically

significant positive association between uranium and TPOAb was observed in the parsimonious (β=3.702, p=0.040; adjusted for age, sex, race/ethnicity, urinary creatinine, and PIR) and full (β=3.237, p=0.045) models. There were no statistically significant associations between urinary uranium and TSH, FT_4, TT_3, FT_3, Tg, and TgAb in any of the models.

Table 5 and **Figure 2** show the odds ratios and 95% confidence intervals of urinary uranium in relation to categories of thyroid hormone and antibody levels. For serum TSH, which was categorized as low (<0.3 uIU/mL), normal (0.3-3.0 uIU/mL), or high (>3.0 uIU/mL), the odds ratio for participants with urinary uranium concentration in the third quintile was marginally statistically significant (OR=1.55, 95% CI: 1.05-2.31) compared to the first quintile (≤0.0030 ug/L), after adjusting for urinary creatinine only. The odds ratios remained statistically significant in the core (OR=1.56, 95% CI: 1.05-2.31), parsimonious (OR=1.57, 95% CI: 1.05-2.36; adjusted for age, sex, race/ethnicity, urinary creatinine, and BMI), and full (OR=1.60, 95% CI: 1.06-2.42) models. For serum TT_3 concentration, categorized as below the median (<112 ng/dL) or above the median (≥112 ng/dL), the odds ratios were marginally statistically significant for only the fifth quintile of urinary uranium for the creatinine-only adjusted (OR=0.64, 95% CI: 0.45-0.91), core (OR=0.68, 95% CI: 0.45-0.98), parsimonious (OR=0.66, 95% CI: 0.45-0.98), and full (OR=0.66, 95% CI: 0.44-0.98) models compared to the first quintile. For TgAb, participants were categorized as negative or positive for TgAb in their blood (≤4.0 IU/mL or >4.0 IU/mL, respectively). The odds ratios for TgAb were statistically significant for the second quintile of urinary uranium compared to the first quintile in the creatinine-only adjusted model (OR=2.01, 95% CI: 1.21-3.34). Odds ratio for TgAb remained significant in the core (OR=1.86, 95% CI: 1.12-3.09), parsimonious (OR=1.86, 95% CI: 1.12-

3.09; adjusted for age, sex, race/ethnicity, and urinary creatinine), and full (OR=1.92, 95% CI: 1.15-3.19) models.

The tests for trend for urinary uranium quintiles in the logistic regression models for all of the outcomes were statistically non-significant (**Table 5**). The odds ratios for interquartile range change in urinary uranium were also statistically non-significant for all outcomes (**Table 5**).

DISCUSSION

In this study, we investigated the association between urinary uranium levels and thyroid hormones and antibodies in the U.S. general population using data from the National Health and Nutrition Examination Survey (2007-2010).

The mean creatinine-corrected urinary uranium concentration was 0.01 ug/g creatinine, which was similar to the mean concentrations found in other studies. One study collected spot urine specimen from a sample of Gulf War veterans who were not exposed to depleted uranium (N=23) and found that their mean urinary uranium level was 0.02 ug/g creatinine. [1]. Another study that investigated the relationship between metals in urine and blood and thyroid function using data from NHANES 2007-2008 found that the median urinary uranium concentration was 0.006 ug/L, before correcting for urinary creatinine [17]. In this study, the median uncorrected urinary uranium concentration was also 0.006 ug/L.

Previous research has suggested that exposure to environmental heavy metals may affect thyroid function in humans [1,2,3,23]. Animal models have suggested that exposure to uranium may have adverse effects on reproductive and thyroid function [13,14,15,16]. Our study observed a negative linear relationship between urinary uranium and serum TT_4 levels, although the association was not seen in the logistic regression models. For serum TSH, those with high serum TSH levels versus the combined normal and low TSH levels had 60% greater odds of having been exposed to moderate levels of environmental uranium (third quintile). In addition, those who had serum TT_3 levels above the median had 34% lower odds of having been exposed to high levels of uranium (quintile 5). Finally, our study observed a positive linear relationship between uranium exposure and serum Tg antibodies. This association was also seen in the logistic regression model where those who tested positive for Tg antibodies had 92% greater

odds compared to those who tested negative for Tg antibodies of having been exposed to moderately low levels of uranium (quintile 2). Results of the parsimonious models were similar to that of full models, indicating that adjusting for age, sex, race/ethnicity, urinary creatinine, PIR, BMI, urinary iodine, and serum cotinine in the relationship between urinary uranium and serum thyroid hormones and antibodies was appropriate.

Abnormal thyroid hormone and antibody levels in the blood have important clinical implications, although interpretation of these levels can be complicated. There has been much controversy over the suitability of diagnostic testing and treatment for subclinical thyroid disease because its clinical significance is uncertain [5]. However, subclinical hyperthyroidism and hypothyroidism can progress to overt thyroid disease that can significantly affect the quality of life for patients. Those who have overt hyperthyroidism have an overactive thyroid, which can cause sudden weight loss, fatigue, goiter, increased sweating, and rapid or irregular heartbeat [27]. Patients who have hypothyroidism often suffer from slow metabolism, weight gain, fatigue, joint and muscle pain, and decreased sweating [28].

This study has several limitations. Because of the cross-sectional nature of NHANES, we were not able to assess a causal relationship between environmental uranium exposure and abnormal thyroid function. Furthermore, urinary uranium concentration was based on a single urine sample, which may only represent recent exposure. Therefore, effect of chronic exposure to environmental uranium is difficult to derive from this analysis. We were unable to categorize total and free thyroxine and total and free triiodothyronine serum concentrations as low, normal, and high because there were too few observations in the low and high categories. Instead, we used the median value for each of these outcomes to categorize them as below or above the

median. Because we did not use clinical reference ranges for these outcomes, the results may be challenging to interpret.

Urinary dilution correction by using specific gravity or osmolality has been proposed as alternatives to using urinary creatinine adjustment [29]. However, we used urinary creatinine adjustment because other measures of urinary dilution were not available in the NHANES dataset. Next, because the covariates included in our models were initially selected based on existing literature on the effect of environmental chemical exposure on thyroid function, we did not use a specific statistical threshold for inclusion of the covariates in our models. Instead, in the parsimonious model for each outcome, backward elimination method was used to eliminate statistically nonsignificant ($p>0.1$) covariates.

Despite the limitations, this study has several strengths. A strength associated with using data from NHANES is that it contains data for many important potential confounders that we were able to adjust for in our models. Furthermore, because NHANES uses a multistage probability sampling design, the results of this study are generalizable to the entire civilian, non-institutionalized U.S. adult population.

In addition, this study has advantages over a previous study that investigated the relationship between metals in blood and urine and thyroid function [17]. Combining data from two NHANES cycles—2007-2008 and 2009-2010—in this study allowed for a large sample size (N=2,934). The study by Christensen et al used one NHANES cycle (2007-2008) and therefore had a sample size that was approximately half of that which was used in this study. Finally, our study only included urinary uranium concentration as the primary exposure variable rather than including other serum or urinary heavy metal variables in order to avoid confounding or collinearity among the exposures.

In conclusion, we observed a negative linear relationship between urinary uranium and serum TT_4 levels and a positive linear relationship between urinary uranium and serum thyroglobulin antibodies. Furthermore, subjects with elevated serum TSH had higher odds and those with elevated serum TT_3 had lower odds of having been exposed to environmental uranium compared to those with lower serum TSH and TT_3, respectively. More research is needed to identify specific mechanisms by which environmental uranium affects thyroid function. Moreover, future studies should explore the uranium-thyroid association in populations with higher levels of exposures such as occupationally exposed individuals or among residents of western United States since health effects of uranium may occur at exposure levels higher than experienced by the U.S. general population.

References

[1] Williams, G. R. (2008). Neurodevelopmental and neurophysiological actions of thyroid hormone. *Journal of neuroendocrinology*, *20*(6), 784-794.

[2] Yazbeck, C. F., & Sullivan, S. D. (2012). Thyroid disorders during pregnancy.*Medical Clinics of North America*, *96*(2), 235-256.

[3] Klein, I., & Ojamaa, K. (2001). Thyroid hormone and the cardiovascular system. *New England Journal of Medicine*, *344*(7), 501-509.

[4] Stathatos, N. (2012). Thyroid physiology. *Medical Clinics of North America*,*96*(2), 165-173.

[5] Cooper, D. S., & Biondi, B. (2012). Subclinical thyroid disease. *The Lancet*, *379*(9821), 1142-1154.

[6] Surks, M. I., Ortiz, E., Daniels, G. H., Sawin, C. T., Col, N. F., Cobin, R. H., ... & Weissman, N. J. (2004). Subclinical thyroid disease: scientific review and guidelines for diagnosis and management. *Jama*, *291*(2), 228-238.

[7] Spencer, C. A., Takeuchi, M., Kazarosyan, M., Wang, C. C., Guttler, R. B., Singer, P. A., ... & Nicoloff, J. T. (1998). Serum Thyroglobulin Autoantibodies: Prevalence, Influence on Serum Thyroglobulin Measurement, and Prognostic Significance in Patients with Differentiated Thyroid Carcinoma 1. *Journal of Clinical Endocrinology & Metabolism*, *83*(4), 1121-1127.

[8] Hollowell, J. G., Staehling, N. W., Flanders, W. D., Hannon, W. H., Gunter, E. W., Spencer, C. A., & Braverman, L. E. (2002). Serum TSH, T4, and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). *Journal of Clinical Endocrinology & Metabolism*, *87*(2), 489-499.

[9] Boas, M., Feldt-Rasmussen, U., Skakkebæk, N. E., & Main, K. M. (2006). Environmental chemicals and thyroid function. *European Journal of Endocrinology*, *154*(5), 599-611.

[10] Pearce, E. N., & Braverman, L. E. (2009). Environmental pollutants and the thyroid. *Best Practice & Research Clinical Endocrinology & Metabolism*, *23*(6), 801-813.

[11] Environmental Medicine and Educational Services. (2009). Case Studies in Environmental Medicine (CSEM) Uranium Toxicity [PDF document]. *Agency for Toxic Substances and Disease Registry*, 1-51. Retrieved from http://www.atsdr.cdc.gov/csem/uranium/docs/uranium.pdf.

[12] (2013). Health Effects. In Toxicological Profile for Uranium. *Agency for Toxic Substances and Disease Registry*, 39-262. Retrieved from http://www.atsdr.cdc.gov/toxprofiles/tp150-c3.pdf.

[13] Arfsten, D. P., Still, K. R., & Ritchie, G. D. (2001). A review of the effects of uranium and depleted uranium exposure on reproduction and fetal development.*Toxicology and industrial health*, *17*(5-10), 180-191.
[14] Maynard EA, Downs WL, Hodge HC (1953). Oral toxicity of uranium compounds. In: Voegtlin C, Hodge HC, eds. Pharmacology and toxicology of uranium compounds. Chronic inhalation and other studies. New York, NY, *McGraw-Hill*, 1121–1369.
[15] Llobet JM et al. (1991). Influence of chronic exposure to uranium on male reproduction in mice. Fundamental and Applied Toxicology, *16*:821–829.
[16] Malenchenko AF, Barkun NA, Guseva GF (1978). Effect of uranium on the induction and course of experimental autoimmune ochitis and thyroiditis. Journal of Hygiene, Epidemiology, Microbiology and Immunology, *22*(3):268–277.
[17] Yorita Christensen KL. (2013). Metals in blood and urine, and thyroid function among adults in the United States 2007-2008. Int J Hyg Environ Health, *216*(6):624-32.
[18] Centers for Disease Control and Prevention (CDC). (2014). About the National Health and Nutrition Examination Survey. Retrieved from http://www.cdc.gov/nchs/nhanes/about_nhanes.htm.
[19] Centers for Disease Control and Prevention (CDC). (2009). 2007-2008 Data Documentation, Codebook, and Frequencies: Urinary Heavy Metals. *National Center for Health Statistics (NCHS) National Health and Nutrition Examination Survey*. Retrieved from http://www.cdc.gov/nchs/nhanes/nhanes2007-2008/UHM_E.htm.
[20] Centers for Disease Control and Prevention (CDC). (2011). 2009-2010 Data Documentation, Codebook, and Frequencies: Urinary Heavy Metals. *National Center for Health Statistics (NCHS) National Health and Nutrition Examination Survey*. Retrieved from http://www.cdc.gov/nchs/nhanes/nhanes2009-2010/UHM_F.htm.
[21] Centers for Disease Control and Prevention (CDC). (2009). 2007-2008 Data Documentation, Codebook, and Frequencies: Thyroid Profile. *National Center for Health Statistics (NCHS) National Health and Nutrition Examination Survey*. Retrieved from http://www.cdc.gov/nchs/nhanes/nhanes2007-2008/THYROD_E.htm.
[22] Centers for Disease Control and Prevention (CDC). (2011). 2009-2010 Data Documentation, Codebook, and Frequencies: Thyroid Profile. *National Center for Health Statistics (NCHS) National Health and Nutrition Examination Survey*. Retrieved from

http://www.cdc.gov/nchs/nhanes/nhanes2009-2010/THYROD_F.htm.
[23] Chen, A., Kim, S. S., Chung, E., & Dietrich, K. N. (2013). Thyroid Hormones in Relation to Lead, Mercury, and Cadmium Exposure in the National Health and Nutrition Examination Survey, 2007–2008. *Environmental health perspectives*,*121*(2), 181.
[24] Centers for Disease Control and Prevention (CDC). Using Blood Lipid or Urine Creatinine Adjustments of Environmental Chemical Data. Retrieved from http://www.cdc.gov/nchs/tutorials/environmental/critical_issues/adjustments/Info1.htm.
[25] Hukkanen, J., Jacob, P., & Benowitz, N. L. (2005). Metabolism and disposition kinetics of nicotine. *Pharmacological reviews*, *57*(1), 79-115.
[26] McDiarmid, M. A., Hooper, F. J., Squibb, K., & McPhaul, K. (1999). The utility of spot collection for urinary uranium determinations in depleted uranium exposed Gulf War veterans. *Health physics*, *77*(3), 261-264.
[27] Hyperthyroidism. (2013). In *MedlinePlus Medical Encyclopedia*. Retrieved from http://www.nlm.nih.gov/medlineplus/ency/article/000356.htm.
[28] Hypothyroidism. (2013). In *MedlinePlus Medical Encyclopedia*. Retrieved from http://www.nlm.nih.gov/medlineplus/ency/article/000353.htm.
[29] Barber, T. E., & Wallis, G. (1986). Correction of urinary mercury concentration by specific gravity, osmolality, and creatinine. *Journal of Occupational and Environmental Medicine*, *28*(5), 354-359.

Table 1. Referenced ranges for thyroid hormones[a] and antibodies[b]

Thyroid Hormones	**Low**	**Normal**	**High**
TSH (μIU/mL)	<0.4	0.4-6.0	>6.0
Total T_4 (ng/dL)	<4.5	4.5-12.6	>12.6
Free T_4 (ng/dL)	<0.7	0.7-1.8	>1.8
Total T_3 (ng/dL)	<80	80-220	>220
Free T_3 (pg/mL)	<2.0	2.0-5.0	>5.0
Tg (ng/mL)	<5	5-25	>25
Anti-thyroid antibodies	**Negative**	**Positive**	
TgAb (IU/mL)	<4.0	≥4.0	
TPOAb (IU/mL)	<9.0	≥9.0	

[a] Garber, J. R., Cobin, R. H., Gharib, H., Hennessey, J. V., Klein, I., Mechanick, J. I., ... & Woeber, K. A. (2012). Clinical practice guidelines for hypothyroidism in adults: cosponsored by the American Association of Clinical Endocrinologists and the American Thyroid Association. *Endocrine Practice*,*18*(6), 988-1028.

[b] Gallagher, C. M., & Meliker, J. R. (2012). Mercury and thyroid autoantibodies in US women, NHANES 2007–2008. *Environment international*, 40, 39-43.

Table 2. Summary of selection and treatment of independent variables in multiple linear regression and multiple logistic regression models

Outcome variables	Creatinine-only adjusted model	Core model[a]	Parsimonious model[b]	Full model
Linear regression				
TSH	Uranium (cont.) Creatinine (cont.)	+Age (cont.) +Sex (cat.) +Race/ethnicity (cat.)	+PIR (cont.) +BMI (cont.)	Uranium (cont.) Creatinine (cont.) Age (cont.) Sex (cat.) Race/ethnicity (cat.) PIR (cont.) BMI (cont.) Cotinine (cont.) Iodine (cont.)
TT_4			+BMI (cont.) +Iodine (cont.)	
FT_4			+Iodine (cont.)	
TT_3			+PIR (cont.) +BMI (cont.) +Cotinine (cont.) +Iodine (cont.)	
FT_3			+PIR (cont.) +BMI (cont.) +Cotinine (cont.) +Iodine (cont.)	
Tg			+BMI (cont.) +Cotinine (cont.) +Iodine (cont.)	
TgAb			+Iodine (cont.)	
TPOAb			+PIR (cont.)	
Logistic regression				
TSH	Uranium (cat.) Creatinine (cont.)	+Age (cont.) +Sex (cat.) +Race/ethnicity (cat.)	+BMI (cont.)	Uranium (cat.) Creatinine (cont.) Age (cont.) Sex (cat.) Race/ethnicity (cat.) PIR (cont.) BMI (cont.) Cotinine (cat.) Iodine (cont.)
TT_4			+PIR (cont.) +BMI (cont.)	
FT_4			+None	
TT_3			+PIR (cont.) +BMI (cont.) +Iodine (cont.)	
FT_3			+BMI (cont.) +Cotinine (cat.) +Iodine (cont.)	
Tg			+BMI (cont.) +Cotinine (cat.) +Iodine (cont.)	
TgAb			+None	
TPOAb			+BMI (cont.)	

[a] In addition to independent variables listed in the creatinine-only adjusted model

[b] In addition to independent variables listed in the creatinine-only adjusted and core models

Table 3. Descriptive characteristics of NHANES 2007-2010 participants aged 20 and older with no missing values for THs, uranium, and covariates who are not currently pregnant and do not currently have a thyroid problem (N=2,934)

	Adults (ages 20+)				
Characteristic	**N**	**Column %**	**Weighted Mean**	**Weighted 95% CI**	**NHANES Range**
Age			46.02	44.94-47.10	20-80
20-39	1014	38.75			
40-59	963	39.03			
≥60	957	22.22			
Sex					
Male	1543	52.59			
Female	1391	47.41			
Race/ethnicity					
White (non-Hispanic)	1405	47.89			
Black (non-Hispanic)	566	19.29			
Mexican American or Hispanic	815	27.78			
Other	148	5.04			
BMI (kg/m2)			28.44	28.12-28.76	13.18-67.71
<25	858	29.24			
25-30	1017	34.66			
≥30	1059	36.09			
Poverty-to-income ratio (PIR)			2.99	2.86-3.13	0.00-5.00
<1	630	21.47			
1-1.98	779	26.55			
1.99-3.78	757	25.80			
≥3.79	768	26.18			
Serum cotinine (ng/mL)			63.21	54.83-71.58	0.01-1160.00
<3	2097	71.47			
≥3	837	28.53			
Urinary iodine (ug/L)			218.17	206.36-264.00	7.70-34017.00
Urinary creatinine (mg/dL)			119.56	115.67-123.46	8.00-528.00
Serum thyroid hormone levels					
TSH (uIU/mL)			1.89	1.82-1.95	0.01-69.84
Low (<0.3)	35	1.19			
Normal (0.3-3.0)	2541	86.61			
High (>3.0)	358	12.20			
TT_4 (ug/dL)			7.83	7.72-7.93	2.40-16.80
Below median (<7.8)	1455	49.59			
Above median (≥7.8)	1479	50.41			
FT_4 (ng/dL)			0.78	0.77-0.79	0.20-3.40
Below median (<0.8)	1372	46.76			
Above median (≥0.8)	1562	53.24			
TT_3 (ng/dL)			114.96	113.11-116-80	38.00-401.00
Below median (<112)	1410	48.06			
Above median (≥112)	1524	51.94			
FT_3 (pg/mL)			3.20	3.18-3.23	1.80-28.84
Below median (<3.12)	1466	49.97			
Above median (≥3.12)	1468	50.03			

Tg (ng/mL)			15.35	14.25-16.46	0.07-1210.90
Low (<5)	554	18.88			
Normal (5-25)	1976	67.35			
High (>25)	404	13.77			
TgAb (IU/mL)			9.23	5.03-13.43	0.60-1614.50
Negative (≤4)	2763	93.89			
Positive >4)	171	6.11			
TPOAb (IU/mL)			18.26	14.84-21.67	0.10-2376.00
Negative (≤9.0)	2654	89.78			
Positive (>9.0)	280	10.22			
Urinary uranium (ug/L)			0.013	0.009-0.016	0.00-1.82
Quintile 1 (≤0.0030)	614	20.93			
Quintile 2 (0.0030-0.0052)	567	19.33			
Quintile 3 (0.0053-0.0086)	594	20.25			
Quintile 4 (0.0087-0.0151)	576	19.63			
Quintile 5 (>0.0152)	583	19.87			

Table 4. Regression coefficients, standard errors, and p-values of ln-transformed urinary uranium levels in relation to ln-transformed THs for creatinine-only adjusted, core, parsimonious, and full models

Thyroid hormones/ antibodies[e]	Unadjusted[a]			Core model[b]			Parsimonious model[c]			Full model[d]		
	β Estimate	SE	P-Value	β Estimate	SE	P-Value	β Estimate	SE	P-Value	β Estimate	SE	P-Value
TSH	0.016	0.017	0.355	0.011	0.018	0.564	0.017	0.018	0.371	0.020	0.020	0.318
TT_4	-0.012	0.007	0.109	-0.016	0.007	0.031	-0.015	0.007	0.037	-0.016	0.007	0.030
FT_4	0.001	0.006	0.824	0.001	0.006	0.922	0.001	0.006	0.923	0.000	0.006	0.978
TT_3	-0.013	0.006	0.052	-0.011	0.007	0.129	-0.012	0.007	0.080	-0.012	0.007	0.080
FT_3	-0.002	0.004	0.603	0.002	0.004	0.689	0.001	0.004	0.847	0.001	0.004	0.847
Tg	0.010	0.034	0.779	0.019	0.035	0.596	-0.002	0.033	0.958	-0.002	0.033	0.954
TgAb	3.229	2.997	0.290	2.949	2.901	0.317	2.949	2.901	0.317	2.881	3.040	0.350
TPOAb	3.637	1.812	0.053	3.226	1.792	0.081	3.702	1.729	0.040	3.237	1.548	0.045

a Adjusted for urinary creatinine only
b Adjusted for age, gender, race/ethnicity, and urinary creatinine
c Adjusted for covariates selected through backward elimination (eliminated if p>0.1), different covariates selected for each outcome
d Adjusted for age, gender, race/ethnicity, urinary creatinine, PIR, BMI, serum cotinine, and urinary iodine
e Ln-transformed

Table 5. Odds ratios and 95% confidence intervals of urinary uranium quintiles in relation to THs for creatinine-only adjusted, core, parsimonious and full models

Thyroid hormones/ antibodies[e]	Uranium[f]	Unadjusted model[a]			Core model[b]			Parsimonious model[c]			Full model[d]		
		OR	Lower 95% CI	Upper 95% CI	OR	Lower 95% CI	Upper 95% CI	OR	Lower 95% CI	Upper 95% CI	OR	Lower 95% CI	Upper 95% CI
TSH	Quintile												
	Quintile 1	1.00 (referent)			1.00 (referent)			1.00 (referent)			1.00 (referent)		
	Quintile 2	1.31	0.88	1.94	1.27	0.86	1.87	1.24	0.84	1.84	1.27	0.85	1.90
	Quintile 3	1.55	1.05	2.31	1.56	1.05	2.31	1.57	1.05	2.36	1.60	1.06	2.42
	Quintile 4	1.19	0.79	1.78	1.16	0.77	1.76	1.18	0.77	1.79	1.22	0.79	1.88
	Quintile 5	1.48	0.92	2.37	1.39	0.86	2.25	1.43	0.87	2.34	1.48	0.90	2.45
	p-trend	0.20			0.30			0.24			0.19		
	Continuous												
	IQR (0.01)	1.00	0.99	1.02	1.00	0.99	1.01	1.00	0.99	1.02	1.00	0.99	1.02
TT_4	Quintile												
	Quintile 1	1.00 (referent)			1.00 (referent)			1.00 (referent)			1.00 (referent)		
	Quintile 2	0.89	0.67	1.18	0.83	0.61	1.12	0.81	0.60	1.09	0.81	0.60	1.09
	Quintile 3	1.04	0.77	1.40	0.94	0.70	1.28	0.94	0.69	1.29	0.95	0.70	1.28
	Quintile 4	0.88	0.62	1.24	0.79	0.55	1.12	0.79	0.55	1.12	0.79	0.55	1.14
	Quintile 5	0.92	0.61	1.39	0.83	0.54	1.26	0.83	0.55	1.26	0.84	0.55	1.29
	p-trend	0.72			0.41			0.45			0.49		
	Continuous												
	IQR (0.01)	0.99	0.97	1.01	0.99	0.97	1.01	0.99	0.97	1.01	0.99	0.97	1.01
FT_4	Quintile												
	Quintile 1	1.00 (referent)			1.00 (referent)			1.00 (referent)			1.00 (referent)		
	Quintile 2	0.90	0.69	1.18	0.89	0.67	1.19	0.89	0.67	1.19	0.89	0.67	1.18
	Quintile 3	1.00	0.70	1.42	0.98	0.68	1.42	0.98	0.68	1.42	0.98	0.68	1.41
	Quintile 4	1.02	0.76	1.36	0.98	0.73	1.31	0.98	0.73	1.31	0.97	0.73	1.29
	Quintile 5	0.99	0.72	1.36	0.95	0.69	1.32	0.95	0.69	1.32	0.95	0.69	1.31
	p-trend	0.79			0.99			0.99			0.98		
	Continuous												
	IQR (0.01)	1.02	1.00	1.04	1.02	1.00	1.04	1.02	1.00	1.04	1.02	1.00	1.04
TT_3	Quintile												
	Quintile 1	1.00 (referent)			1.00 (referent)			1.00 (referent)			1.00 (referent)		
	Quintile 2	0.77	0.57	1.05	0.81	0.62	1.07	0.80	0.59	1.08	0.80	0.59	1.08
	Quintile 3	0.79	0.61	1.03	0.82	0.63	1.06	0.82	0.62	1.07	0.81	0.62	1.07
	Quintile 4	0.84	0.60	1.18	0.87	0.62	1.21	0.87	0.62	1.22	0.86	0.61	1.22
	Quintile 5	0.64	0.45	0.91	0.67	0.45	0.98	0.66	0.45	0.98	0.66	0.44	0.98
	p-trend	0.06			0.11			0.12			0.12		
	Continuous												

	IQR (0.01)	0.96	0.92	1.01	0.96	0.91	1.02	0.96	0.91	1.02	0.96	0.91	1.02
	Quintile												
	Quintile 1	1.00 (referent)			1.00 (referent)			1.00 (referent)			1.00 (referent)		
FT_3	Quintile 2	0.96	0.71	1.29	1.10	0.82	1.46	1.07	0.80	1.43	1.07	0.80	1.43
	Quintile 3	0.88	0.67	1.16	0.99	0.73	1.34	0.99	0.73	1.34	0.98	0.72	1.34
	Quintile 4	1.08	0.83	1.42	1.23	0.96	1.56	1.21	0.93	1.57	1.21	0.93	1.56
	Quintile 5	1.11	0.81	1.51	1.36	0.93	2.00	1.36	0.93	1.99	1.34	0.91	1.98
	p-trend	0.36			0.09			0.10			0.11		
	Continuous												
	IQR (0.01)	1.02	1.00	1.03	1.02	1.00	1.04	1.02	1.00	1.04	1.02	1.00	1.04
	Quintile												
	Quintile 1	1.00 (referent)			1.00 (referent)			1.00 (referent)			1.00 (referent)		
Tg	Quintile 2	1.23	0.89	1.69	1.23	0.91	1.67	1.15	0.86	1.54	1.15	0.86	1.54
	Quintile 3	1.29	0.94	1.78	1.32	0.94	1.84	1.25	0.91	1.72	1.25	0.91	1.72
	Quintile 4	1.08	0.76	1.55	1.14	0.79	1.65	1.03	0.71	1.48	1.03	0.71	1.48
	Quintile 5	1.31	0.90	1.93	1.39	0.95	2.05	1.25	0.86	1.81	1.25	0.86	1.81
	p-trend	0.37			0.23			0.48			0.49		
	Continuous												
	IQR (0.01)	1.00	0.99	1.01	1.00	0.99	1.01	1.00	0.99	1.01	1.00	0.99	1.01
	Quintile												
	Quintile 1	1.00 (referent)			1.00 (referent)			1.00 (referent)			1.00 (referent)		
TgAb	Quintile 2	2.01	1.21	3.34	1.86	1.12	3.09	1.86	1.12	3.09	1.92	1.15	3.19
	Quintile 3	1.21	0.68	2.14	1.12	0.64	1.95	1.12	0.64	1.95	1.15	0.66	2.02
	Quintile 4	1.80	1.02	3.17	1.63	0.92	2.87	1.63	0.92	2.87	1.70	0.94	3.07
	Quintile 5	1.59	0.87	2.94	1.38	0.72	2.64	1.38	0.72	2.64	1.45	0.78	2.68
	p-trend	0.26			0.54			0.54			0.42		
	Continuous												
	IQR (0.01)	1.01	0.99	1.02	1.00	0.99	1.02	1.00	0.98	1.02	1.00	0.98	1.02
	Quintile												
	Quintile 1	1.00 (referent)			1.00 (referent)			1.00 (referent)			1.00 (referent)		
TPOAb	Quintile 2	1.17	0.71	1.93	1.08	0.65	1.79	1.08	0.65	1.80	1.10	0.65	1.84
	Quintile 3	1.11	0.61	2.02	1.00	0.55	1.82	0.99	0.54	1.80	1.00	0.55	1.85
	Quintile 4	1.42	0.80	2.51	1.28	0.71	2.32	1.27	0.70	2.30	1.29	0.70	2.37
	Quintile 5	1.56	0.95	2.55	1.35	0.79	2.31	1.32	0.78	2.24	1.37	0.80	2.36
	p-trend	0.14			0.31			0.35			0.30		
	Continuous												
	IQR (0.01)	1.01	0.99	1.04	1.01	0.99	1.03	1.00	0.98	1.02	1.00	0.99	1.01

a Adjusted for urinary creatinine only.
b Adjusted for age, gender, race/ethnicity, and urinary creatinine.

c Adjusted for covariates selected through backward elimination (eliminated if p>0.1), different covariates selected for each outcome.
d Adjusted for age, gender, race/ethnicity, urinary creatinine, PIR, BMI, serum cotinine, and urinary iodine.
e Treated as ordinal or binary: TSH (low vs. normal vs. high); TT4 (below vs. above median); FT4 (below vs. above median), TT3 (below vs. above median); FT3 (below vs. above median); Tg (low vs. normal vs. high); TgAb (negative vs. positive); TPOAb (negative vs. positive).
f Urinary uranium levels were treated as categorical (quintiles, where referent group was quintile 1) and as continuous (where the unit of change was the interquartile range of uranium, 0.01 ug/L).

Figure 1. Analytic sample selection from NHANES 2007-2010

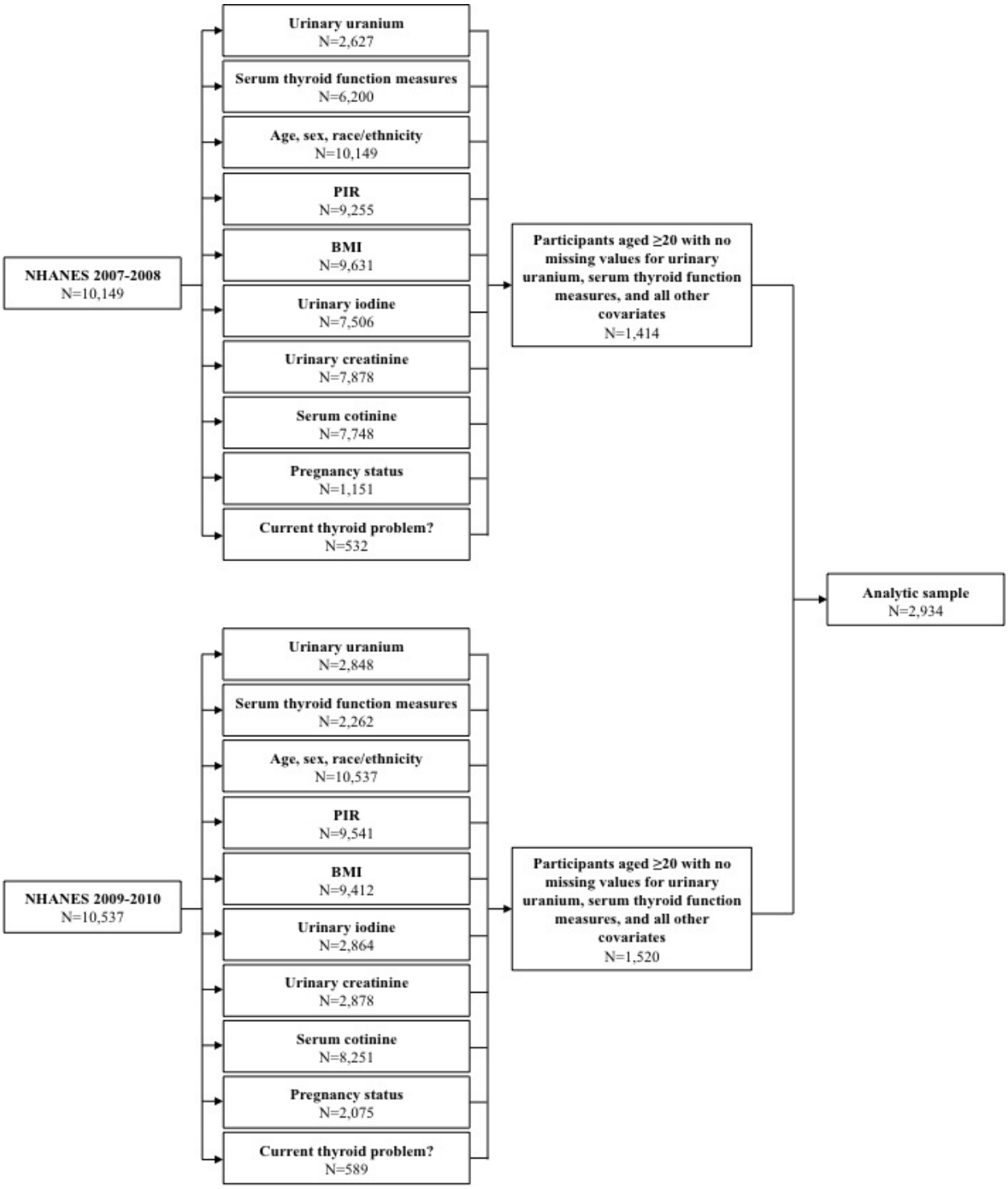

Figure 2. Estimated odds ratios and 95% confidence intervals for thyroid hormones and antibodies according to urinary uranium quintiles, adjusted for age, sex, race/ethnicity, urinary creatinine, PIR, BMI, serum cotinine, and urinary iodine