

Perchlorate Exposure is Associated with Oxidative Stress and Indicators of Serum Iron Homeostasis Among NHANES 2005–2008 Subjects

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ABSTRACT: Perchlorate (ClO_4^-), an oxidizing agent, is a ubiquitous environmental pollutant. Several studies have investigated its thyroid hormone disrupting properties. Its associations with other biological measures are largely unknown. This study, combining 2005–2008 National Health and Nutrition Examination Surveys, investigated associations between urinary perchlorate and biomarkers of iron homeostasis, lipids, blood cell counts, and glucose metabolism. Healthy males ($n = 3705$), non-pregnant females ($n = 2967$), and pregnant females ($n = 356$), aged 12–59 years, were included in the linear regression models, which showed significant positive (+) and negative (–) associations for both males and non-pregnant females with serum uric acid (–), serum iron (–), RBC count (–), blood urea nitrogen (+), and lymphocyte count (+). Other significant associations were observed for either males or non-pregnant females. Among pregnant females, perchlorate was significantly associated with blood urea nitrogen (+) and serum iron (–). These associations may be indicators of perchlorate's potential effect on several biological systems, which when considered in total, may implicate perturbation of iron homeostasis.

KEYWORDS: perchlorate, epidemiology, biomarkers, iron homeostasis

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Introduction

Biomonitoring data, including those collected as part of the National Health and Nutrition Examination Survey (NHANES), can contribute insight into how xenobiotics affect biological systems by providing information both on their presence or their metabolites in blood and urine, and on levels of many biological measures. Establishing an association between internal pollutants and disturbance of biochemical and physiological processes may help establish an adverse outcome pathway for a particular pollutant or a group of pollutants.

Perchlorate (ClO_4^-) is a widespread environmental contaminant.¹ It occurs naturally in mostly arid regions in the

form of fertilizer and potash ore. Man-made sources include fireworks, rocket propellants, and explosives. Perchlorate can be found in vegetation, ground, and surface water. Human beings are exposed to it through food and water. Perchlorate is known to compete with iodide uptake² and has been shown to be associated with thyroid hormone (TH) levels at low environmental exposures.^{3,4} A previous study involving NHANES 2001–2002 data observed decreased levels of hemoglobin (HGB) and high-density lipoprotein cholesterol (HDL) in association with urinary perchlorate.⁵ This study involving data from combined NHANES 2005–2006 and NHANES 2007–2008 (NHANES 2005–2008) is a continuation of the



previous NHANES 2001–2002 study. Use of a larger dataset provides the opportunity to further investigate if exposure to perchlorate can be linked to changes in the level of biomarkers that are known or suspected to be associated with adverse health outcomes, such as cardiovascular disease, impaired glucose metabolism, and anemia. These outcomes have been associated with hypothyroidism.^{6–8}

Materials and Methods

Data source and exclusion criteria. The NHANES are designed to assess the health and nutritional status of the civilian, noninstitutionalized US population. These surveys include household interviews on health conditions, physical examinations, and collection of blood and spot urine samples. Subjects are selected based on a complex multistage probability sampling design. Participants aged 6 years and older in the combined NHANES 2005–2006 and NHANES 2007–2008 were tested for perchlorate. Urine samples from both the surveys were frozen (–20 °C) until shipment on dry ice to the CDC Division of Environmental Health Laboratory Sciences. Urinary perchlorate was determined by ion chromatography tandem mass spectrometry. NHANES 2005–2008 participants with known urinary perchlorate levels were selected for this study. They represented 75% of the total NHANES 2005–2008 population. To avoid potential interference of previous diseases, subjects were excluded if in the past they had been diagnosed with cancer, heart failure, coronary heart disease, angina, myocardial infarction, stroke, thyroid problems, diabetes, or prediabetes, defined as having impaired glucose tolerance or elevated fasting glucose. Subjects were also excluded if they were on at least one of the several classes of medications categorized as antineoplastic, cardiovascular, hormonal, or metabolic, taken in the month prior to the interview. Using such broad criteria for exclusion of medication assured us that most subjects with relevant medical problems would be excluded from the study. In addition, underweight subjects (body mass index [BMI] <18.5), which could be an indication of nutritional deficiencies, were also excluded.

The standard biochemistry profile was available only for subjects aged ≥ 12 years. Pregnant and nonpregnant females were analyzed separately. Pregnancy was determined by the human chorionic gonadotropin-based urine test.

Some laboratory tests were performed only on specific groups of subjects/samples. For example, some tests were performed only on morning (fasting) samples, NHANES 2005–2006 or NHANES 2007–2008 samples, or samples from females only. Where necessary, this information is shown in the tables. Collection procedures and laboratory methods have been previously described in detail.⁹

Statistical analyses. Urinary perchlorate was determined for 15,326 of the 20,497 subjects in NHANES 2005–2008. Subjects aged 6–11 years ($n = 2,197$) were excluded from this study due to the lack of biochemistry profile data. The remaining subjects were further divided into age groups 12–59 years

($n = 9,877$) and ≥ 60 years ($n = 3,252$). The reason for this division is that older subjects may be more susceptible to oxidative stress and inflammation¹⁰ and may therefore react differently to xenobiotic exposures. After excluding subjects with previous diseases or on current prescriptions from age groups 12–59 years (23%, $n = 2,297$) and ≥ 60 years (83%, $n = 2,705$), the remaining numbers of subjects were 7,580 in the 12–59 years age group and 547 in the ≥ 60 years age group. The ≥ 60 years age group (316 males and 231 females) was considered too small for gender-specific analyses and was excluded from further study. Among 12–59 years age group, an additional number of 552 underweight subjects were excluded, resulting in 7,028 subjects, aged 12–59 years (3,705 males and 3,323 females) available for analysis.

A correction for urinary concentration was applied by dividing urinary perchlorate concentration ($\mu\text{g/L}$) by urinary creatinine concentration (mg/dL). This method is used commonly to adjust for urine volume status.¹¹ Nonparametric Spearman correlations, plots, and linear regression analyses were used to investigate the association between the exposure variables and several biological measures as the dependent variable.¹² Smoothed interpolations of the unadjusted observations were used for the plots. The regressions were based on Proc Surveyreg (SAS), which performs linear regressions on observations from non-randomly selected study populations, with adjustment for potential correlations between sampling clusters and strata. Because we were focused on population subsets, and not on developing national estimates, NHANES sample weights were not included. Not including NHANES sample weights, but including demographics in the regression as done here, is regarded as a reasonable compromise between efficiency and bias.¹³

The following biomarkers of interest available in NHANES 2005–2008 were tested as the dependent variables in regression analyses for their association with perchlorate: serum uric acid; blood urea nitrogen (BUN); serum iron; erythrocyte number; HGB; ferritin; total iron binding capacity; percent transferrin saturation; C-reactive protein (CRP); number and percent of neutrophils, monocytes, lymphocytes, and platelets; total cholesterol; high-density lipoprotein (HDL); non-HDL; and fasting subsample including low-density lipoprotein (LDL), apolipoprotein-B (Apo-B), triglycerides, glucose tolerance (2 hours, 75 g of glucose), glucose, and insulin. These measures were natural log-transformed in order to reduce skewness. These biological measures were selected for two reasons: (1) they are known to be associated with several interrelated biological systems and processes, including oxidative stress, kidney function, iron homeostasis, inflammation, lipid homeostasis, and glucose metabolism, and (2) their values were available in the NHANES 2005–2008 data.

Besides the biomarkers of interest and the main exposure variable, the regression models included several explanatory variables that adjusted for individual characteristics: age



(continuous); race/ethnicity: non-Hispanic White (reference), Hispanic American, non-Hispanic Black, other race/ethnicity; BMI (continuous, log-transformed); serum cotinine: low (<0.015 ng/mL, referent), medium (0.015–10 ng/mL), high (>10 ng/mL); poverty index, the ratio of family income to poverty threshold (dichotomous, <1 versus ≥ 1); hours of fasting (log-transformed, continuous); daily kilocalorie intake (continuous, natural log-transformed); an indicator variable for the NHANES dataset with value 0 for 2005–2006 and 1 for 2007–2008.

The regression analyses produced parameter estimates β [95% confidence interval (CI), *P* value] representing the change of the log-transformed dependent variable associated with a unit change of the log-transformed urinary-creatinine-adjusted perchlorate [$\log(\mu\text{g}/10\text{ mg})$], with adjustment for other explanatory variables. Significant associations are defined as a CI excluding the value zero and a *P* value ≤ 0.05 . To facilitate interpretation of the regression models, the percent change for selected biomarkers was calculated based on the estimate for the β , and assuming a 10-fold increase of the exposure measure, eg, from 0.01 to 0.10 urinary perchlorate ($\mu\text{g}/\text{L}$)/urinary creatinine (mg/L), holding all other variables constant, according to the following formula: $(10^\beta - 1) \times 100$.¹⁴

We selected not to use Bonferroni adjustments for multiple comparisons. As long as new findings are submitted to further scrutiny in order to rule out chance findings, it should not make a difference if one is investigating many associations in as many studies or combined in a single study.^{15,16}

Results

Gender-specific demographics and biomarker levels are presented in Table 1.

Regression analysis for males and females aged 12–59 years, excluding pregnant women (n = 6672). These analyses involving 3,705 males and 2,967 non-pregnant females formed the main body of this study, which investigated associations of perchlorate with the biochemistry profile biomarkers and blood counts.

Regression-based associations between creatinine-adjusted perchlorate and biomarkers are presented in Table 2. For both males and females, serum uric acid, serum iron, and red blood cell (RBC) count have a strong negative association with perchlorate, while BUN and lymphocyte number have a positive association with perchlorate. The unadjusted associations between perchlorate and these biomarkers are shown in Figures 1A–1E. At least 99% of the values for serum uric acid, BUN, and lymphocyte count were within normal range. Regarding serum iron, 16% of the males and 36% of the females were below the lower limit of the reference range (60 $\mu\text{g}/\text{dL}$). Regarding RBC counts, 8.9% of the males and 23% of the females were below their respective reference levels of 4.6×10^9 and 4.2×10^9 .

The percent changes of these biomarkers for males/females based on the estimates for the betas, and assuming

a 10-fold change of the exposure measure “urinary perchlorate ($\mu\text{g}/\text{L}$)/urinary creatinine (mg/L)”, were as follows: serum uric acid, $-7.3/-6.0$; BUN $6.9/10.9$; serum iron $-13.7/-11.5$; RBC $-1.8/-1.4$; lymphocyte count $4.5/7.2$.

Other significant associations were observed among males for HGB (strongly negative); total cholesterol, LDL, and apolipoprotein-B (negative); platelets, insulin, and glucose (positive); and it was observed among females for transferrin saturation (negative), monocyte number, and lymphocyte percent (positive) (Table 2).

Initial Spearman correlation analyses showed that several of the outcome measures of interest were strongly associated with serum iron, but showed only a weak or no association with perchlorate (data not shown). This was confirmed by repeating the regression analyses using serum iron as the main explanatory variable instead of perchlorate (Table 3). Examples of biomarkers that were weakly or not associated with perchlorate, but significantly associated with serum iron, are measures related to iron homeostasis for females; total cholesterol, HDL, CRP, monocytes, platelets, and insulin for males and females; and LDL, apolipoprotein and triglycerides, and lymphocyte percent for males. Taking this one step further by using HDL as the main explanatory variable in the regression analyses, it is shown that HDL, which is not associated with perchlorate, is strongly associated with apolipoprotein-B, triglycerides, CRP, WBC count, neutrophil and lymphocyte count, and the glucose metabolism measures for both males and females (Table 4). These results may reflect the different metabolic stages of the subjects. In other words, exposure to perchlorate and potentially other stressors may be associated with serum iron levels, which in turn may be associated with HDL, which in turn may be associated with other markers.

Pregnant women (n = 356). Due to the small sample size, restricted analyses were performed for the pregnant females. Only those biomarkers that had been shown to be significantly associated with perchlorate among both males and non-pregnant females (serum uric acid, BUN, serum iron, RBC, and number of lymphocytes) were analyzed. Among the 305 pregnant women with non-missing regression covariates, significant associations were observed for BUN ($\beta = 0.063$ [0.000, 0.126], *P* = 0.0497) and serum iron ($\beta = -0.063$ [-0.122, -0.005], *P* = 0.035). These estimates are compatible with those observed for the non-pregnant females (Table 2). No associations were observed for serum uric acid, RBC, or lymphocyte number (data not shown).

Dietary supplements among 12–59-year-old males and nonpregnant females, and among pregnant females. To assess whether intake of dietary supplements during 30 days prior to the interview influenced the association between perchlorate and serum iron, a term for dietary supplement intake (yes/no) was included in the regression model for serum iron. Supplements were used by 26% of the males, 30% of the non-pregnant females, and 76% of the pregnant

**Table 1.** Characteristics and biomarker levels of subjects, age 12–59, NHANES 2005–2008.

CHARACTERISTICS	MALES (n = 3705)		FEMALES (n = 3323 ^a)	
	PERCENTAGE OR MEDIAN (RANGE)	NUMBER MISSING	PERCENTAGE OR MEDIAN (RANGE)	NUMBER MISSING
Age				
12–19,	37		38	
20–39,	38		40	
40–59,	25		22	
BMI	26.0 (18.5–71.8)		26.0 (18.5–76.1)	
Ethnicity				
White%	37		32	
Hispanic American%	34		37	
NonHispanic Black%	25		26	
Other ethnicity %	4		5	
Cotinine (ng/mL)		232		234
<0.015	11		21	
0.015–10	58		62	
>10	31		17	
Below poverty index (%)	23	256	26	216
Daily kcal intake	2512 (149–13509)	139	1792 (89–10436)	125
Hours of fasting	6 (0.5–64)	90	5 (0.5–86)	79
Urinary perchlorate (ng/mL)	4.28 (0.18–348)		3.53 (0.12–74)	
Urinary creatinine (mg/dL)	153 (6–678)		116 (5–510)	
Serum uric acid (mg/dL)	5.8 (1.4–11.7)	244	4.3 (1.2–9.8)	241
Blood urea nitrogen (mg/dL)	11 (2–27)	243	9 (1–57)	241
Serum iron (refrig.) (μg/dL)	89 (15–315)	244	71 (7–382)	241
RBC (10 ⁶ /μL)	5.08 (3.16–7.08)	221	4.47 (2.44–6.67)	208
HGB (g/dL)	15.4 (8.8–18.8)	221	13.3 (6.2–18.3)	208
Ferritin (ng/mL)	NA	3705	32 (2–611)	473
TIBC ^b (μg/dL)	NA	3705	368 (224–721)	1542
Percent transferrin saturation ^b (%)	NA	3705	19.1 (1.4–95.0)	1542
CRP (< = 3) (mg/dL)	0.10 (0.01–3.00)	229	0.14 (0.01–2.99)	224
Total cholesterol (mg/dL)	179 (78–458)	230	179 (87–411)	235
HDL (mg/dL)	46 (7–128)	230	55 (15–155)	235
Non-HDL (mg/dL)	130 (24–420)	230	121 (34–314)	235
LDL ^c (mg/dL)	105 (19–344)	2063	101 (22–253)	1900
Apolipoprotein-B ^c (mg/dL)	84 (22–318)	2013	80 (24–179)	1883
Triglycerides ^c (mg/dL)	99 (14–1600)	2023	87 (18–693)	1886
WBC (10 ³ /μL)	6.9 (1.5–19.2)	221	7.3 (2.3–55.9)	208
Neutrophil number (10 ³ /μL)	3.8 (0.4–17.8)	225	4.3 (0.7–16.3)	224
Neutrophil percent	55.8 (18.3–92.7)	225	58.9 (9.1–90.4)	224
Monocyte number (10 ³ /μL)	0.6 (0.1–1.8)	225	0.5 (0.1–1.9)	224
Monocyte percent	8.1 (0.6–39.1)	225	7.2 (0.8–26.3)	224
Lymphocyte number (10 ³ /μL)	2.2 (0.5–6.6)	225	2.2 (0.6–48.4)	224
Lymphocyte percent	31.9 (5.8–64.8)	225	30.3 (7.2–86.6)	224
Platelets (10 ³ /μL)	263 (56–562)	221	287 (4–1000)	208
Glucose tolerance test ^c (mg/dL)	98 (33–491)	2277	100 (32–403)	2228
Fasting insulin ^c (μU/mL)	9.0 (1–151)	2029	10.2 (1–231)	1893
Fasting glucose ^c (mg/dL)	98 (63–334)	2009	93 (38–250)	1870

Notes: ^aamong the 3323 females, 356 (11%) were pregnant. ^bonly available for women in NHANES 2005–2006. ^cAM samples.

Abbreviation: NA, not available.

**Table 2.** Association of selected biomarkers with urinary perchlorate/creatinine for males and non-pregnant females, age 12–59, BMI ≥ 18.5 , NHANES 2005–2008.

BIOMARKER (NATURAL LOG TRANSFORMED)	MALES			FEMALES		
	N	ESTIMATE (95% CI)	P-VALUE	N	ESTIMATE (95% CI)	P-VALUE
Oxidative stress, kidney function, iron homeostasis						
Serum uric acid (mg/dL)	3135	-0.033 (-0.044, -0.023)	<0.0001	2500	-0.027 (-0.038, -0.016)	<0001
Blood urea nitrogen (mg/dL)	3136	0.029 (0.011, 0.046)	0.003	2500	0.045 (0.025, 0.065)	<0.0001
Serum iron ($\mu\text{g}/\text{dL}$)	3135	-0.064 (-0.085, -0.043)	<-0.0001	2500	-0.053 (-0.081, -0.025)	0.0006
RBC ($10^6/\mu\text{L}$)	3145	-0.008 (-0.013, -0.004)	0.0005	2503	-0.006 (-0.010, -0.002)	0.006
HGB (g/dL)	3145	-0.011 (-0.015, -0.007)	<0.0001	2503	-0.000 (-0.005, 0.005)	0.998
Ferritin (ng/mL) age 12–49	NA	NA	NA	2294	-0.022 (-0.064, 0.020)	0.287
TIBC ($\mu\text{g}/\text{dL}$) ^a	NA	NA	NA	1381	0.003 (-0.008, 0.014)	0.537
Transferrin saturation (%) ^a	NA	NA	NA	1381	-0.060 (-0.106, -0.013)	0.016
Lipids						
Total cholesterol (mg/dL)	3148	-0.011 (-0.020, -0.002)	0.022	2505	0.002 (-0.008, 0.013)	0.648
HDL (mg/dL)	3148	-0.006 (-0.017, 0.005)	0.252	2505	0.010 (-0.006, 0.026)	0.218
Nonhdl (mg/dL)	3148	-0.012 (-0.025, 0.000)	0.058	2505	-0.001 (-0.014, 0.012)	0.921
LDL (mg/dL) ^b	1492	-0.021 (-0.039, -0.004)	0.019	1131	0.009 (-0.010, 0.028)	0.323
Apolipoprotein-B (mg/dL) ^b	1533	-0.017 (-0.032, -0.001)	0.038	1140	0.004 (-0.016, 0.024)	0.676
Triglycerides (mg/dL) ^b (excl 9 obs >900)	1521	-0.021 (-0.049, 0.007)	0.142	1137	-0.001 (-0.046, 0.043)	0.954
CRP, WBC, platelets						
CRP (excl obs >3) (mg/dL)	3128	-0.045 (-0.097, 0.006)	0.084	2491	-0.059 (-0.123, 0.005)	0.071
WBC ($1000/\mu\text{L}$)	3145	0.009 (-0.007, 0.024)	0.253	2503	0.011 (-0.003, 0.025)	0.130
Neutrophil # ($1000/\mu\text{L}$)	3143	0.003 (-0.020, 0.026)	0.793	2496	-0.000 (-0.023, 0.023)	0.994
Neutrophil (%)	3143	-0.006 (-0.015, 0.004)	0.258	2496	-0.011 (-0.023, 0.001)	0.071
Monocytes # ($1000/\mu\text{L}$)	3143	0.007 (-0.008, 0.022)	0.368	2496	0.020 (0.004, 0.036)	0.014
Monocytes (%)	3143	-0.005 (-0.017, 0.008)	0.452	2496	0.009 (-0.009, 0.028)	0.311
Lymphocytes # ($1000/\mu\text{L}$)	3143	0.019 (0.004, 0.034)	0.016	2496	0.030 (0.011, 0.048)	0.002
Lymphocytes (%)	3143	0.010 (-0.004, 0.024)	0.149	2496	0.019 (0.001, 0.037)	0.043
Platelets ($1000/\mu\text{L}$)	3145	0.014 (0.003, 0.025)	0.018	2503	-0.005 (-0.019, 0.010)	0.522
Glucose metabolism						
Glucose tolerance ^b (mg/dL, 2 hrs)	1323	-0.004 (-0.024, 0.016)	0.706	992	-0.010 (-0.033, 0.013)	0.366
Insulin ($\mu\text{U}/\text{mL}$) ^b	1519	0.044 (0.003, 0.084)	0.034	1130	0.048 (-0.008, 0.104)	0.088
Glucose (mg/dL) ^b	1534	0.010 (0.003, 0.018)	0.006	1140	0.02 (-0.007, 0.010)	0.715

Notes: Estimates are adjusted for age, BMI, ethnicity, cotinine, poverty index, daily kcal intake, fasting duration, NHANES survey. ^aNHANES 2005–2006 only. ^bAM sample. Significant positive (+) and negative (-) associations with creatinine-adjusted perchlorate for both males and females were observed for serum uric acid (-), serum iron (-), RBC count (-), blood urea nitrogen (+), and lymphocyte number. Additional associations among males were observed for hemoglobin (-), total cholesterol (-), LDL (-), apolipoprotein (-), platelets (+), insulin (+), glucose (+), and among females for transferrin saturation (-), monocyte number (+), lymphocyte percent (+).

females. Among the males ($n = 3135$), the use of supplements was not significantly associated with serum iron: $\beta = 0.009$, 95% CI (-0.031, 0.049), $P = 0.66$. Among the non-pregnant females ($n = 2499$), supplement intake was borderline significantly associated with serum iron: $\beta = 0.044$, 95% CI (-0.002, 0.091), $P = 0.06$. Among the 305 pregnant females, the association was significantly positively associated: $\beta = 0.128$, 95% CI (0.005, 0.250), $P = 0.042$. However, the use of dietary supplement had no effect on the negative association between serum iron and urinary perchlorate among pregnant women.

Association with THs among 12–59-year-old males and nonpregnant females. A previous study observed significant associations between urinary perchlorate and THs among NHANES 2007–2008 subjects.⁴ Although the purpose of our study was to investigate associations between perchlorate and biomarkers other than THs, it was of interest to determine if the biomarkers, such as serum uric acid, BUN, serum iron, RBC count, and lymphocyte count, correlated with THs, thyroid peroxidase antibodies, and thyroglobulin among the NHANES 2007–2008 healthy males ($n = 1603$) and non-pregnant females ($n = 1255$). Spearman correlations (r)

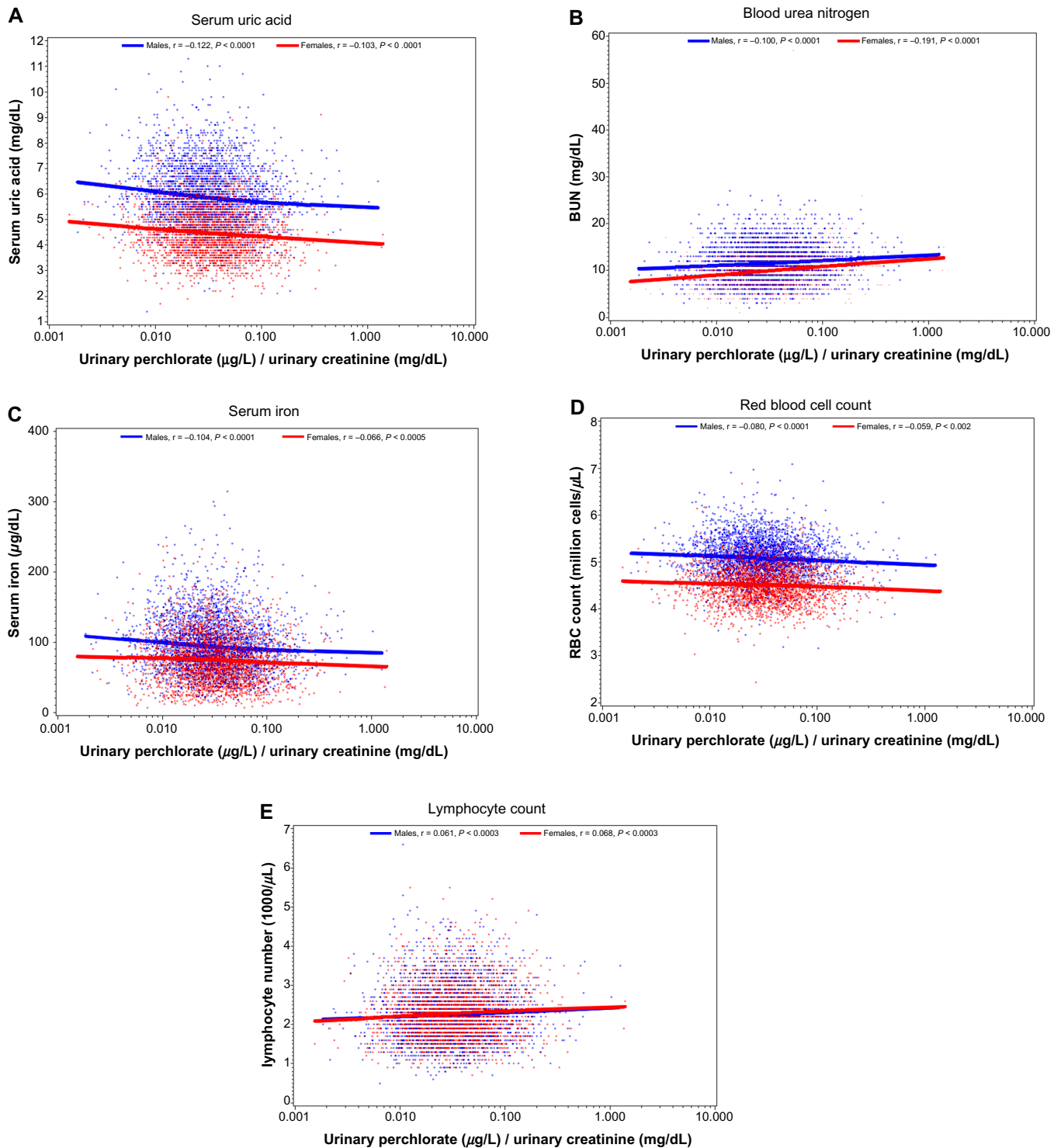


Figure 1. Trends and Spearman correlations between urinary perchlorate and selected biomarkers among healthy NHANES 2005–2008 males and non-pregnant females, age 12–59 years. (A) serum uric acid; (B) blood urea nitrogen; (C) serum iron; (D) red blood cells; (E) lymphocyte number. The y-axis represents the biomarker levels. The x-axis represents urinary creatinine-adjusted perchlorate levels.

with $P < 0.0001$ (**) or < 0.01 (*) are presented here. Serum uric acid was correlated with TSH (males, $r = 0.07^*$; females, $r = 0.09^*$). BUN was correlated with thyroglobulin (males, $r = -0.11^{**}$), TSH (females, $r = 0.10^*$), and T3 (males, $r = -0.11^{**}$; females, $r = -0.15^{**}$). Serum iron was correlated with thyroid peroxidase antibodies (males, $r = 0.09^*$) and

TSH (males, $r = -0.07^*$). RBC count was correlated with T4 (males, $r = 0.12^{**}$; females, $r = 0.13^{**}$) and T3 (males, $r = 0.07^*$; females, $r = 0.14^{**}$). Lymphocyte count was correlated with TSH (males, $r = 0.12^{**}$; females, $r = 0.09^*$), T4 (males, $r = 0.09^*$), and T3 (males, $r = 0.14^{**}$; females, $r = 0.11^*$).

**Table 3.** Association of selected biomarkers with serum iron for males and non-pregnant females, age 12–59, BMI ≥ 18.5 , NHANES 2005–2008.

BIOMARKER (NATURAL LOG TRANSFORMED)	MALES			FEMALES		
	N	ESTIMATE (95% CI)	P-VALUE	N	ESTIMATE (95% CI)	P-VALUE
Oxidative stress, kidney function, iron homeostasis						
Serum uric acid (mg/dL)	3135	0.078 (0.057, 0.099)	<0.0001	2500	0.051 (0.035, 0.066)	<0.0001
Blood urea nitrogen (mg/dL)	3135	0.007 (–0.018, 0.032)	0.587	2500	0.038 (0.013, 0.064)	0.005
RBC ($10^6/\mu\text{L}$)	3130	0.011 (0.004, 0.019)	0.006	2496	0.011 (0.004, 0.018)	0.005
HGB (g/dL)	3130	0.048 (0.041, 0.055)	<0.0001	2496	0.085 (0.073, 0.097)	<0.0001
Ferritin (ng/mL) age 12–49	NA	NA	NA	2287	0.746 (0.659, 0.833)	<0.0001
TIBC ($\mu\text{g/dL}$) ^a	NA	NA	NA	1376	–0.058 (–0.079, –0.037)	<0.0001
Transferrin saturation (%) ^a	NA	NA	NA	1376	1.049 (1.023, 1.075)	<0.0001
Lipids						
Total cholesterol (mg/dL)	3134	0.030 (0.011, 0.049)	0.003	2500	0.015 (–0.000, 0.029)	0.051
HDL (mg/dL)	3134	0.068 (0.044, 0.091)	<0.0001	2500	0.044 (0.025, 0.063)	<0.0001
Nonhdl (mg/dL)	3134	0.018 (–0.008, 0.044)	0.175	2500	0.003 (–0.018, 0.023)	0.796
LDL (mg/dL) ^b	1486	0.059 (0.032, 0.086)	0.0001	1127	–0.003 (–0.037, 0.030)	0.845
Apolipoprotein-B (mg/dL) ^b	1527	0.044 (0.017, 0.071)	0.003	1136	–0.004 (–0.037, 0.028)	0.793
Triglycerides (mg/dL) ^b (excl 9 obs >900)	1515	0.075 (0.011, 0.138)	0.023	1133	0.019 (–0.033, 0.071)	0.466
CRP, WBC, platelets						
CRP (excl obs >3) (mg/dL)	3360	–0.876 (–1.361, –0.391)	0.028	2484	–0.371 (–0.485, –0.257)	<0.0001
WBC ($1000/\mu\text{L}$)	3130	–0.113 (–0.135, –0.091)	<0.0001	2496	–0.013 (–0.036, 0.010)	0.252
Neutrophil # ($1000/\mu\text{L}$)	3128	–0.157 (–0.188, –0.126)	<0.0001	2489	–0.004 (–0.034, 0.027)	0.800
Neutrophil (%)	3128	–0.045 (–0.060, –0.029)	<0.0001	2489	0.008 (–0.004, 0.021)	0.165
Monocytes # ($1000/\mu\text{L}$)	3128	–0.073 (–0.107, –0.039)	0.0001	2489	–0.047 (–0.077, –0.017)	0.003
Monocytes (%)	3128	0.040 (0.011, 0.068)	0.007	2489	–0.031 (–0.051, –0.010)	0.004
Lymphocytes # ($1000/\mu\text{L}$)	3128	–0.020 (–0.051, 0.011)	0.199	2489	–0.003 (–0.026, 0.020)	0.805
Lymphocytes (%)	3128	0.092 (0.059, 0.126)	<0.0001	2489	0.009 (–0.010, 0.029)	0.339
Platelets ($1000/\mu\text{L}$)	3130	–0.041 (–0.066, –0.016)	0.002	2496	–0.068 (–0.088, –0.048)	<0.0001
Glucose metabolism						
Glucose tolerance ^b (mg/dL, 2 hrs)	1321	0.007 (–0.034, 0.049)	0.723	989	0.004 (–0.028, 0.037)	0.786
Insulin ($\mu\text{U/mL}$) ^b	1517	–0.110 (–0.190, –0.031)	0.008	1128	–0.105 (–0.169, –0.042)	0.002
Glucose (mg/dL) ^b	1527	–0.013 (–0.028, 0.001)	0.071	1134	–0.007 (–0.017, 0.002)	0.134

Notes: Estimates are adjusted for age, BMI, ethnicity, cotinine, poverty index, daily kcal intake, fasting duration before blood sample, NHANES survey. ^aNHANES 2005–2006 only. ^bAM sample. NA: not available. Significant positive (+) and negative (–) associations with serum iron for both males and females were observed for serum uric acid (+), RBC count (+), HGB (+), total cholesterol (+), HDL (+), CRP (–), monocyte # (–), platelets (–), insulin (–). Additional associations among males were observed for LDL (+), apolipoprotein-B (+), triglycerides (+), WBC (–), neutrophil # (–), neutrophil % (–), monocyte % (+), lymphocyte % (+), and among females ferritin (+), TIBC (–), transferrin saturation (+), monocyte % (–).

Discussion

Although the TH disrupting characteristics of perchlorate have been studied, less is known about effects on other human biological processes. Perchlorate has a short half-life (6–8 hours) and is eliminated from the body unchanged. The perchlorate anion, recognized as an oxidizing agent, has limited reactivity due to strong chlorine–oxygen bonds. Degradation by chemical reduction is therefore not very successful, although microbial degradation under anaerobic conditions can reduce perchlorate to chloride.¹ Given perchlorate's short half-life and low reactivity, it is not immediately obvious how to interpret the observed associations with biomarkers such as

levels of RBC counts, serum iron, uric acid, BUN levels, and lymphocyte count in both genders. Comparison of the regressions that use perchlorate (Table 2) and serum iron (Table 3) as the main explanatory variable shows that serum iron is associated with several biological measures in contrast to perchlorate with fewer associations. We may be dealing with a stepwise process, where serum iron levels represent the combined influences from several exposures including perchlorate. Vineis et al.¹⁷ explained that the traditional toxicology model of studying single agents, ie, one at a time, at different doses, does not work any longer when dealing with multiple, environmental exposures. Therefore, “acquired vulnerability”

**Table 4.** Association of selected biomarkers with HDL for males and non-pregnant females, age 12–59, BMI \geq 18.5, NHANES 2005–2008.

BIOMARKER (NATURAL LOG TRANSFORMED)	MALES			FEMALES		
	N	ESTIMATE (95% CI)	P-VALUE	N	ESTIMATE (95% CI)	P-VALUE
Lipids						
LDL (mg/dL) ^b	1492	0.015 (–0.063, 0.093)	0.700	1131	–0.070 (–0.168, 0.028)	0.154
Apolipo protein-B (mg/dL) ^b	1533	–0.182 (–0.235, –0.129)	<0.0001	1140	–0.167 (–0.245, –0.089)	0.0001
Triglycerides (mg/dL) ^b (excl 9 obs >900)	1521	–0.987 (–1.107, –0.867)	<0.0001	1137	–0.739 (–0.897, –0.580)	<0.0001
CRP, WBC, platelets						
CRP (excl obs >3) (mg/dL)	3375	–0.404 (–0.408, –0.401)	0.0004	2489	–0.427 (–0.586, –0.268)	<0.0001
WBC (1000/ μ L)	3143	–0.111 (–0.158, –0.065)	<0.0001	2501	–0.129 (–0.167, –0.091)	<0.0001
Neutrophil # (1000/ μ L)	3141	–0.074 (–0.140, –0.008)	0.030	2494	–0.113 (–0.165, –0.061)	0.0001
Neutrophil (%)	3141	0.038 (0.012, 0.064)	0.005	2494	0.011 (–0.016, 0.037)	0.416
Monocytes # (1000/ μ L)	3141	–0.020 (–0.074, 0.034)	0.452	2494	–0.068 (–0.119, –0.017)	0.010
Monocytes (%)	3141	0.089 (0.039, 0.140)	0.001	2494	0.051 (0.004, 0.099)	0.036
Lymphocytes # (1000/ μ L)	3141	–0.180 (–0.218, –0.142)	<0.0001	2494	–0.137 (–0.182, –0.092)	<0.0001
Lymphocytes (%)	3141	–0.069 (–0.114, –0.024)	0.004	2494	–0.013 (–0.060, 0.033)	0.556
Platelets (1000/ μ L)	2143	0.013 (–0.018, 0.044)	0.407	2501	–0.044 (–0.085, –0.002)	0.040
Glucose metabolism						
Glucose tolerance ^b (mg/dL, 2 hrs)	1322	–0.142 (–0.206, –0.078)	<0.0001	992	–0.119 (–0.202, –0.036)	0.007
Insulin (μ U/mL) ^b	1518	–0.645 (–0.794, –0.496)	<0.0001	1130	–0.479 (–0.630, –0.328)	<0.0001
Glucose (mg/dL) ^b	1532	–0.041 (–0.076, –0.005)	0.026	1138	–0.047 (–0.071, –0.024)	0.0003

Notes: Estimates are adjusted for age, BMI, ethnicity, cotinine, poverty index, daily kcal intake, fasting duration before blood sample, NHANES survey. ^bAM sample. Significant positive (+) and negative (–) associations with HDL for both males and females were observed for apolipoprotein-B (–), triglycerides (–), CRP (–), WBC count (–), neutrophil count (–), monocyte % (+), lymphocyte count (–), glucose tolerance (–), insulin (–), glucose (–). Additional associations among males were observed for neutrophil %(+), lymphocyte percent (–), and among females for monocyte count (–), platelets (–).

should be considered, which represents the cumulative effects contributed by different exposures, which can lead to increased biological changes and predispose to disease. The problem is finding a biomarker that may represent acquired vulnerability. If we are correct in assuming that the level of serum iron is affected by multiple exposures that perturb iron homeostasis, then serum iron represents the endpoint of acquired vulnerability of an exposed subject and perchlorate is a contributor to this endpoint.

Because serum iron is an important health indicator, studying xenobiotics that may potentially affect its levels is important. Serum iron as a measure of iron availability is recognized to impact RBC counts, with greater metal concentrations increasing cell numbers and numerous erythrocytic indices.¹⁸ Accordingly, perchlorate and its association with decreased serum iron in our study (Table 2) could affect changes in the RBC counts (Table 3). Similarly, concentrations of uric acid correlate with iron. Such correlations have been previously demonstrated between serum urate and indices of total body iron.¹⁹ The only enzymatic source of urate is xanthine oxidoreductase. In cultured cells, the activity and expression of xanthine oxidoreductase have been demonstrated to increase after exposures to iron.²⁰ In further support of a possible association between levels of iron and urate, exposures of both rodents^{21,22} and humans²³ to elevated concentrations

of this metal increase urate. Concordantly, uric acid decreased with exposures to perchlorate (Table 2) and diminished iron availability (Table 3). The association between perchlorate exposure and BUN among females can reflect a potential biological effect of the metal on renal function (Table 3).²⁴ Other significant correlations between perchlorate exposure and biomarkers could result from an association of the endpoint with indices of iron homeostasis. This includes the relationships between perchlorate and indices of lipids, glucose metabolism, and populations of white blood cells in males or females.^{25–32}

In the human, dietary iron absorption requires that iron traverse both the apical and basolateral membranes of the intestinal epithelial cells to reach the blood where it is incorporated into and transported by transferrin.³³ The transport of iron across membranes occurs in the ferrous (Fe^{2+}) state via the divalent metal transporter 1, the major intestinal iron importer. However, dietary iron exists mainly in ferric form (Fe^{3+}) and subsequently must be reduced to the ferrous state prior to its uptake. The protein duodenal cytochrome B, participates in the chemical reduction of iron (ie, ferrireduction) for intracellular uptake, and is localized on the apical membrane of intestinal enterocytes. Perchlorate potentially diminishes iron uptake in the human intestine following either a direct interaction of perchlorate with iron or an indirect depletion of systemic reductants. There are at least two possible



direct interactions with perchlorate. First, perchlorate might complex iron in the gastrointestinal tract, producing iron III and iron II perchlorates, $\text{Fe}^{3+}(\text{ClO}_4^-)_3$ and $\text{Fe}^{2+}(\text{ClO}_4^-)_2$, respectively. Such metal complexes would decrease the capacity of iron to support electron transport and therefore preclude uptake of the metal in a reduced valence state by the intestinal epithelium. Such perchlorate and iron complexes have been proposed as a methodology by which ClO_4^- can be removed from potable water.¹ Second, perchlorate has some capacity to directly oxidize Fe^{2+} to Fe^{3+} .³⁴ This would decrease uptake of the metal in the intestine since iron in the reduced valence state would be diminished, and it is the Fe^{2+} which is transported across the membrane.

Alternatively, human iron homeostasis may be disrupted by perchlorate in a manner comparable to an acute phase reaction albeit without obvious evidence of inflammation, eg, an elevated C-reactive protein (Table 2). In an acute phase reaction, there is a response to an exposure (eg, infectious agent), which prompts the host to decrease availability of iron by impacting the hepcidin–ferroportin interaction. There is an isolation of host iron in the reticulo-endothelial system and serum iron subsequently decreases. Such a response to an exposure is postulated to be beneficial in that iron is not available for the infectious agent. Infections are absolutely dependent on an availability of host iron and the isolation of this metal in the reticulo-endothelial system would be beneficial in that proliferation of the microbe would be controlled. It can be proposed that such a pathway might also be functioning with perchlorate. Comparable to microbes, perchlorate has a capacity to bind iron and diminish concentrations available to the host. The host may conclude that this is a threat/challenge and initiate the same pathway.³⁵

An additional interpretation is based on the capacity of the perchlorate anion to have a high binding affinity for transferrin combined with the ability to displace iron from this transport protein.³⁶ These characteristics may contribute to the observed decrease of serum iron levels among both males and females and the decrease of transferrin saturation among females (Table 2). Transferrin saturation values for males were not available. Most importantly, iron may become a poor ligand in this process. It is known that reactive oxygen species naturally occurring in the human body can produce the highly reactive hydroxyl radical in the presence of poorly bound iron. The hydroxyl radical has been implicated in tissue damage and organ failure.³⁷

Regardless of the mechanistic pathway, interactions between perchlorate and iron could assist in understanding the observed associations of this pollutant with the end points measured in this investigation. Perchlorate diminishes iron levels and availability, which will successively impact numerous other indices that are metal dependent. Concentrations of urate, cholesterol, and HGB are included among such end points.

Associations between the perchlorate-related biomarkers and THs in this study were modest, but significant. These

associations are supported by the published literature. The relationship between iron status and THs may be bidirectional.³⁸ Iron deficiency has been reported to impair thyroid metabolism by potentially lowering heme-dependent thyroid peroxidase activity.³⁹ Subclinical hypothyroidism is associated with impaired iron utilization and/or transport. Erdogan et al.⁴⁰ reported that among all organ systems, the hematopoietic system is the most seriously affected.

The number of pregnant women available for analyses in this NHANES dataset was small. However, given the concern regarding adverse fetal development due to environmental exposures, we decided to include our limited observations. In common with the 12–59-year-old males and non-pregnant females, increased BUN and decreased serum iron levels were observed in association with perchlorate among the pregnant females. The decreased serum iron levels are of concern. It has been reported that maternal sub-optimal iron stores may adversely affect fetal iron levels.⁴¹ A rat study showed that iron deficiency can cause hypothyroxinemia in pregnant rats.⁴² Another rat study reported that fetal/neonatal iron and TH deficiencies are associated with similar brain developmental abnormalities.⁴³ Again, the biological effects of perchlorate may reflect an impact on iron homeostasis.

Conclusion

In general, studies involving environmental chemicals use changes in TH levels (ie, TH function) as a measure of suspected TH disruption. However, other changes in association with xenobiotics are possible without changes in TH levels, eg, changes in TH signaling (ie, TH action).⁴⁴ Although previous studies investigated changes in TH function (TH levels), the current study shows that perchlorate is associated with several other biomarkers besides THs. The results indicate how NHANES biomonitoring data can identify associations between a surrogate of perchlorate exposure, ie, urinary perchlorate, and several biochemical changes. These associations can serve as hypotheses for controlled *in vitro/in vivo* studies investigating if exposure to environmental perchlorate is indeed the cause of oxidative stress and perturbation of iron homeostasis. Examples would include studies using exposures of relevant cell types to perchlorate with measurement of oxidant generation and indices of iron homeostasis such as iron import/export, ferritin concentration, and RNA for proteins participating in metal transport.

Abbreviations

- Apo-B: apolipoprotein-B
- BMI: body mass index
- BUN: blood urea nitrogen
- CI: confidence interval
- CRP: C-reactive protein
- HDL: high-density lipoprotein
- LDL: low-density lipoprotein



NHANES: National Health and Nutrition Examination Survey

RBC: red blood cells

T3: triiodothyronine

T4: thyroxine

TH: thyroid hormones

TSH: thyroid stimulating hormone

WBC: white blood cells.

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Author Contributions

Conceived and designed the experiments: DS, AG, JS, MW. Analyzed the data: DS. Wrote the first draft of the manuscript: DS. Contributed to the writing of the manuscript: DS, AG, JS. Agree with manuscript results and conclusions: DS, AG, JS, MW. Jointly developed the structure and arguments for the paper: DS, AG, JS, MW. Made critical revisions and approved final version: AG. All authors reviewed and approved of the final manuscript.

Human Subjects Disclaimer

Publicly available NHANES data were the source for this study; thus, requirements for informed consent have been met.

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