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Association between Perchlorate and Indirect Indicators of Thyroid Dysfunction in NHANES 2001–2002, a Cross-Sectional, Hypothesis-Generating Study

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Abstract

Background: A previous study based on NHANES 2001–2002 observed that increased levels of urinary perchlorate were associated with increased levels of thyroid stimulating hormone among all women, and with decreased levels of thyroxine among women with low urinary iodine. No associations were observed for men.

Methods: Using the same NHANES 2001–2002 data, associations of urinary perchlorate with indirect biomarkers of thyroid hormone disruption were investigated. Decreased levels of hemoglobin (HGB), hematocrit (HCT), and high density lipoprotein (HDL) have been observed among subjects with subclinical hypothyroidism. To investigate the suitability of these indicators for use in observational studies, subjects were divided into six groups: boys, age 6–19; men, age 20–85; girls, age 6–14; non-pregnant women, age 15–49; women, age 50–85; and pregnant women. Use of perchlorate quintiles (Q1-Q5) and continuous log-transformed perchlorate in the regression models allowed investigation of both non-linear and linear associations. Adjustments were made for age, urinary creatinine, race/ethnicity, body mass index, cotinine, poverty index, hours of fasting, thiocyanate, nitrate, daily kcal intake, C-reactive protein. Adjustment for alcohol consumption depended on availability. Adjustment for prescription drugs (beta-blockers, sex hormones, antihyperlipidemic and antidiabetic drugs) was made if it changed the perchlorate estimate by ≥10%.

Results: Statistically significant decreases were observed for HGB and HCT among boys, men, women age 15–49, and pregnant women, and for HDL among men.

Conclusions: Although the mean response biomarkers were within normal range, their association with urinary perchlorate is of interest. HGB and HCT among pregnant women showed a stronger association with urinary perchlorate than non-pregnant women age 15–49. Statistically significant associations were observed for individual perchlorate quintiles. Assumption of linearity of log-transformed perchlorate may result in underestimation of some associations.

Keywords: perchlorate, biomarkers, endocrine disruption, hypothyroidism, HDL, haemoglobin, hematocrit

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Background

Presence of perchlorate (ClO_4^-) in the environment is widespread due to both natural processes and human activities.[1] Exposure of humans, which occurs primarily through ingestion of contaminated water and food, is a current national concern, because perchlorate is a known thyroid toxicant. It prevents iodide from entering the thyroid, which may lead to diminished production of thyroid hormones. Several studies have discussed the risks of environmental exposures to perchlorate, especially the potential adverse effects on thyroid hormone function.[1–4]

Urinary perchlorate levels can be used as non-invasive surrogate biological markers of exposure, because perchlorate is not metabolized in the human body.[1] Thyroid stimulating hormone (TSH) and thyroxine (T4) are measured as direct biological markers of effect on thyroid economy, as for example in newborn screening and adult testing for hypo- and hyperthyroidism. A recent study based on the National Health and Nutrition Examination Survey (NHANES) 2001–2002, assessed associations of urinary perchlorate with TSH and T4.[2] Results showed that increased levels of urinary perchlorate were associated with increased levels of TSH for all women, and decreased levels of T4 for women with low urinary iodine (<100 μg/L). No association was observed for men. Another study among women in the same NHANES 2001–2002 data observed that smoking and thiocyanate interact with perchlorate in diminishing thyroid function.[3] These studies brought attention to the gender-specific effect of perchlorate on the thyroid.[5]

Thyroid hormones are a requisite for proper neurodevelopment and physiological function throughout life. Levels of thyroid hormones (THs) serve as direct indicators of thyroid hormone dysfunction. Examples are increased levels of TSH associated with normal or decreased levels of T4, or decreased levels of T4 without a change of TSH, which may occur when T4 is displaced from its binding protein by a xenobiotic. Another type of disruption occurs when endocrine disrupting chemicals displace THs from their receptors, which can change the structure and the signaling of the TH receptor without necessarily changing the TH levels.[5–8] Signaling pathways of different nuclear receptors, including TH receptors, can cross-talk with each other, and disrupt the TH transcription process with unknown biological consequences.[6,7] Chemicals that are known to cause adverse changes in circulating TH levels have been classified as thyroid toxicants.[6] In contrast, endocrine disrupting chemicals that cause perturbation of TH action through their receptors, such as signaling, without causing a change in TH levels, are not included in the definition of thyroid toxicants.[6] Given that environmental pollutants can affect THs in different ways, it may be useful to investigate if in addition to the routinely used "direct" indicators, "indirect" biomarkers of TH dysfunction can be identified, in order to expand the available number of biomarkers measuring the level of TH dysfunction in observational human studies. Therefore, to expand observations of adverse effects associated with TH, both changes in TH levels and TH action may have to be considered. The problem is how to determine perturbation of TH action. Thyroid hormones play an important role in many functions of the body, such as lipid metabolism, enzyme activity, neurologic, endothelial, and cardiac functions.[9,10] Interference of TH action by environmental pollutants may adversely affect one or more of these systems, which may be indicated by effect biomarkers, such as decreased levels of HDL, HGB or HCT ("indirect" markers of thyroid dysfunction). High density lipoprotein (HDL), a major factor in the reverse cholesterol transport (RCT) mechanism, removes excess cholesterol from peripheral tissues to the liver for excretion.[11–15] A decrease of HDL may indicate a perturbation of the RCT mechanism, the key enzymes of which are under control of TH.[16,17] The erythrocyte-related indices haemoglobin (HGB) and hematocrit (HCT) may be potential indicators of TH changes. Among subjects with overt or subclinical hypothyroidism, decreased HGB and HCT have been observed, which are thought to be associated with decreased TH-controlled hematopoiesis.[18–20]

Because the traditionally used direct biomarkers of thyroid status (TSH and T4) were not associated with perchlorate among men,[2] the purpose of this study is to investigate if HDL, HGB, and HCT are suitable indirect indicators of TH dysfunction among both men and women.

Methods

Data source and exclusion criteria

The data source for this study was NHANES 2001–2002, one of several publicly available survey datasets collected by the Centers for Disease

Control (CDC).[21] The purpose of these surveys is to obtain information on the nutritional and health status of the US general population. Because these datasets do not contain identifiable private information, studies based on these data are exempt from IRB approval. The total number of subjects in the subpopulation selected for perchlorate analysis of spot urine samples was 2942 with an age range of 6–85, consisting of 47% males (n = 1374) and 53% females (n = 1568). The current study excluded subjects based on the following criteria: missing perchlorate values; previous diagnosis of diseases in which TH dysfunction might have played a role, such as congestive heart failure, coronary heart disease, angina pectoris, acute myocardial infarction, stroke, cancer, diabetes mellitus, thyroid problems and use of prescribed thyroid hormones. Values judged to be outliers, such as TSH > 200 IU/L, or CRP > 10 mg/dL, were also excluded.

Variables and statistical analyses

The analyses were applied to six groups of subjects: males, ages 6–19 and ages 20–85; girls, ages 6–14; women of reproductive age, but non-pregnant, ages 15–49; women, ages 50–85; and pregnant women as determined by test. The reason for categorizing men and women by different age groups is the following. Alcohol consumption, which is an important variable, was available only for subjects ages 20 and over. Therefore men were categorized by age groups <20 and ≥20, so that alcohol could be adjusted for in those at age 20 years and older. However, women were categorized by reproductive status, which was thought to be more meaningful with regard to potential associations with perchlorate.

Regression analysis was used to assess the association of levels of urinary perchlorate as the exposure surrogate with the biological measures HDL, HGB, and HCT. HDL was natural-log transformed in order to reduce skewness. HGB and HCT were approximately normally distributed. The exposure variable of interest, urinary perchlorate, was used in several ways: (1) as an indicator variable representing individual quintiles (Q1 through Q5), with Q1 as the referent (<1.9, 1.9–3.1, 3.2–4.8, 4.9–7.3, ≥7.4 μg/L); (2) grouping of contiguous perchlorate quintiles, eg, combined Q2 and Q3 (Q2-3), combined Q4 and Q5 (Q4-5), and combined Q3, Q4, and Q5 (Q3-5) with Q1 as the referent; and (3) continuous perchlorate (natural log transformed). Use of these different perchlorate measures allows identification of both nonlinear and linear associations.

Most of the explanatory variables selected for this study are the same as those used by Blount et al.[2] The following variables were included in all regression analyses: age (years, continuous), urinary creatinine (mg/dL, continuous, log transformed); race/ethnicity: non-Hispanic White (referent), Hispanic American, non-Hispanic Black, other race/ethnicity; body mass index (BMI, continuous, log-transformed), cotinine: low, (<0.015 ng/mL referent), medium (0.015–10 ng/mL), and high (>10 ng/mL), based on Steinmaus and Miller;[3] poverty index, the ratio of family income to poverty threshold (dichotomous, <1 versus ≥1); hours of fasting (log-transformed, continuous), thiocyanate (mg/L, continuous, log-transformed); nitrate (mg/L, continuous, log-transformed); daily kcal intake (continuous, log-transformed), which is closely related to dietary iron intake;[22] C-reactive protein (CRP, mg/dL, continuous, log-transformed). Thiocyanate (mg/L) and nitrate (mg/L) were included because, like perchlorate, they may impair thyroid function by inhibiting iodide uptake by the thyroid.

Additional adjustments were made for some groups. Information on alcohol consumption (none, referent; low, 1–10 drinks per week; high, 11 or more drinks per week) was available only for subjects ages 20 and over, and was included in the regressions for men, age 20 and over, women age 50 and over, and women age 15–49 with alcohol use for the 15–19 year old set to "none". Two separate indicator variables were created for the regression analyses, indicating low alcohol use (1 = yes, 0 = otherwise) and high alcohol use (1 = yes, 0 = otherwise). Adjustments were made for use of the following prescription drugs: beta blockers, sex hormones (contraceptives, androgens, estrogens, progestins), antihyperlipidemic agents; antidiabetic agents) for men age 20 and over, women age 15–49, and women age 50 and over. Indicator variables were created for each of the prescriptions with values 1 = yes (taking this prescription) or 0 (not taking this prescription). Non-pregnant women age 15–49 were also adjusted for having been pregnant in the past year (1 = yes, 0 = no) and for having a menstrual period (1 = yes, 0 = no) at time of sampling. Regression analyses for girls age 6–14 were adjusted

for menarchal status by an indicator variable with values 1 (= first period had occurred before interview) or 0 (= otherwise). Because no information was available for girls age 6–11, they were assumed to be premenarche. Among the 12–14 year old girls, 105 out of 167 (63%) were post-menarche. No additional adjustments were included for pregnant women because of their low sample size.

Laboratory methods have been described in detail previously.[2,21] Whole blood and spot urine samples were collected and stored cold or frozen. HDL was measured using a heparin-manganese precipitation method. HGB and HCT were obtained from a complete blood count on a Beckman Coulter counter. Urinary perchlorate, thiocyanate, and nitrate were analytically measured by ion chromatography tandem mass spectrometry.

Regression analyses were performed using the SAS procedure PROC SURVEYREG.[23] This procedure applies linear regression to a non-randomly selected study population by accounting for correlations between sampling clusters and by assigning weights to subjects, so that outcomes are representative of the US general population. The Wilcoxon rank sum test was used for the comparison of the dependent variables between subject groups. The Spearman correlation test was used to measure correlations between variables.

The parameter estimates β and 95% confidence interval (CI) represent the change of the dependent variable associated with a perchlorate quintile or combined quintiles for non-linear associations, or a unit of continuous log-transformed perchlorate, indicating a linear association, with adjustment for other characteristics.

Results

The total number of excluded subjects was 848 due to: missing perchlorate values (n = 122); thyroid associated diseases (n = 489); use of prescribed thyroid hormones (n = 321); and TSH and CRP outliers (n = 5). The remaining number of subjects was 2094 consisting of 1010 (48%) men and 1084 (52%) women. Characteristics of the resulting study population are presented in Table 1. Distribution characteristics of urinary perchlorate for the six study groups are presented in Table 2. The highest perchlorate concentrations were observed for the younger male and female subjects, followed by the pregnant women. Table 3 presents unadjusted means and standard errors for the three outcome variables (HGB, HCT, and HDL) by quintiles of urinary perchlorate for each of the six study groups. Although the means by perchlorate quintile were within normal range, both linear and non-linear associations with urinary perchlorate were observed. An example of a possible non-linear relationship was the decrease observed for HDL, which dropped at the 2nd and 3rd perchlorate quintile for men, age 20–85 and 6–19, respectively, and continued to stay at a lower level. Other variables showed a more direct linear decrease with increasing perchlorate concentration, eg, HGB and HCT among pregnant women.

Regression-based associations between urinary perchlorate and the three outcome variables of interest are presented in Table 4A and B.

Boys, age 6–19 (Table 4A)

The average level of association with HGB and HCT showed a significant decrease with coefficients of −0.23 (−0.41, −0.04) and −0.57 (−1.10, −0.03), respectively. No significant association was observed with HDL.

Men, age 20–85 (Table 4A)

Associations with HGB and HCT were not clearly linear, although the highest values were observed in Q5. The coefficients associated with log-transformed perchlorate for HGB and HCT were −0.18 (−0.33, −0.02) and −0.47 −0.92, −0.01), respectively. The regression model for log-transformed HDL showed a significant decrease based on the coefficient of −0.09 (−0.17, −0.01) associated with Q2-3. This agreed with the "drop off" observed for the unadjusted data (Table 3). At higher concentrations of urinary perchlorate HDL started to increase.

Girls, age 6–14 (Table 4A)

No significant associations with perchlorate were observed for HGB, HCT, or HDL.

Women, age 15–49, non-pregnant (Table 4B)

Among the 491 women ages 15–49 (reproductive age), 213 (43%) were under age 20. Information on alcohol consumption was not available for this age group.

Table 1. Characteristics of subjects.

Characteristics	Men (n = 1010)			Women (n = 1084)		
	Missing (n)	%	Mean (SE)	Missing (n)	%	Mean (SE)
Age (yrs)						
6–19		43			42	
20–85		57			58	
Race/ethnicity						
Non-Hispanic White		43			43	
Non-Hispanic Black		25			23	
Hispanic American (Mexican, other)		28			30	
Other ethnicity		4			4	
Below poverty threshold	99	20		109	25	
BMI	66			81		
<25		51			53	
25–30		30			23	
≥30		19			23	
Serum cotinine	117			135		
Low, nondetectable, <0.015 ng/mL		15			21	
Medium, 0.015–10 ng/mL		61			64	
High, >10 ng/mL		24			15	
Use of prescription drugs						
Beta blockers		14			15	
Sex hormones		18			20	
Lipid lowering drugs		20			20	
Anti diabetic drugs		14			14	
Alcoholic drinks/week, ages ≥ 20	42			84		
None		52			74	
1–10/week		34			21	
>10/week		13			4	
Other predictor variables						
Urinary perchlorate (μg/L)			5.99 (0.20)			5.03 (0.16)
Urinary creatinine (mg/dL)			156.3 (2.7)			122.5 (2.4)
Nitrate (mg/L)			71.23 (1.65)	1		58.43 (1.40)
Thiocyanate (mg/L)			2.84 (0.10)	2		2.02 (0.07)
Hours of fasting	25		9.81 (0.16)	25		9.81 (0.17)
Daily kcal intake	39		2509 (35)	45		1950 (25)
CRP (mg/dL)	68		0.22 (0.01)	70		0.32 (0.02)
Outcome variables						
Hemoglobin (g/dL)	59		14.95 (0.04)	58		13.27 (0.04)
Hematocrit (%)	59		44.07 (0.13)	58		39.07 (0.10)
HDL (mg/dL)	71		48.50 (0.41)	82		55.55 (0.46)

To check if the value "no alcohol use" could be assigned to these women, the following analysis was done. Among the women age 20–49, 69% did not use alcohol, 27% were low alcohol users, and 4% were high users in the last 12 months. Regression models for women age 20–49 showed that the estimated effect of alcohol consumption on the dependent variables of interest in this study was no more than 1%. This, and the fact that alcohol consumption is either none or low for most women, are reasons to believe setting alcohol consumption for 15–19 year old women to zero is reasonable. The strongest decrease for HGB and HCT was observed in Q3, followed by an increase. The coefficients for HGB and HCT in Q3 were –0.67 (–1.36, 0.02) and –2.02 (–3.74, –0.30), respectively. No associations were observed for HDL.

Women, age 50–85 (Table 4B)

No associations were observed for HGB, HCT, or HDL.

Pregnant women, age 16–38 (Table 4B)

In spite of the low number of observations, statistically significant decreases were observed for HGB

Table 2. Distribution of urinary perchlorate by study group.

Subjects	n	Urinary perchlorate (µg/L)	
		Mean ± SE	Median
Men			
Age 6–19	501	6.20 ± 0.25	4.90
Age 20–85	509	5.79 ± 0.31	4.20
Women			
Age 6–14	332	6.34 ± 0.33	4.80
Age 15–49, not pregnant	491	4.33 ± 0.19	3.20
Age 50–85	168	3.94 ± 0.31	2.70
Age 16–38, pregnant	93	5.94 ± 0.82	3.70

and HCT. Coefficients for combined Q3-5 were –0.59 (–1.01, –0.17), and –1.73 (–2.89, –0.58), for HGB and HCT respectively. In contrast to the non-pregnant women of reproductive age, estimates for the individual perchlorate quintiles among pregnant women showed that the association between perchlorate and HGB, HCT was monotonic, resulting in a significant estimate for the overall perchlorate association. This was also observed in the non-adjusted data (Table 3). No significant associations were observed for HDL.

Association between direct and indirect indicators

To determine if direct indicators (TSH and T4) and indirect indicators (HDL, HGB, and HCT) of TH disruption were associated, partial age-adjusted Spearman correlations were performed for the combined groups of men and the combined groups of women, ages 12–85 (Table 5). Thyroid hormone values were not available for those under age 12. The following significant correlations were observed among men: TSH and HDL (r = –0.070, $P = 0.05$); T4 and HGB (r = 0.093, $P = 0.01$); T4 and HCT (r = 0.089, $P = 0.01$). Among women,T4 and HDL were correlated (r = 0.066, $P = 0.05$). No significant correlations were observed among subjects with urinary iodine < 100 µg/L.

Table 3. Indicators of thyroid dysfunction by quintiles of urinary perchlorate (mean ± SE).

Urinary perchlorate	Boys age 6–19	Men age 20–85	Girls age 6–14	Women age 15–49 not pregnant	Women age 50–85	Women age 16–38 pregnant
Hemoglobin (g/dL)						
	n = 464	n = 487	n = 300	n = 476	n = 164	n = 86
Q1	14.66 ± 0.20	15.53 ± 0.16	13.32 ± 0.17	13.61 ± 0.10	13.49 ± 0.16	12.71 ± 0.22
Q2	14.68 ± 0.17	15.38 ± 0.13	13.13 ± 0.16	13.35 ± 0.15	13.68 ± 0.18	12.67 ± 0.21
Q3	14.51 ± 0.15	15.51 ± 0.10	13.13 ± 0.14	13.01 ± 0.13	13.69 ± 0.22	12.39 ± 0.21
Q4	14.47 ± 0.14	15.32 ± 0.12	13.36 ± 0.11	13.24 ± 0.14	13.18 ± 0.28	12.43 ± 0.24
Q5	14.37 ± 0.12	15.18 ± 0.11	13.41 ± 0.10	13.37 ± 0.13	13.23 ± 0.16	12.12 ± 0.23
mean	14.51 ± 0.07	15.38 ± 0.05	13.28 ± 0.06	13.33 ± 0.06	13.50 ± 0.09	12.48 ± 0.10
Hematocrit (%)						
	n = 464	n = 487	n = 300	n = 476	n = 164	n = 86
Q1	43.38 ± 0.56	45.74 ± 0.45	39.07 ± 0.47	40.08 ± 0.27	39.72 ± 0.44	36.99 ± 0.58
Q2	43.16 ± 0.49	45.37 ± 0.37	38.48 ± 0.41	39.40 ± 0.37	40.28 ± 0.53	37.07 ± 0.62
Q3	42.82 ± 0.44	45.66 ± 0.27	38.43 ± 0.38	38.54 ± 0.36	40.63 ± 0.63	36.21 ± 0.48
Q4	42.53 ± 0.42	45.19 ± 0.35	39.24 ± 0.30	39.17 ± 0.38	39.11 ± 0.77	36.18 ± 0.67
Q5	42.30 ± 0.36	44.90 ± 0.31	39.20 ± 0.29	39.46 ± 0.37	39.09 ± 0.49	35.53 ± 0.72
mean	42.73 ± 0.20	45.35 ± 0.15	38.92 ± 0.16	39.37 ± 0.16	39.86 ± 0.25	36.44 ± 0.28
High density lipoprotein (mg/dL)						
	n = 456	n = 483	n = 289	n = 467	n = 160	n = 86
Q1	53.16 ± 1.69	52.55 ± 1.89	49.03 ± 1.47	55.51 ± 1.08	61.81 ± 2.44	62.11 ± 2.87
Q2	52.62 ± 1.56	46.27 ± 1.28	51.15 ± 1.77	56.64 ± 1.31	64.39 ± 2.81	67.45 ± 3.69
Q3	49.08 ± 1.08	43.92 ± 1.01	50.71 ± 1.55	51.98 ± 1.29	58.93 ± 3.92	67.94 ± 3.61
Q4	49.49 ± 1.24	46.32 ± 1.13	51.54 ± 1.24	53.11 ± 1.35	61.18 ± 3.56	64.13 ± 3.82
Q5	49.01 ± 1.09	46.87 ± 1.19	50.87 ± 1.14	52.93 ± 1.91	69.00 ± 5.44	63.00 ± 3.29
mean	50.18 ± 0.58	46.91 ± 0.58	50.79 ± 0.63	54.31 ± 0.60	62.69 ± 1.49	65.02 ± 1.54

Notes: Urinary perchlorate quintiles (µg/L): Q1, <1.9; Q2, 1.9–3.1; Q3, 3.2–4.8; Q4, 4.9–7.3; Q5, ≥7.4.

Table 4A. Markers of thyroid dysfunction and association with urinary perchlorate. Regression estimates by gender and age.

Urinary perchlorate (quintiles and continuous)	Estimate, 95% CI, *P*-value		
	Boys, age 6–19	**Men, age 20–85**	**Girls, age 6–14**
Hemoglobin (g/dL)			
Q1 = referent	n = 410	n = 422	n = 268
Q2	0.16 (–0.11, 0.42), *P* = 0.22	–0.20 (–0.59, 0.18), *P* = 0.28	0.21 (–0.25, 0.68), *P* = 0.34
Q3	–0.25 (–0.78, 0.28), *P* = 0.33	–0.18 (–0.49, 0.14), *P* = 0.26	0.22 (–0.24, 0.68), *P* = 0.32
Q4	–0.22 (–0.71, 0.27), *P* = 0.35	–0.14 (–0.55, 0.27), *P* = 0.48	0.13 (–0.39, 0.65), *P* = 0.59
Q5	–0.35 (–0.77, 0.08), *P* = 0.10	–0.46 (–0.87, –0.06), *P* = 0.03	0.46 (–0.10, 1.01), *P* = 0.10
Q2-3	–0.04 (–0.41, 0.34), *P* = 0.84	–0.18 (–0.51, 0.15), *P* = 0.26	0.18 (–0.25, 0.62), *P* = 0.38
Q4-5	–0.25 (–0.63, 0.13), *P* = 0.19	–0.30 (–0.64, 0.04), *P* = 0.08	0.27 (–0.27, 0.81), *P* = 0.31
Q3-5	–0.27 (–0.71, 0.17), *P* = 0.21	–0.23 (–0.53, 0.07), *P* = 0.12	0.23 (–0.24, 0.71), *P* = 0.31
log(perchlorate)	–0.23 (–0.41, –0.04), *P* = 0.02	–0.18 (–0.33, –0.02), *P* = 0.03	0.14 (–0.05, 0.33), *P* = 0.13
Hematocrit (%)			
Q1 = referent	n = 410	n = 422	n = 268
Q2	–0.01 (–0.87, 0.86), *P* = 0.99	–0.57 (–1.66, 0.53), *P* = 0.29	0.17 (–1.29, 1.63), *P* = 0.81
Q3	–0.70 (–2.21, 0.82), *P* = 0.34	–0.60 (–1.46, 0.26), *P* = 0.16	0.13 (–1.39, 1.65), *P* = 0.86
Q4	–0.70 (–2.10, 0.69), *P* = 0.30	–0.34 (–1.51, 0.83), *P* = 0.55	0.30 (–1.28, 1.87), *P* = 0.69
Q5	–1.01 (–2.23, 0.21), *P* = 0.10	–1.29 (–2.41, –0.17), *P* = 0.03	0.89 (–0.82, 2.60), *P* = 0.28
Q2-3	–0.33 (–1.44, 0.78), *P* = 0.54	–0.55 (–1.45, 0.34), *P* = 0.21	0.09 (–1.30, 1.47), *P* = 0.90
Q4-5	–0.80 (–1.92, 0.33), *P* = 0.15	–0.79 (–1.77, 0.19), *P* = 0.11	0.55 (–1.05, 2.16), *P* = 0.48
Q3-5	–0.80 (–2.05, 0.46), *P* = 0.20	–0.68 (–1.48, 0.12), *P* = 0.09	0.33 (–1.16, 1.83), *P* = 0.64
log(perchlorate)	–0.57 (–1.10, –0.03), *P* = 0.04	–0.47 (–0.92, –0.01), *P* = 0.04	0.34 (–0.23, 0.90), *P* = 0.22
High density lipoprotein (mg/dL, log–transformed)			
Q1 = referent	n = 409	n = 421	n = 264
Q2	–0.01 (–0.15, 0.13), *P* = 0.90	–0.09 (–0.18, 0.00), *P* = 0.06	–0.07 (–0.20, 0.06), *P* = 0.26
Q3	–0.03 (–0.13, 0.07), *P* = 0.52	–0.09 (–0.20, 0.01), *P* = 0.07	–0.05 (–0.15, 0.06), *P* = 0.36
Q4	–0.02 (–0.16, 0.12), *P* = 0.76	–0.04 (–0.13, 0.05), *P* = 0.32	–0.04 (–0.14, 0.06), *P* = 0.39
Q5	–0.03 (–0.16, 0.11), *P* = 0.69	–0.02 (–0.12, 0.09), *P* = 0.75	–0.02 (–0.15, 0.10), *P* = 0.69
Q2-3	–0.02 (–0.13, 0.10), *P* = 0.73	–0.09 (–0.17, –0.01), *P* = 0.03	–0.06 (–0.14, 0.02), *P* = 0.13
Q4-5	–0.02 (–0.15, 0.11), *P* = 0.74	–0.03 (–0.12, 0.06), *P* = 0.50	–0.04 (–0.13, 0.06), *P* = 0.44
Q3-5	–0.03 (–0.14, 0.09), *P* = 0.64	–0.06 (–0.15, 0.03), *P* = 0.15	–0.04 (–0.13, 0.05), *P* = 0.33
log(perchlorate)	0.01 (–0.03, 0.06), *P* = 0.54	–0.01 (–0.05, 0.04), *P* = 0.78	0.02 (–0.04, 0.07), *P* = 0.57

Notes: The regression coefficients associated with the individual or combined perchlorate quintiles represent the estimated average change compared to the first perchlorate quintile. The regression coefficients associated with log(perchlorate) represent the estimated average change per one unit increase of natural log-transformed perchlorate. Perchlorate effects are adjusted for age, urinary creatinine, ethnicity, cotinine, BMI, poverty index, total kcal intake, hours of fasting, urinary nitrates, urinary thiocyanates, CRP. Additional adjustments: Men, age 20–85: alcohol consumption, prescriptions for betablocker, sex hormones, lipid lowering drugs, antidiabetic drugs. Girls, age 6–14: postmenarche status.

Discussion

In addition to changes of TSH and T4 levels as direct indicators of TH disruption in association with perchlorate exposure, results from this study support the hypothesis that HDL, HGB and HCT levels may serve as indirect indicators. Although the means of these indirect effect biomarkers are within normal range (Table 3), their negative association with urinary perchlorate is of interest, such as decreased HDL among men and decreased HGB and HCT among both men and women. Reports have been published on decreased levels of HDL among subjects with subclinical hypothyroidism.[7,9,11,18–20,24,25] The herbicide 2,4-dichlorophenoxy acetic acid (2,4-D) which is known to displace T4 from its binding site on the carrier protein transthyretin[26] and thus may be involved in TH dysfunction, was shown to be associated with decreased levels of HDL.[27] Regarding associations between direct and indirect indicators of thyroid dysfunction, statistically significant negative associations have been observed between HDL and values of TSH within the reference range.[28,29] Among older subjects, TSH and HGB were significantly inversely correlated among those with hypothyroidism and anemia.[30] Patients with chronic stable heart failure showed that those that developed hypothyroidism, subclinical

Table 4B. Markers of thyroid dysfunction and association with urinary perchlorate. Regression estimates by gender and age.

Urinary perchlorate (quintiles and continuous)	Estimate, 95% CI, *P*-value		
	Women, age 15–49 (non-pregnant)	Women, age 50–85	Women, pregnant
Hemoglobin (g/dL)			
Q1 = referent	n = 416	n = 134	n = 80
Q2	–0.20 (–0.73, 0.33), *P* = 0.44	0.09 (–0.48, 0.65), *P* = 0.75	0.17 (–0.15, 0.48), *P* = 0.28
Q3	–0.67 (–1.36, 0.02), *P* = 0.06	0.10 (–0.52, 0.71), *P* = 0.75	–0.47 (–1.04, 0.12), *P* = 0.11
Q4	–0.49 (–0.98, 0.01), *P* = 0.05	–0.35 (–1.65, 0.95), *P* = 0.57	–0.59 (–1.10, –0.08), *P* = 0.03
Q5	0.37 (–0.95, 0.20), *P* = 0.19	–0.75 (–2.17, 0.67), *P* = 0.28	–0.77 (–1.73, 0.18), *P* = 0.11
Q2-3	–0.37 (–0.89, 0.15), *P* = 0.15	0.11 (–0.40, 0.61), *P* = 0.66	–0.11 (–0.49, 0.28), *P* = 0.57
Q4-5	–0.41 (–0.86, 0.05), *P* = 0.08	–0.51 (–1.66, 0.64), *P* = 0.36	–0.64 (–1.15, –0.14), *P* = 0.02
Q3-5	–0.56 (–1.00, –0.13), *P* = 0.01	–0.09 (–0.81, 0.63), *P* = 0.80	–0.59 (–1.01, –0.17), *P* = 0.01
log(perchlorate)	–0.24 (–0.49, 0.02), *P* = 0.07	–0.22 (–0.67, 0.23), *P* = 0.31	–0.26 (–0.45, –0.08), *P* = 0.01
Hematocrit (%)			
Q1 = referent	n = 416	n = 134	n = 80
Q2	–0.66 (–2.12, 0.79), *P* = 0.35	0.03 (–1.41, 1.47), *P* = 0.96	0.54 (–0.45, 1.52), *P* = 0.26
Q3	–2.02 (–3.74, –0.30), *P* = 0.02	0.43 (–1.13, 1.98), *P* = 0.57	–1.33 (–2.54, –0.11), *P* = 0.04
Q4	–1.35 (–2.92, 0.21), *P* = 0.09	–1.06 (–4.59, 2.47), *P* = 0.53	–1.57 (–3.00, –0.15), *P* = 0.03
Q5	–0.82 (–2.58, 0.94), *P* = 0.34	–2.25 (–6.28, 1.79), *P* = 0.25	–2.48 (–5.39, 0.43), *P* = 0.09
Q2-3	–1.16 (–2.54, 0.23), *P* = 0.10	0.24 (–1.05, 1.52), *P* = 0.70	–0.30 (–1.30, 0.71), *P* = 0.54
Q4-5	–1.04 (–2.51, 0.44), *P* = 0.16	–1.56 (–4.85, 1.72), *P* = 0.33	–1.90 (–3.57, –0.23), *P* = 0.03
Q3-5	–0.79 (–1.59, 0.01), *P* = 0.05	–0.17 (–2.14, 1.79), *P* = 0.85	–1.73 (–2.89, –0.58), *P* = 0.01
log(perchlorate)	–0.55 (–1.28, 0.18), *P* = 0.13	–0.64 (–1.97, 0.69), *P* = 0.32	–0.86 (–1.44, –0.29), *P* = 0.01
High density lipoprotein (mg/dL, log-transformed)			
Q1 = referent	n = 411	n = 132	n = 80
Q2	0.01 (–0.08, 0.09), *P* = 0.86	0.11 (–0.02, 0.24), *P* = 0.09	0.08 (–0.12, 0.27), *P* = 0.40
Q3	–0.01 (–0.07, 0.06), *P* = 0.77	–0.01 (–0.15, 0.14), *P* = 0.93	0.10 (–0.05, 0.24), *P* = 0.19
Q4	0.06 (–0.03, 0.15), *P* = 0.16	0.03 (–0.17, 0.23), *P* = 0.75	0.12 (–0.04, 0.27), *P* = 0.14
Q5	–0.05 (–0.17, 0.07), *P* = 0.39	0.17 (–0.08, 0.41), *P* = 0.17	–0.02 (–0.18, 0.13), *P* = 0.78
Q2-3	0.00 (–0.07, 0.07), *P* = 0.89	0.06 (–0.04, 0.16), *P* = 0.22	0.07 (–0.06, 0.21), *P* = 0.26
Q4-5	0.02 (–0.08, 0.11), *P* = 0.74	0.10 (–0.09, 0.28), *P* = 0.29	0.05 (–0.09, 0.20), *P* = 0.44
Q3-5	0.00 (–0.07, 0.07), *P* = 0.91	0.02 (–0.11, 0.14), *P* = 0.78	0.07 (–0.01, 0.15), *P* = 0.09
log(perchlorate)	0.00 (–0.06, 0.07), *P* = 0.88	0.02 (–0.07, 0.11), *P* = 0.64	0.01 (–0.04, 0.06), *P* = 0.68

Notes: The regression coefficients associated with the individual or combined perchlorate quintiles represent the estimated average change compared to the first perchlorate quintile. The regression coefficients associated with log(perchlorate) represent the estimated average change per one unit increase of natural log-transformed perchlorate. Perchlorate effects are adjusted for age, urinary creatinine, ethnicity, cotinine, BMI, poverty index, total kcal intake, hours of fasting, urinary nitrates, urinary thiocyanates, CRP. Additional adjustments: Women, age 15–49: alcohol consumption, prescriptions for betablocker, sex hormones, lipid lowering drugs, antidiabetic drugs, pregnancy in past year, having a period at time of sampling. Women, age 50–85: alcohol consumption, prescriptions for betablocker, sex hormones, lipid lowering drugs, antidiabetic drugs.

hypothyroidism, or euthyroid syndrome (normal TSH, decreased T3 with/without decreased T4) had lower HGB and HCT.[31] Although in the current study subjects with thyroid related diseases had been excluded, nevertheless some associations between direct and indirect indicators were observed, such as TSH and HDL among men, T4 and HDL among women, T4 and HGB, HCT among men (Table 5).

Use of HDL levels as an indirect measure of thyroid hormone dysfunction may not be valid for any study population. The purpose of this study was to investigate associations with perchlorate among *healthy* subjects, in order to observe initial effects associated with TH perturbation. In general, plasma concentrations of HDL as a measure of HDL functionality depends on health status.[32] The atheroprotective characteristics of HDL are based on the reverse cholesterol transport mechanism (RCT), inhibition of LDL oxidation, and anti-inflammatory properties. In susceptible subjects, HDL may become dysfunctional, lose its RCT promoting and antioxidant characteristics, and even become pro-inflammatory. In the current study, subjects with previous chronic diseases were excluded from the analyses. Therefore, the observed association between urinary perchlorate and HDL among men in this relatively healthy subpopulation may reflect an

Table 5. Comparison of direct (TSH, T4) and indirect (HDL, HGB, HCT) indicators of thyroid hormone disruption among men and women, age 12–85.

Urinary iodine	Indicators		Spearman correlations (r), *P*-value (*P*) and number of subjects (n), age-adjusted					
			Men			Women		
			r	*P*	n	r	*P*	n
All values	TSH	HDL	–0.070	0.050	788	–0.055	0.105	856
		HGB	–0.005	0.890	788	0.024	0.489	856
		HCT	–0.019	0.595	788	0.003	0.932	856
	T4	HDL	–0.034	0.338	788	0.066	0.054	858
		HGB	0.093	0.009	788	–0.034	0.322	858
		HCT	0.089	0.012	788	–0.039	0.256	858
<100 µg/L	TSH	HDL	–0.057	0.483	153	–0.070	0.237	288
		HGB	0.034	0.674	153	0.032	0.591	288
		HCT	0.034	0.673	153	0.004	0.951	288
	T4	HDL	0.027	0.737	153	0.081	0.170	288
		HGB	0.109	0.182	153	0.056	0.343	288
		HCT	0.088	0.279	153	0.040	0.505	288

early indication of interference of perchlorate with TH action.

Unexpected results were observed for the group of women aged 50–85. These women seemed to be largely unaffected by perchlorate in contrast to the younger women. However, this assumption may be incorrect. A large percentage of the women in this age group was excluded from the study (56%, 214 out of 382), because of previous diseases or because they were prescribed thyroid hormones, with the result that the 50–85 year old women remaining in the study were generally healthy. In comparison, the following percentages were excluded from the other groups: 14% (52 out of 384) for girls ages 6–14; 19% (135 out of 719) for women, ages 15–49, including pregnant women; 12% (71 out of 572) for boys, age 6–19; 33% (252 out of 761) for men, age 20–85.

Comparison of the non-pregnant women of reproductive age and the pregnant women based on the unadjusted data (Table 3) showed that with regard to HGB and HCT, the non-pregnant women showed a U-shaped pattern based on perchlorate quintiles, while the pregnant women showed a more linear decreasing association. Before one can conclude that perchlorate is adversely associated with HGB and HCT among pregnant women, the following information should be considered. It has been shown that both high and low maternal HGB is associated with poor pregnancy outcomes, such as increased rates of preterm births and stillbirths. The optimal values of this U-shaped pattern of HGB among pregnant women with regard to adverse birth outcomes, is represented by the bottom part of the "U", with a reported range of 9–11 g/dL for the lowest maternal HGB recorded during the pregnancy, and a range of 11–13 g/dL for the first recorded HGB value in another study.[33,34] This decrease in HGB is thought to be due to a healthy plasma volume expansion associated with pregnancy. Therefore, interpretation of the decreasing HGB values among pregnant women associated with perchlorate becomes more complicated. A larger study group of pregnant women is needed to solve the question if perchlorate exposure is associated with enhancement of the plasma volume expansion (non-adverse effect), or with decreased erythropoiesis (adverse effect). A recent study involving pregnant women only, observed that perchlorate exposure did not affect thyroid hormones in either euthyroid or hypothyroid women.[35] An editorial on the impact of perchlorate exposure during early pregnancy speculated that perchlorate may have more subtle effects than changes of maternal thyroid hormones, or may have an effect on the fetus without having an effect on maternal thyroid hormones.[36]

Results from the regression analyses showed that use of a continuous exposure variable, such as log-transformed urinary perchlorate, which represents the average population response, can be misleading when the association is nonlinear. Nonlinear responses associated with low-dose exposures are a

characteristic of hormone-like chemicals. The initial receptor-mediated responses are linear at low saturation or occupation of the receptors. With increasing exposure, ie, higher receptor saturation, the effects weaken. No effect may be observed at the highest exposures. This may lead to a U- or inverted U-shaped response curve.[37] Such nonlinear responses can be quantified by including a quadratic term for the exposure in the regression. This allows for an increase/decrease of the response followed by a decrease/increase at higher exposures. However, a regression model using exposure quantiles is easier to interpret and has been used in other studies. For example, an observational study on the association of perfluorooctanoic acid on thyroid disease showed that responses were not necessarily linear for increasing quartiles of the exposure variable.[38] A nonlinear association was observed between persistent organic pollutants (POPs) and type-2 diabetes.[39] In this study, the second sextile of the combined POPs showed the strongest association. To measure the response occurring at low levels of exposure, the referent group should be based on non-exposed subjects or subjects with very low levels of exposure. The choice of quantiles depends on the size of the study population. In the current study, for example, an association of HDL with perchlorate among men, ages 20–85 was observed in the non-adjusted data (Table 3), where a decrease in HDL was observed in the second and third quintiles, followed by an increase in the fourth and fifth quintiles. The adjusted data for men, ages 20–85 (Table 4A) show a similar pattern with stronger decreases in the 2nd and 3rd quintiles (−0.088 and −0.094, respectively) followed by weaker associations in the 4th and 5th quintiles. Combining the 2nd and 3rd quintiles produced statistical significance compared to the lowest quintile. No such association was observed when the lowest quartile instead of quintile was used as the referent.

Conclusions

This cross-sectional study has generated hypotheses for mechanistic studies on the effects of perchlorate exposure. Its results indicate that adverse effects associated with perchlorate may be underestimated when only circulating levels of TH (direct measures) are considered. Additional use of indirect biomarkers, such as HDL, HGB, and HCT, may contribute to a more complete picture of initial biological responses to perchlorate. Future studies need to confirm these results in healthy subjects. The effects seen for pregnant women should be investigated in a larger dataset. In addition, use of these indirect biomarkers associated with perchlorate, should be studied in already compromised subjects.

Abbreviations

BMI, Body mass index; CDC, Centers for Disease Control and Prevention; CI, confidence interval; CRP, C-reactive protein; HCT, hematocrit; HDL, high density lipoprotein; HGB, hemoglobin; IRB, Institutional Review Board; NHANES, National Health and Nutrition Examination Survey; POP, persistent organic pollutants; RCT, reverse cholesterol transport mechanism; TH, thyroid hormones; TSH, thyroid stimulating hormone; T4, thyroxine.

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Competing Interests

The author declares that she has no competing interests.

Authors' Contributions

The author conducted the literature review, performed the analyses, interpreted the results, and wrote the manuscript.

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test subjects. If this article contains identifiable human subject(s) author(s) were required to supply signed patient consent prior to publication. Author(s) have confirmed that the published article is unique and not under consideration nor published by any other publication and that they have consent to reproduce any copyrighted material. The peer reviewers declared no conflicts of interest.

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