On Arsenic, Diabetes, Creatinine, and Multiple Regression Modeling

A Response to the Commentaries on Our Reanalysis

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The original Navas-Acien et al article[1] and their subsequent commentary[2] do not provide valid evidence of an association between low levels of arsenic exposure and diabetes. The reported odds ratios near 2.0–4.0 are inconsistent with those near one we obtained, and result from errors in multiple regression analyses (including inappropriate adjustments for arsenobetaine and creatinine).

Arsenobetaine, present in seafood, is considered nontoxic. Since arsenobetaine is part of total arsenic and the 2 are highly correlated, entering both into the same model can cause unstable results and the spuriously high odds ratios reported by Navas-Acien et al The best way to remove the influence of arsenobetaine is to subtract it from total arsenic. In our paper, we showed that such adjustment produced a result close to the null.[3] When we include the more recent NHANES data (2005–2006), this odds ratio becomes 1.07 (0.58–1.98), even closer to the null and with a narrower confidence interval.

In their commentary, Navas-Acien et al "control" for nontoxic organic arsenic by restricting their analyses to people with no detectable arsenobetaine. In this analysis, their odds ratio comparing the upper and lower quintiles of total arsenic is 4.26 (0.83–21.8), they use to justify their previous results. This finding depends on a questionable adjustment in their multiple regression modeling.

Multiple regression models provide powerful tools for epidemiologists, but like most powerful tools, they are potentially dangerous. A cardinal rule is to examine both unadjusted and adjusted relative risk estimates. If the 2 differ, it is imperative to determine the variables that caused the difference. In this instance, the unadjusted odds ratio is close to 1.0, but changes with adjustment to 4.26. The next step should be to consider the adjustment variables one at a time. Age, sex, ethnicity, and obesity (BMI) are risk factors for type 2 diabetes. As shown in the lower part of the Table (2003–2006 data),[2] the odds ratio estimates remain close to 1.0 when these variables are entered. However, when urinary creatinine is added as a continuous variable, the odds ratio dramatically increases. This change occurs whether or not the NHANES sampling weights are used, or whether cotinine, hypertension medications, or education are entered into the model. Given this large change, one must assess whether adding creatinine to the model is valid.

Adjustment for creatinine is done to account for urine dilution, but there is an expanding body of literature demonstrating that such adjustment can cause problems.[4] Many factors other than urinary dilution can affect urinary creatinine concentrations (eg, diabetes, muscle mass, genetics, diet).[5] In NHANES 2003–2006, for example, the range in urine creatinine concentrations was over 100-fold (7–768 mg/dL)—well beyond any

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variation possibly due to urine dilution. In addition, arsenic and creatinine concentrations in urine have been found to be correlated, even after adjusting for urine dilution measured by specific gravity.[4] Other evidence also suggests that differences in urine creatinine levels between patients with diabetes and nondiabetics are not due to urine volume.[6] Because of all these factors, adjusting for creatinine to account for urine dilution can distort results. We found this in our analyses. When we adjusted for creatinine, some people with very low arsenic concentrations (ie, <1.6 μg/L) ended up in the highest quintile of arsenic exposure. Because of small sample size, even small changes in the numbers of patients with diabetes in each exposure category can have profound effects on odds ratio estimates.

Further evidence of this distortion comes from the fact that the high odds ratios reported by Navas-Acien et al occur only when creatinine is entered as a continuous variable. One method of dealing with the over-dispersion in creatinine is to divide creatinine into quartiles so that outliers are merely in the appropriate quartile. When we enter creatinine as a categorical variable (ie, indicator variables for each quartile of creatinine concentration), the odds ratio for high arsenic becomes 1.19 (0.10–14.0) (Table). One might expect small changes in the odds ratios following adjustment for urine dilution, or small changes when entering creatinine as a categorical rather than as a continuous variable. However, the very large changes reported by Navas-Acien et al are a statistical artifact.

In his commentary, Matthew Longnecker suggests that the odds ratios near 1.0 that we reported could be due to negative confounding by dimethylarsinate (DMA) from seafood.[7] However, the degree of confounding needed to change an odds ratio near 4.0 to an odds ratio near 1.0 would have to be extraordinarily large. In addition, since most ingested inorganic arsenic is metabolized to DMA, there is already DMA in urine from inorganic arsenic exposure. DMA is known to be toxic. The idea that some additional DMA from fish (or arsenosugar) protects one from diabetes, and causes confounding strong enough to completely mask the odds ratios near 3.0–4.0 reported by Navas-Acien et al, is not plausible.

The odds ratios near 1.0 we report for diabetes with very low (ie, background) levels of arsenic in NHANES are consistent with previous studies showing only modestly increased relative risks at exposure levels up to around 50 times higher than seen in NHANES.[3] Our findings highlight the importance of biologic plausibility and the need for caution in conducting multiple regression modeling. Contrary to the statements of Navas-Acien et al, there is no evidence that persons with low arsenic exposure in the United States are at increased risk of diabetes.

TABLE. Association of Total Urine Arsenic Concentrations (Comparing Subjects in the Upper Quintile to Subjects in the Lower Quintile) With Diabetes

	OR (95% CI)
NHANES 2003–2004: all subjects	
Model 1: Adjusted for sex, age, and race	0.97 (0.48–1.94)
Model 2: Further adjusted for education, BMI, serum cotinine, and hypertension medication	0.88 (0.39–1.97)
Model 3a: Further adjusted for arsenobetaine	3.57 (1.28–9.95)
Model 3b: Model 2 with arsenobetaine subtracted from total arsenic	1.15 (0.53–2.50)
NHANES 2003–2006: Subjects with undetectable arsenobetaine concentrations	
Model 1: Unadjusted	1.06 (0.46–2.43)
Model 2: Adjusted for sex and age	1.24 (0.49–3.16)
Model 3: Further adjusted for race	1.03 (0.39–2.75)
Model 4: Further adjusted for BMI	1.03 (0.38–2.80)
Model 5a: Further adjusted for urine creatinine as continuous variable	2.32 (0.51–10.5)[a]
Model 5b: Model 4 further adjusted for urine creatinine as a categorical variable (in quartiles)	1.19 (0.10–14.0)

[a]With NHANES sampling weights, further adjustment (for education, serum cotinine, and hypertension medication), and using slightly different quintile cut-off points, Navas-Acien et al[1] reported this odds ratio as 4.26 (0.83–21.8).

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