Published in final edited form as:

Environ Res. 2018 April ; 162: 211–218. doi:10.1016/j.envres.2018.01.007.

Relationship between serum Trimethylamine N-oxide and exposure to dioxin-like pollutants

Michael C Petriello^{1,2,3,4}, Richard Charnigo⁵, Manjula Sunkara^{1,4}, Sony Soman^{1,4}, Marian Pavuk⁶, Linda Birnbaum⁷, Andrew J Morris^{1,3,4,*}, and Bernhard Hennig^{1,2,*}

¹Superfund Research Center, University of Kentucky, Lexington, KY 40536

²Department of Animal and Food Sciences, College of Agriculture, Food and Environment, University of Kentucky, Lexington, KY 40536

³Lexington Veterans Affairs Medical Center, Lexington, Kentucky, USA

⁴Division of Cardiovascular Medicine, College of Medicine, University of Kentucky, Lexington, KY 40536

⁵Department of Biostatistics, College of Public Health, University of Kentucky, Lexington, KY

⁶CDC Agency for Toxic Substances and Disease Registry, Atlanta, GA, USA

⁷NCI at NIEHS, Research Triangle Park, NC 27709

Abstract

Trimethylamine N-oxide (TMAO) is a diet and gut microbiota-derived metabolite that has been linked to cardiovascular disease risk in human studies and animal models. TMAO levels show wide inter and intra individual variability in humans that can likely be accounted for by multiple factors including diet, the gut microbiota, levels of the TMAO generating liver enzyme Flavin-containing monooxygenase 3 (FMO3) and kidney function. We recently found that dioxin-like (DL) environmental pollutants increased FMO3 expression to elevate circulating diet-derived TMAO in mice, suggesting that exposure to this class of pollutants might also contribute to inter-individual variability in circulating TMAO levels in humans. To begin to explore this possibility we examined the relationship between body burden of DL pollutants (reported by serum lipid concentrations) and serum TMAO levels (n=340) in the Anniston, AL cohort, which was highly exposed to polychlorinated biphenyls (PCBs). TMAO concentrations in archived serum samples from the Anniston Community Health Survey (ACHS-II) were measured, and associations of TMAO with 28 indices of pollutant body burden, including total dioxins toxic equivalent (TEQ), were quantified. Twenty-three (22 after adjustment for multiple comparisons) of the 28 indices were significantly positively associated with TMAO. Although the design of ACHS-II does not enable quantitative assessment of the contributions of previously known determinants of TMAO

*Correspondence should be directed to: Bernhard Hennig and Andrew Morris, 900 S. Limestone Street, Superfund Research Center, University of Kentucky, Lexington, KY 40536, USA. Tel.: +1 859-218-1343; fax: +1 859-257-1811; email: bhennig@uky.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

The authors declare that there are no competing financial interests.

variability to this relationship, limited multivariate modeling revealed that total dioxins TEQ was significantly associated with TMAO among females (except at high BMIs) but not among males. Our results from this cross-sectional study indicate that exposure to DL pollutants may contribute to elevated serum TMAO levels. Prospective longitudinal studies will be required to assess the joint relationship between DL pollutant exposures, other determinants of TMAO, and health outcomes.

Keywords

TMAO; FMO3; dioxin; cardiovascular disease; Anniston

INTRODUCTION

A diet-derived molecule, trimethylamine-N-oxide (TMAO), has been identified as a circulating prospective predictor of cardiovascular disease risk in humans (Tang et al. 2013). Accordingly, while the mechanisms linking elevated TMAO to cardiovascular disease risk are not well understood (and may be complex), a better understanding of factors that determine the observed wide inter- and intra-individual variation in circulating TMAO levels is needed to establish the broader value of this biomarker. TMAO is formed from dietary methylamine-containing precursors, primarily choline containing phospholipids and L-carnitine, which are abundant in many foods including dairy products and meat. The gut microbiota generate trimethylamine (TMA) from these dietary precursors, and the liver enzyme flavin-containing monooxygenase 3 (FMO3) oxidizes TMA to form TMAO. In addition to dietary intake of TMAO precursors, genetic and/or environmental factors (e.g., diet and use of antibiotics) that alter components of this pathway can impact circulating levels of TMAO (Tang et al. 2013; Ussher et al. 2013). Also, pathologies such as kidney disease have been linked to increased levels of TMAO perhaps through alterations of TMAO excretion (Stubbs et al. 2016). In addition to the well-established importance of diet and gut microbiota on TMAO formation, we recently showed in preclinical models that exposure to environmental pollutants known as dioxin-like polychlorinated biphenyls (PCBs) elevate TMAO production from dietary precursors by increasing expression of hepatic FMO3 (Petriello et al. 2016). Exposure to dioxin-like pollutants has previously been linked to increased risk of cardiovascular diseases and cardiorenal injury, but more work needs to be completed to elucidate if increased FMO3 and/or TMAO plays a critical role in these pathologies (Aragon et al. 2008; ATSDR 2000; Kataria et al. 2015). We have now attempted to determine if our observed association between dioxin-like pollutants and increased circulating TMAO is evident in humans.

Although most individuals are continuously exposed to persistent organic pollutants such as dioxins, at relatively low background levels, cross sectional epidemiological studies of more highly exposed sample populations, for example individuals living in Anniston, Alabama (a major site of North American PCB manufacturing) provide an opportunity to identify associations between POP exposures and disease risk biomarkers that are broadly relevant to the entire US population. PCBs, especially higher chlorinated congeners (e.g., PCB 153, PCB 118, and PCB 126) are found in the plasma of most US residents. Although intentional

production of PCBs was halted in the United States by the late 1970's, due to their chemical stability they have remained in aqueous and terrestrial ecosystems. PCB exposure remains a public health concern because these lipophilic toxicants can biomagnify through the food chain leading to dietary exposures when consuming primarily animal-derived foods (Kvalem, et al. 2009). The Anniston Community Health Survey follow up study (ACHS-II) is an accessible and relevant cohort for our study because high-quality, technically challenging, measurements of multiple dioxin-like chemicals are available along with some limited information about other known determinants of variability in TMAO levels. This study conducted eight years after the initial ACHS focused on a subset of the original population, was designed to quantify the missing dioxin-like pollutants including coplanar polychlorinated biphenyls (PCBs), polychlorinated dibenzodioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs) (Birnbaum et al. 2016). The purpose of the present study was to analyze data of archived serum samples from ACHS-II to test the hypothesis that individuals with higher pollutant body burden of dioxin-like compounds (normalized as dioxin toxic equivalents, TEQs) would have higher circulating levels of TMAO. A secondary goal of this study was to examine how the relationship between circulating TMAO and TEQ was modified by other known determinants of circulating TMAO levels.

METHODS

Cohort description and pollutant measurements

Details of The Anniston Community Health Survey: Follow-Up and Dioxin Analyses (ACHS-II) have been previously reported (Birnbaum et al. 2016). THE ACHS-II protocol was reviewed by the Institutional Review Boards at the Centers for Disease Control and Prevention (CDC) and University of Alabama at Birmingham (UAB). This study was not primarily designed to study the effect of dioxins on biomarkers of CVD (e.g., TMAO), but is useful to make initial associations due to the high quality of pollutant measurements. Briefly, 359 of the 766 participants who took part in the original ACHS-I (114 deceased, 69 moved away from study area; 483 contacted) were recruited to ACHS-II and provided an additional fasted blood sample and responded to an interviewer-administered questionnaire detailing current health and demographic information. To complete the interview, the participants of the ACHS-II study were required to bring in all medication bottles; current (last two weeks') medication name and dosage were abstracted by the interviewer-nurse which was the basis for all drug-related variables (e.g., antimicrobial medications). Serum concentrations of the following environmental pollutants were determined by the CDC's National Center for Environmental Health (NCEH): Seven 2,3,7,8-substituted polychlorinated dibenzo-dioxins, 10 dibenzo-furans, and three non-ortho PCBs (congeners 81, 126, 169) were measured using high resolution GC/MS (Turner et al. 1997) as well as 35 ortho-substituted PCBs (congeners 28, 44, 49, 52, 66, 74, 87, 99, 101,105, 110, 118, 128, 138–158, 146, 149, 151, 153, 156, 157, 167, 170, 172, 177, 178, 180,183, 187, 189, 194, 195, 196–203, 199, 206, and 209; same congeners measured in the original ACHS; Sjodin et al. 2004). Pesticides, polybrominated diphenyl ethers, and heavy metals were not part of the present analyses.

Toxic equivalency (TEQ) analyses

Toxic equivalency factors (TEFs/TEQs) are a weighted quantity measure based on the toxicity of each of the dioxin-like compounds relative to the most toxic member of the category. TEQs enable evaluation of cumulative exposure for risk assessment and health outcomes research. We therefore calculated TEQs for four groups of the dioxin-like pollutants – dioxins (PCDD), furans (PCDF), non-ortho PCBs, and mono-ortho PCBs (Vandenberg et al. 2006). Toxic equivalency factors (TEFs) were determined using the 2005 World Health Organization's guidelines. Summary measures of those congener groups (e.g., sum of seven dioxin congeners- PCDD TEQ and Total dioxins TEQ) were calculated from lipid adjusted pollutant concentrations. TEQs were also created for individual dioxin-like congeners (e.g., PCB 126). TEQ Total was used as the primary summary measurement for most of the statistical analyses reported here. The total toxic equivalency is the weighted sum of the product of the TEF multiplied by concentration for each relevant dioxin-like compound.

TMAO and choline extraction and quantitation

TMAO has been reported to be stable in human serum/plasma stored at -80°C for up to 5 years (Wang et al. 2014). TMAO and choline were extracted from serum and analyzed by HPLC electrospray ionization tandem mass spectrometry as described previously with minor modifications (Petriello et al. 2016). Briefly, 25 μl of serum was extracted with acetonitrile in 96 well Impact Protein Precipitation plates (Phenomenex). Deuterated TMAO (d9-TMAO) and choline (d9-choline) were used as internal standards, and each extraction plate contained blank samples, quality controls and a range of quantitative standards to enable absolute quantitation of TMAO and choline by stable isotope dilution methods. TMAO and choline were measured using a Shimadzu HPLC coupled with an AB Sciex 6500-QTRAP hybrid linear ion trap triple quadrupole mass spectrometer operated in multiple reaction monitoring (MRM) mode. Samples were separated on a Primesep 100, 3 μm , 2.1×100 mm column (SIELC Technologies, Wheeling IL) and MRM transitions used for quantitation were as follows: 76/59.1 for TMAO, 85.1/66.0 for d9-TMAO, 104.2/60.1 for choline, and 113.1/69.1 for d9-choline. Data was processed using ABSciex Multiquant software, and TMAO/choline concentrations (micromolar) were determined for each available serum sample. The University of Kentucky Superfund Research Center was provided with stored frozen serum samples from 340 of the ACHS-II participants for TMAO analysis from ATSDR in 2016.

Dietary intake questionnaire

Each of the ACHS-II participants responded to an interviewer-administered questionnaire detailing dietary patterns. This questionnaire was not developed to quantitatively study the intake of dietary methylamines, however, some of the questions were at least tangentially relevant to TMAO formation and were included in this study. Questions possibly related to intake of TMAO precursors (e.g., choline, L-carnitine, etc.) included, "Overall, when you think about the foods you ate over the past 12 months, would you say your diet was high, medium, or low in fat" and "Have you eaten fish or shellfish in the past week". Other possibly relevant questions included "Have you ever eaten local eggs", "Have you ever eaten

local dairy products like milk or cheese”, and “Have you ever eaten local beef or beef products”. Responses for these questions were coded as Yes/No/Don’t Know. Two additional, more semi-quantitative questions were included in analyses. They were phrased, “Think about your eating habits over the past 12 months. About how often did you eat or drink each of the following foods? Remember breakfast, lunch, dinner, snacks, and eating out. For each food, select one choice either Never, <1 time per month, 1–3 per month, 1–2 per week, 3–4 per week, 5–6 per week, 1 per day, 2 per day, or don’t know”. Specifically, the food variables 1) “Eggs, fried or scrambled in margarine, butter, or oil”, and 2) “Cheese or cheese spread, regular-fat” were investigated and were tested for significant associations with TMAO as described below.

Data analyses and statistical modeling

The data presented were generated using the following statistical methods: For each of the 28 pollutant TEQ exposure indices, we calculated its Pearson correlation with TMAO and a corresponding p-value. Regarding the p-values, a modified Benjamini-Hochberg adjustment for multiple comparisons was applied (Benjamini and Yekutieli 2001) in view of there being 28 indices. For clarity, subsequent analyses focused primarily on the mixture index “total dioxins TEQ”, whose correlation with TMAO was at a level typical of those exhibited in Table 2 and which best summarizes body burden of dioxin-like pollutants. To identify significant covariates, we examined correlations between TMAO and numerous auxiliary variables (Pearson correlations for numeric variables and point biserial correlations for binary variables; see Table 2 row headings for variables of interest) and determined corresponding p-values. We then used linear regression modeling to assess the association of total dioxins TEQ with TMAO when adjusted for the auxiliary variable and whether the auxiliary variable interacted with total dioxins TEQ; in other words, did the strength of association between total dioxins TEQ and TMAO differ according to the value of the auxiliary variable (see Table S1)?

We next fit a multivariate linear regression model, using backward stepwise elimination, to quantify adjusted associations of total dioxins TEQ with TMAO in strata defined by covariates interacting with total dioxins TEQ. The initial version of this model, before the backward steps, contained all auxiliary variables significantly ($p < 0.05$) or close to significantly ($0.05 < p < 0.10$) associated with TMAO and all significant ($p < 0.05$) or close to significant ($0.05 < p < 0.10$) interactions previously identified; the initial version of the model and all backward steps were also required to satisfy the constraint that any covariate whose interaction term appeared in the model also had to appear in the model itself. The resulting regression model focused on total dioxins TEQ, sex, diabetes status, BMI, race, kidney disease status, and interactions of total dioxins TEQ with sex and BMI (see Table 3). We also fit similar regression models in which total dioxins TEQ was replaced by one of the other TEQs (see Table S2). Some variables were log transformed to reduce non-normality (one was added before taking the logarithm), as indicated in the tables. The SAS (SAS Institute Inc., Cary NC USA), R (R Foundation for Statistical Computing, Vienna Austria), and Microsoft Excel (Microsoft Corporation, Redmond WA) environments were used for data analysis and visualization.

RESULTS

The median serum concentration of TMAO in this sample was 4.26 μM with a maximum of 86.32 μM (Q1=2.87 μM ; Q3=6.95 μM). Subjects were older than the national median and there was an obvious gender imbalance (see Table 1 for demographics). Table 2 shows the bivariate analyses associating 28 exposure indices with TMAO. Of the 28 indices, including all mixtures examined, 22 were significantly and positively related to TMAO after adjustment for multiple comparisons. The dioxin equivalencies most strongly related to TMAO were 1,2,3,6,7,8- HCDD, 1,2,3,4,7,8,- HCDD, 1,2,3,6,7,8- HCDF, and the sum of 7 PCDDs, all with correlation coefficients above 0.25. Most single congener TEQs for the dioxin-like PCBs, although still significantly correlated, had slightly weaker bivariate associations with TMAO than other indices. Total dioxins TEQ, a widely used summary measure of dioxin toxicity was used primarily for other analyses herein.

Other groups have identified multiple covariates that may relate with or alter the inter-individual variability of TMAO formation. To identify relevant covariates in the ACHS-II population that may impact TMAO levels independently in further analyses, we next examined bivariate associations of TMAO with multiple auxiliary variables including sex, BMI, age, self-reported kidney disease, self-reported diabetes status, self-reported stroke, race, dietary intake information, antimicrobial medications, glucose, insulin, triglycerides, and VLDL cholesterol (see lower portion of Table 2). Many of the variables examined have been reported to be associated with TMAO in other studies (Randrianarisoa et al. 2016; Wang et al. 2011; Zheng et al. 2015). We determined that self-reported diabetes status, age, race, glucose, self-reported stroke, triglycerides, self-reported kidney disease, and VLDL cholesterol were all significantly associated with TMAO. Of these additional covariates investigated, self-reported kidney disease and self-reported diabetes status exhibited two of the strongest associations with TMAO. Next, since diet has been shown to be a major modulator of TMAO production, and also has been shown in previous studies of this cohort to be an important predictor of pollutant exposure, association analyses between TMAO and answers from a diet-recall questionnaire were completed (Pavuk et al. 2014). None of the diet-related covariates significantly associated with TMAO and were therefore excluded from further analyses (local dairy was closest, but with an unexpected negative correlation; $p=0.056$). Importantly, the questionnaire was not designed to investigate quantitatively the impact of methylamine-containing foods on TMAO levels. In an attempt to better elucidate diet's impact on TMAO formation in the ACHS-II population, we also quantitated, via LC-MS/MS, serum choline levels. Similarly, serum choline levels were not significantly associated with TMAO ($p=0.113$). Self-reported diabetes status, age, Caucasian race, and self-reported kidney disease were all positively significantly associated with TMAO; self-reported diseases were coded 1=yes, 2=no. In Table S1, we also document the covariate-adjusted associations of total dioxins TEQ with TMAO.

In our bivariate models we observed a significant positive association with TMAO and multiple dioxin-like pollutants (Table 2). Next, we examined if the strength of the association between TMAO and total dioxins TEQ might be modified by the covariate factors previously shown to associate with or modulate circulating TMAO (Table S1). In fact, multiple significant interactions were identified between covariates of interest and total

dioxins TEQ. Interestingly, the relationship between total dioxins TEQ and TMAO diminished as BMI increased. Some evidence of a sex/exposure interaction was also observed ($p=0.071$), with a significant relationship between TMAO and total dioxins TEQ among females but not males. These interactions are illustrated in Figure S1A–B.

Obtaining a multivariate linear regression model via backward elimination (see Table 3A,B), we found that self-reported diabetes, race, and self-reported kidney disease were significant predictors of TMAO and that there were significant interactions of total dioxins TEQ with sex and BMI. The multivariate regression model containing interactions explained 22.3% of the variation in TMAO. Adding in age post-hoc (which was not significantly associated in the model and removed by the backward elimination process; $p=0.201$), either of the two semi-quantitative dietary recall variables, or serum choline concentrations did not eliminate the observed significant association of TMAO and TEQ total. We also adjusted for the ratio of TMAO:Choline, and again, the observed significant association of TMAO and TEQ total was not eliminated. Table 4 presents the associations of total dioxins TEQ with TMAO for males and females at selected values of BMI (20, 30, and 40). Dioxin exposure was significantly and positively associated with TMAO in less obese females (e.g., with BMI = 20 and BMI = 30); significance was nearly attained for females with BMI = 40, but significance was never attained for males.

Ultimately, Table S2 shows results from 28 different multivariate linear regression models in which total dioxins TEQ is replaced by various indices of exposure. After adjustment for multiple comparisons, exposure was positively and significantly associated with TMAO in females at BMI = 20 and BMI = 30 for 23 out of 28 indices examined; however, there were no exposure indices significantly associated with TMAO in females at BMI = 40 or in males at any one of the indicated BMIs. The TEQ indices whose models yielded the strongest overall R^2 values were the sum of mono-ortho PCBs (0.251), PCB 156 (0.248), PCB 167 (0.248), and PCB 157 (0.245).

DISCUSSION

In our previous preclinical study we showed that exposure to dioxin-like PCBs increased circulating TMAO, and here we found that most indices of dioxin exposure significantly associated with increased circulating levels of TMAO. Although we believe FMO3 activity/expression may play an important role in these observed associations, multiple other sources of inter-individual variability in circulating TMAO have been previously described (e.g., diet, alterations of gut microbiota, and kidney function). Using this cross-sectional study, it is however difficult to ascertain any mechanistic insight in to why this association may exist especially since no information related to FMO3 genetic polymorphisms, microbiota, or accurate measures of kidney function were collected. Diet is thought to be a primary influencer on the production of TMAO and the ACHS-II participants were allowed to maintain their regular dietary patterns prior to the fasting blood draw. Although we attempted to examine the impact of diet on inter-individual differences of TMAO, no associations were observed which is most likely due to the format of the questionnaire. Diet is also a source of exposure to PCBs in most individuals including the ACHS-II subjects. The lack of any positive association between TMAO and self-reported dietary intake of

animal derived foods that are likely sources of pollutants may suggest that the observed relationships between TMAO and dioxin like pollutants cannot be explained by a shared dietary source. We also failed to identify a significant association between serum choline and TMAO in the ACHS-II population. Although unexpected, we are not the first to find no correlation between choline and TMAO. In a preclinical feeding study using a phospholipid-protein complex it was determined that “There was a close correlation between plasma TMAO and carnitine, trimethyllysine, and γ -butyrobetaine, but not between TMAO and choline (Bjørndal et al. 2015).” Future studies specifically investigating phosphatidylcholines instead may lead to a significant association with TMAO. However, the observed positive associations between total dioxins TEQ and TMAO, after adjustments for numerous covariates (Table S1), are intriguing and deserve replication in a more controlled prospective study with stronger diet and adverse outcomes data available.

When allowing sex to be an effect modifier, we found that the association between TMAO and pollutant exposure was restricted to female subjects (Table 4, Supplemental table 2, and Supplemental Figure 1A). Although approximately 70% of the ACHS-II cohort is female, there is some evidence from previous reports to support the idea that this sex-specific association is genuine. In a previous ACHS-related study investigating PCB exposure, there was an observed positive association with diabetes in women (increased odds ratio), but a negative association was seen in men (Silverstone et al. 2012). A group investigating dioxin-like pollutants (PCDDs and PCDFs) determined that a statistically significant association with increased rates of hypertension was evident for women but not men (Ha et al. 2009). Also, it has been reported that diabetic males may be more likely to have less circulating TMAO compared to diabetic females (Tang et al. 2016). Here, we show the strongest associations between TMAO and pollutant exposure are observed in women and especially in less obese individuals (see Figures S1:A-B). There is a growing body of evidence showing that increased adiposity may actually protect against pollutant toxicity. This may be because lipophilic pollutants can sequester within adipose tissue limiting their burden on other target organs (e.g., vascular tissue, kidneys, and liver). In leaner individuals, a greater percentage of the overall body burden may be accessible to the liver to increase FMO3 expression activity, modulate gut microbiota and/or alter kidney function. There is some precedent for these observations as some epidemiological studies have observed an inverse relationship with BMI and relative risk of pollutant-associated diseases. Using the NHANES dataset from 1994-2004, researchers showed that, in elderly individuals with low fat mass, PCBs were positively associated with CVD mortality, but this association was lost as BMI increased (Kim et al. 2015). Also, preclinical studies have been able to recapitulate these observed BMI effects supporting the hypothesis that increased adipose expansion may protect against certain lipophilic pollutant-induced diseases (Baker et al., 2013; La Merrill et al., 2013; Tuomisto et al., 1999). In our current study, we did not observe a significant association between TMAO and BMI ($p=0.42$) which is in agreement with other reports (Rohrmann et al. 2016).

Circulating TMAO levels are a predictor for myocardial infarction, which is a result of coronary artery disease. A seminal paper described a clinical-outcomes study in which TMAO was analyzed at baseline for 4007 volunteers; after a 3-year follow-up, TMAO levels predicted major cardiovascular events even after adjustment for covariate determinants of

cardiovascular disease risk (Tang et al. 2013). Identifying novel biomarkers of cardiovascular disease, such as TMAO, is critical to better predict detrimental health outcomes, especially in an evolving world of personalized medicine. This positive link between TMAO and CVD has been replicated in other studies with clinical end points ranging from heart failure to atherosclerosis (Randrianarisoa et al. 2016; Senthong et al. 2016; Shafi et al. 2016; Suzuki et al. 2016). TMAO is now emerging as a potential biomarker of other diseases related to CVD, including type 2 diabetes and kidney disease (Dambrova et al. 2016; Kim et al. 2016; Missailidis et al. 2016; Stubbs et al. 2016). In the most recent meta-analysis, female gender was examined as an effect modifier for the primary outcome of all-cause mortality and was determined to be insignificant (Schiattarella et al. 2017). However, most prospective studies have predominately been made up of male subjects. In animal studies, female mice fed a high choline diet produce ~10 times more TMAO than male mice and this may be due to the sexual dimorphic nature of FMO3 expression in rodents (Wang et al. 2015). However, definitive conclusions about the joint relationships between dioxin like pollutants, TMAO, and risk of myocardial infarction cannot be made from this cross-sectional study.

Currently, it is unknown if TMAO is a causative agent of human disease, or more broadly a biomarker of FMO3 activity. Dietary supplementation with TMAO accelerates atherosclerosis in mouse models, and blood and vascular cells treated with TMAO display pro-inflammatory signaling responses (Chen et al. 2016; Seldin et al. 2016). However, some evidence supports an alternative hypothesis where FMO3 itself, independently of TMAO, may play a role in cardiometabolic disease, possibly through regulation of hepatic gene expression. For example, in mice FMO3 regulates cholesterol balance and glucose/insulin signaling in mice (Miao et al. 2015; Schugar and Brown 2015; Shih et al. 2015; Warrier et al. 2015). In a mouse model of insulin resistance, loss of FMO3 protects against hyperglycemia, hyperlipidemia, and atherosclerosis (Miao et al. 2015). It is attractive to speculate that the association between elevated TMAO and diabetes observed here is a clinical manifestation of the observations seen in mouse models. More work is required to determine the mechanisms linking TMAO and human disease, but the formation, metabolic fate, and kinetics of TMAO are well established.

TMAO formation has been shown to be dependent on dietary intake of foods containing necessary precursors such as choline-containing phospholipids and carnitine. For example, major dietary sources of choline and L-carnitine include eggs, milk, liver, red meat, poultry, and shell fish (Koeth et al. 2013), all of which are highly consumed in the United States. In humans, direct conversion of dietary choline and L-carnitine into TMAO has been demonstrated, and vegans have less circulating TMAO than omnivores (Koeth et al. 2013; Miller et al. 2014). Also, TMAO has been associated with dairy consumption (a rich source of choline-containing phospholipids) in at least one European population (Rohrmann et al. 2016). The ACHS-II participants underwent an interview, which included many questions related to dietary patterns (see methods section). In our sample, self-reported dairy intake was nearly significantly associated with TMAO, but this association was in the opposite direction of that expected. The other diet-related variables were not significantly associated with TMAO. This interesting result may be due in part to how some of the diet-related questions were formulated; for example specifically asking about *local* dairy products

(potentially contaminated with PCBs), or due to an unknown confounding variable. The primary goal of the questionnaire was to identify possible dietary exposures of pollutants and consisted primarily of questions related to local consumption of foods. Even the more “quantitative” dietary recall questions were worded so specifically that it is not surprising that significant associations with TMAO were not observed. For example, instead of asking generally about frequency of egg consumption, the related question was phrased, “About how often did you eat eggs, fried or scrambled in margarine, butter, or oil?” Well-designed dietary assessments utilize common food photographs that illustrate portion sizes and food frequency questionnaires (FFQs) with responses such as never, per year, per month, per week, or per day (Cho et al. 2006). The most useful dietary assessments can then be compared against currently available food composition databases to estimate total energy consumption and individual nutrient content (e.g., methionine, betaine, etc.) (Zeisel et al. 2003). With the currently available dietary information, it cannot be ignored that circulating dioxin concentrations may be a significant biomarker of consuming foods rich in methylamine precursors. Future studies looking at biomarkers of diet such as odd or branched chain fatty acids found primarily in dairy foods may provide a clearer, more quantitative measure of the dietary patterns of the ACHS-II participants. Measuring other nutritional biomarkers related to TMAO formation including those of red meat may also be useful in future studies. Finally, it would be useful to quantitate concentrations of other well established FMO3 products and substrates to create a value of FMO3 activity. If information of this sort can be quantified and subsequently added in to future statistical models, the associations between TMAO and pollutant exposure may become more persuasive.

In the ACHS-II cohort, exposure to environmental pollutants could positively associate with TMAO concentrations through multiple mechanisms. We began investigating the association between TMAO and dioxins in the ACHS-II cohort because recently we reported that dioxin-like pollutants (PCB 126 and PCB 77) upregulate FMO3, a critical enzyme responsible for production of TMAO. This upregulation can lead to increased TMAO production when mice are challenged with necessary dietary precursors (Petriello et al. 2016). The primary enzymes involved in PCB detoxification are cytochrome P450s (CYPs). Although there is some speculation supporting the hypothesis that FMOs have the ability to detoxify PCBs and PCB metabolites, this would be expected to be a minor pathway compared to CYP1A1 and other CYP-mediated detoxification (Grimm et al. 2015). We previously showed that FMO3 could be induced in rodents by PCB 126 (Petriello et al. 2016) and others have shown that TCDD, 3-methylcholanthrene and benzo[a]pyrene could also induce FMO3 expression in animals and cell lines most likely via an AhR-mediated mechanism (Celius et al. 2008, 2010). In humans, is not well established if FMO3 is overly inducible with most current work focusing on induction through FXR-mediated mechanisms primarily by bile acids (Bennett et al. 2014). Interestingly, recent preclinical work shows dioxin-like pollutants may modulate bile acid production and/or transport which may be another mechanism linking pollutant exposures to increased FMO3/TMAO (Fader et al. 2017). More work is needed to elucidate if exposure to dioxin-like pollutants can upregulate FMO3 in humans. Importantly, pollutants may alter TMAO formation via additional mechanisms, including modulation of gut microbiota. TMAO production requires the metabolism of dietary precursors to TMA by gut microbes, and altering the diversity of flora

may also modulate TMAO production. For example, one group determined that only a subset of the gut microbiota species produced TMA from choline *in vitro* (Romano et al. 2015). One could hypothesize that alterations of the gut flora diversity to favor these and similar strains of bacteria may lead to increased TMAO formation and risk of cardiovascular disease. Importantly for our current human study, multiple groups have now shown that exposures to environmental pollutants, and specifically dioxin-like pollutants, can alter gut microbiota diversity leading to inflammation (Potera 2015; Zhang et al. 2015). Highly exposed cohorts, such as the one from Anniston, may enable investigations of the effects of pollutant exposure on gut flora diversity in future follow-up studies.

There are some notable limitations with this study that may be addressed in future follow-up analyses. In particular, because the study is cross sectional and involves single measurements of pollutants and TMAO, we cannot exclude reverse causality- i.e the possibility that higher TMAO levels alter systemic accumulation of pollutants. Thus, identifying associations between TMAO and dioxin-like pollutants, including other polycyclic aromatic hydrocarbons, in future prospective or longitudinal studies may be useful. Also, it is unclear how generalizable the findings are, due to potential bias due to the selection of a subcohort (many of the original ACHS participants are deceased). Much of the covariate data (e.g., health outcomes and diet) were based solely on questionnaires and interview responses, not medical records, which may introduce recall or social desirability biases (e.g., under-reporting of undesirable behaviors or pathologies). Because of the community based nature of the study, effort was not made to validate the health outcome responses through quantitative measurements of atherosclerosis such as carotid ultrasound and intima media thickness. Participants were instructed to bring their medications with them to the study office to be recorded, which may be an alternative means of validating disease occurrences in future studies. Unfortunately, TMAO has not been quantified in other large datasets (e.g., NHANES) where pollutant measurements are available. A more definitive analysis of the joint relationship among pollutant exposures, TMAO, and coronary artery disease risk will require a prospective longitudinal study with appropriate data collection and endpoints.

CONCLUSION

Our findings support the concept that exposure to dioxin-like pollutants may contribute to inter-individual differences in plasma TMAO levels, but more work is needed to causatively link pollutant exposures, TMAO, and CVD in humans.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding

This work was supported by the National Institute of Environmental Health Sciences at the National Institutes of Health [P42ES007380], the intramural program of the NCI/NIH, the University of Kentucky Agricultural Experiment Station, T32 postdoctoral fellowship, and used resources at the Lexington, KY VA medical center. The

content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or Center for Disease Control and Prevention.

References

- Aragon AC, Goens MB, Carbett E, Walker MK. Perinatal 2,3,7,8-tetrachlorodibenzo-p-dioxin exposure sensitizes offspring to angiotensin II-induced hypertension. *Cardiovasc Toxicol*. 2008; 8:145–54. [PubMed: 18670907]
- ATSDR. Toxicological Profile for Polychlorinated Biphenyls. U.S. Department of Health and Human Services; Agency for Toxic Substances and Disease Registry; Atlanta, GA: 2000. <https://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=142&tid=26>
- Baker NA, Karounos M, English V, Fang J, Wei Y, Stromberg A, et al. Coplanar polychlorinated biphenyls impair glucose homeostasis in lean C57BL/6 mice and mitigate beneficial effects of weight loss on glucose homeostasis in obese mice. *Environmental health perspectives*. 2013; 121:105–10. [PubMed: 23099484]
- Benjamini Y, Yekutieli D. The control of the false discovery rate in multiple testing under dependency. *Ann Statist*. 2001; 29:1165–1188.
- Bennett BJ, de Aguiar Vallim TQ, Wang Z, Shih DM, Meng Y, Gregory J, Allayee H, et al. Trimethylamine-N-oxide, a metabolite associated with atherosclerosis, exhibits complex genetic and dietary regulation. *Cell Metab*. 2013; 17:49–60. [PubMed: 23312283]
- Bjørndal B, Ramsvik MS, Lindquist C, Nordrehaug JE, Bruheim I, Svardal A, et al. A phospholipid-protein complex from antarctic krill reduced plasma homocysteine levels and increased plasma trimethylamine-n-oxide (tmao) and carnitine levels in male wistar rats. *Mar Drugs*. 2015; 13:5706–21. [PubMed: 26371012]
- Birnbaum LS, Dutton ND, Cusack C, Mennemeyer ST, Pavuk M. Anniston community health survey: Follow-up and dioxin analyses (achs-ii)—methods. *Environmental science and pollution research international*. 2016; 23:2014–2021. [PubMed: 25982988]
- Celius T, Roblin S, Harper PA, Matthews J, Boutros PC, Pohjanvirta R, et al. Aryl hydrocarbon receptor-dependent induction of flavin-containing monooxygenase mRNAs in mouse liver. *Drug Metab Dispos*. 2008; 36:2499–505. [PubMed: 18765683]
- Celius T, Pansoy A, Matthews J, Okey AB, Henderson MC, Krueger SK, et al. Flavin-containing monooxygenase-3: induction by 3-methylcholanthrene and complex regulation by xenobiotic chemicals in hepatoma cells and mouse liver. *Toxicol Appl Pharmacol*. 2010; 247:60–9. [PubMed: 20570689]
- Chen ML, Yi L, Zhang Y, Zhou X, Ran L, Yang J, et al. Resveratrol attenuates trimethylamine-n-oxide (tmao)-induced atherosclerosis by regulating tmao synthesis and bile acid metabolism via remodeling of the gut microbiota. *mBio*. 2016; 7
- Cho E, Zeisel SH, Jacques P, Selhub J, Dougherty L, Colditz GA, et al. Dietary choline and betaine assessed by food-frequency questionnaire in relation to plasma total homocysteine concentration in the Framingham Offspring Study. *Am J Clin Nutr*. 2006; 83:905–911. [PubMed: 16600945]
- Dambrova M, Latkovskis G, Kuka J, Strele I, Konrade I, Grinberga S, et al. Diabetes is associated with higher trimethylamine n-oxide plasma levels. *Experimental and clinical endocrinology & diabetes : official journal, German Society of Endocrinology [and] German Diabetes Association*. 2016; 124:251–256.
- Fader KA, Nault R, Zhang C, Kumagai K, Harkema JR, Zacharewski TR. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD)-elicited effects on bile acid homeostasis: Alterations in biosynthesis, enterohepatic circulation, and microbial metabolism. *Sci Rep*. 2017; 7:5921. [PubMed: 28725001]
- Grimm FA, Hu D, Kania-Korwel L, Lehmler HJ, Ludewig G, Hornbuckle KC, et al. Metabolism and metabolites of polychlorinated biphenyls. *Crit Rev Toxicol*. 2015; 45:245–272. [PubMed: 25629923]
- Ha MH, Lee DH, Son HK, Park SK, Jacobs DR Jr. Association between serum concentrations of persistent organic pollutants and prevalence of newly diagnosed hypertension: Results from the national health and nutrition examination survey 1999–2002. *J Hum Hypertens*. 2009; 23:274–286. [PubMed: 18843279]

- Hennig B, Slim R, Toborek M, Robertson LW. Linoleic acid amplifies polychlorinated biphenyl-mediated dysfunction of endothelial cells. *Journal of biochemical and molecular toxicology*. 1999; 13:83–91. [PubMed: 9890193]
- Kataria A, Trasande L, Trachtman H. The effects of environmental chemicals on renal function. *Nat Rev Nephrol*. 2015; 10:610–25.
- Kim SA, Kim KS, Lee YM, Jacobs DR, Lee DH. Associations of organochlorine pesticides and polychlorinated biphenyls with total, cardiovascular, and cancer mortality in elders with differing fat mass. *Environmental research*. 2015; 138:1–7. [PubMed: 25682252]
- Kim RB, Morse BL, Djurdjev O, Tang M, Muirhead N, Barrett B, et al. Advanced chronic kidney disease populations have elevated trimethylamine n-oxide levels associated with increased cardiovascular events. *Kidney international*. 2016; 89:1144–1152. [PubMed: 27083288]
- Kim SA, Kim KS, Lee YM, Jacobs DR, Lee DH. Associations of organochlorine pesticides and polychlorinated biphenyls with total, cardiovascular, and cancer mortality in elders with differing fat mass. *Environmental research*. 2015; 138:1–7. [PubMed: 25682252]
- Koeth RA, Wang Z, Levison BS, Buffa JA, Org E, Sheehy BT, et al. Intestinal microbiota metabolism of l-carnitine, a nutrient in red meat, promotes atherosclerosis. *Nature medicine*. 2013; 19:576–585.
- Kvale HE, Knutsen HK, Thomsen C, Haugen M, Brantsaeter AL, Froshaug M, et al. Role of dietary patterns for dioxin and PCB exposure. *Molecular Nutrition Food Research*. 2009; 53:1438–1451. [PubMed: 19842105]
- La Merrill M, Emond C, Kim MJ, Antignac JP, Le Bizet B, Clement K, et al. Toxicological function of adipose tissue: focus on persistent organic pollutants. *Environmental health perspectives*. 2013; 121:162–9. [PubMed: 23221922]
- Miao J, Ling AV, Manthena PV, Gearing ME, Graham MJ, Crooke RM, et al. Flavin-containing monooxygenase 3 as a potential player in diabetes-associated atherosclerosis. *Nature communications*. 2015; 6:6498.
- Miller CA, Corbin KD, da Costa KA, Zhang S, Zhao X, Galanko JA, et al. Effect of egg ingestion on trimethylamine-n-oxide production in humans: A randomized, controlled, dose-response study. *Am J Clin Nutr*. 2014; 100:778–786. [PubMed: 24944063]
- Missailidis C, Hallqvist J, Qureshi AR, Barany P, Heimbürger O, Lindholm B, et al. Serum trimethylamine-n-oxide is strongly related to renal function and predicts outcome in chronic kidney disease. *PloS one*. 2016; 11:e0141738. [PubMed: 26751065]
- Pavuk M, Olson JR, Wattigney WA, Dutton ND, Sjodin A, Shelton C, et al. Predictors of serum polychlorinated biphenyl concentrations in Anniston residents. *Sci Total Environ*. 2014; 496:624–634. [PubMed: 25115605]
- Perkins JT, Petriello MC, Newsome BJ, Hennig B. Polychlorinated biphenyls and links to cardiovascular disease. *Environmental science and pollution research international*. 2016; 23:2160–2172. [PubMed: 25877901]
- Petriello MC, Newsome BJ, Dziubla TD, Hilt JZ, Bhattacharyya D, Hennig B. Modulation of persistent organic pollutant toxicity through nutritional intervention: Emerging opportunities in biomedicine and environmental remediation. *The Science of the total environment*. 2014; 491-492:11–16. [PubMed: 24530186]
- Petriello MC, Hoffman JB, Sunkara M, Wahlang B, Perkins JT, Morris AJ, et al. Dioxin-like pollutants increase hepatic flavin containing monooxygenase (fmo3) expression to promote synthesis of the proatherogenic nutrient biomarker trimethylamine n-oxide from dietary precursors. *The Journal of nutritional biochemistry*. 2016; 33:145–153. [PubMed: 27155921]
- Pieterse B, Felzel E, Winter R, van der Burg B, Brouwer A. PAH-CALUX, an optimized bioassay for AhR-mediated hazard identification of polycyclic aromatic hydrocarbons (PAHs) as individual compounds and in complex mixtures. *Environ Sci Technol*. 2013; 47:11651–11659. [PubMed: 23987121]
- Potera C. Pops and gut microbiota: Dietary exposure alters ratio of bacterial species. *Environmental health perspectives*. 2015; 123:A187. [PubMed: 26131758]

- Randrianarisoa E, Lehn-Stefan A, Wang X, Hoene M, Peter A, Heinzmann SS, et al. Relationship of serum trimethylamine n-oxide (tmao) levels with early atherosclerosis in humans. *Scientific reports*. 2016; 6:26745. [PubMed: 27228955]
- Rohrmann S, Linseisen J, Allenspach M, von Eckardstein A, Muller D. Plasma concentrations of trimethylamine-n-oxide are directly associated with dairy food consumption and low-grade inflammation in a german adult population. *J Nutr*. 2016; 146:283–289. [PubMed: 26674761]
- Romano KA, Vivas EI, Amador-Noguez D, Rey FE. Intestinal microbiota composition modulates choline bioavailability from diet and accumulation of the proatherogenic metabolite trimethylamine-n-oxide. *mBio*. 2015; 6:e02481. [PubMed: 25784704]
- Schiattarella GG, Sannino A, Toscano E, Giugliano G, Gargiulo G, Franzone A, et al. Gut microbe-generated metabolite trimethylamine-N-oxide as cardiovascular risk biomarker: a systematic review and dose-response meta-analysis. *Eur Heart J*. 2017; 38:2948–2956. [PubMed: 29020409]
- Schugar RC, Brown JM. Emerging roles of flavin monooxygenase 3 in cholesterol metabolism and atherosclerosis. *Current opinion in lipidology*. 2015; 26:426–431. [PubMed: 26218418]
- Seldin MM, Meng Y, Qi H, Zhu W, Wang Z, Hazen SL, et al. Trimethylamine n-oxide promotes vascular inflammation through signaling of mitogen-activated protein kinase and nuclear factor-kappab. *J Am Heart Assoc*. 2016; 5
- Senthong V, Li XS, Hudec T, Coughlin J, Wu Y, Levison B, et al. Plasma trimethylamine n-oxide, a gut microbe-generated phosphatidylcholine metabolite, is associated with atherosclerotic burden. *Journal of the American College of Cardiology*. 2016; 67:2620–2628. [PubMed: 27256833]
- Shafi T, Powe NR, Meyer TW, Hwang S, Hai X, Melamed ML, et al. Trimethylamine n-oxide and cardiovascular events in hemodialysis patients. *J Am Soc Nephrol*. 2016
- Shih DM, Wang Z, Lee R, Meng Y, Che N, Charugundla S, et al. Flavin containing monooxygenase 3 exerts broad effects on glucose and lipid metabolism and atherosclerosis. *Journal of lipid research*. 2015; 56:22–37. [PubMed: 25378658]
- Silverstone AE, Rosenbaum PF, Weinstock RS, Bartell SM, Foushee HR, Shelton C, et al. Polychlorinated biphenyl (pcb) exposure and diabetes: Results from the anniston community health survey. *Environmental health perspectives*. 2012; 120:727–732. [PubMed: 22334129]
- Stubbs JR, House JA, Ocque AJ, Zhang S, Johnson C, Kimber C, et al. Serum trimethylamine-n-oxide is elevated in ckd and correlates with coronary atherosclerosis burden. *J Am Soc Nephrol*. 2016; 27:305–313. [PubMed: 26229137]
- Suzuki T, Heaney LM, Bhandari SS, Jones DJ, Ng LL. Trimethylamine n-oxide and prognosis in acute heart failure. *Heart*. 2016; 102:841–848. [PubMed: 26869641]
- Tang WH, Wang Z, Levison BS, Koeth RA, Britt EB, Fu X, et al. Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. *The New England journal of medicine*. 2013; 368:1575–1584. [PubMed: 23614584]
- Tang WH, Wang Z, Li XS, Fan Y, Li DS, Wu Y, et al. Increased trimethylamine n-oxide portends high mortality risk independent of glycemic control in patients with type 2 diabetes mellitus. *Clinical Chemistry*. 2016; 63:1.
- Tuomisto JT, Pohjanvirta R, Unkila M, Tuomisto J. TCDD-induced anorexia and wasting syndrome in rats: effects of diet-induced obesity and nutrition. *Pharmacology, biochemistry, and behavior*. 1999; 62:735–42.
- Turner W, DiPietro E, Lapeza C, Green V, Gill J, Patterson DG Jr. A fast universal automated cleanup system for the isotope-dilution high-resolution mass spectrometric analysis of PCDDs, PCDFs, coplanar PCBs, PCB congeners, and persistent pesticides from the same serum sample. *Organohalogen Compounds*. 1997; 31:26–31.
- Ussher JR, Lopaschuk GD, Arduini A. Gut microbiota metabolism of l-carnitine and cardiovascular risk. *Atherosclerosis*. 2013; 231:456–461. [PubMed: 24267266]
- Van den Berg M, Birnbaum LS, Denison M, De Vito M, Farland W, Feele M, et al. The 2005 World Health Organization reevaluation of human and Mammalian toxic equivalency factors for dioxins and dioxin-like compounds. *Toxicological Sciences*. 2006; 93:223–41. [PubMed: 16829543]
- Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS, Dugar B, et al. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature*. 2011; 472:57–63. [PubMed: 21475195]

- Wang Z, Levison BS, Hazen JE, Donahue L, Li XM, Hazen SL. Measurement of trimethylamine-N-oxide by stable isotope dilution liquid chromatography tandem mass spectrometry. *Analytical biochemistry*. 2014; 455:35–40. [PubMed: 24704102]
- Wang Z, Roberts AB, Buffa JA, Levison BS, Zhu W, Org E, et al. Non-lethal inhibition of gut microbial trimethylamine production for the treatment of atherosclerosis. *Cell*. 2015; 163:1585–95. [PubMed: 26687352]
- Warrier M, Shih DM, Burrows AC, Ferguson D, Gromovsky AD, Brown AL, et al. The tmao-generating enzyme flavin monooxygenase 3 is a central regulator of cholesterol balance. *Cell Rep*. 2015
- Zeisel SH, Mar MH, Howe JC, Holden JM. Concentrations of choline-containing compounds and betaine in common foods. *J Nutr*. 2003; 133:1302–1307. [PubMed: 12730414]
- Zhang L, Nichols RG, Correll J, Murray IA, Tanaka N, Smith PB, et al. Persistent organic pollutants modify gut microbiota-host metabolic homeostasis in mice through aryl hydrocarbon receptor activation. *Environmental health perspectives*. 2015; 123:679–688. [PubMed: 25768209]
- Zheng H, Yde CC, Clausen MR, Kristensen M, Lorenzen J, Astrup A, et al. Metabolomics investigation to shed light on cheese as a possible piece in the french paradox puzzle. *Journal of agricultural and food chemistry*. 2015; 63:2830–2839. [PubMed: 25727903]

Highlights

- Trimethylamine-N-oxide (TMAO) is a diet and gut microbiota-derived metabolite that has been linked to cardiovascular disease risk in human studies and animal models.
- TMAO concentrations in archived serum samples from the Anniston Community Health Survey (ACHS-II) were measured, and TMAO was significantly positively associated with 22 indexes of dioxin-like pollutant exposures (TEQs).
- Total dioxins TEQ was significantly associated with TMAO among females (except at high BMIs) but not among males.
- Total dioxins TEQ was still significantly associated with TMAO even after adjustment for multiple covariates known to alter or associate independently with TMAO.

Table 1

Demographic information.

Total Sample (n=333) ^a	
Age (Mean + SD)	62.7 + 13.0
Sex	
Male	Total; 91 (27.3%)
BMI=Underweight ^b	0 (0%)
BMI=Normal	15 (4.5%)
BMI=Overweight	31 (9.3%)
BMI=Obese	36 (10.8%)
BMI=Morbidly Obese	8 (2.4%)
Female	Total; 242 (72.7%)
BMI=Underweight	1 (0.3%)
BMI=Normal	49 (14.7%)
BMI=Overweight	65 (19.5%)
BMI=Obese	88 (26.4%)
BMI=Morbidly Obese	39 (11.7%)
Race	
White	162 (48.8%)
Black	170 (51.2%)
Glucose Median (Q1, Q3)	88 (79, 104) mg/dL
Insulin Median (Q1, Q3)	276 (188, 480) pmol/L
Triglycerides Median (Q1, Q3)	110 (79, 157) mg/dL
VLDL cholesterol Median (Q1, Q3)	22, (16, 31) mg/dL
Body mass index (Mean + SD)	31.7 + 8.1
TEQ Total Median (Q1, Q3)	18.0 (16.0, 31.2)
Self-reported diabetes	
Yes	77 (23.5%)
No	251 (76.5%)
Self-reported stroke	
Yes	28 (8.5%)
No	301 (91.5%)
Self-reported kidney disease	
Yes	24 (7.3%)
No	303 (92.7%)
Use of antimicrobial medications	
Yes	17 (5.1%)
No	316 (94.9%)
Consumption of local dairy	
Yes	109 (33.3%)
No	218 (66.7%)

^aSample size may be less for some variables due to missing or suppressed data. Individuals missing TMAO or sex information were altogether excluded from analysis. VLDL – very low density lipoprotein cholesterol

^bUnderweight (BMI<18.5), Normal (BMI between 18.5–24.9), Overweight (BMI between 25.0 and 29.9), Obese (BMI between 30.00–39.9), and Morbidly Obese (BMI ≥40.00)

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 2

Bivariate associations of dioxin-like pollutant exposures and covariates of interest with TMAO.

TEQ index or Covariate	Pearson or point-biserial correlation	p-value
TEQ Mixtures		
sum of non-ortho PCBs TEQ	0.157	0.004
sum of PCDDs TEQ	0.254	<.001
Sum of PCDDs, PCDFs, and coplanar PCBs TEQ	0.215	<.001
sum of PCDFs TEQ	0.214	<.001
sum of mono-ortho PCBs TEQ	0.174	0.001
Total dioxins TEQ	0.207	<.001
Individual TEQs		
2,3,7,8- TCDD TEQ	0.232	<.001
1,2,3,7,8- PCDD TEQ	0.209	<.001
1,2,3,4,7,8- HCDD TEQ	0.272	<.001
1,2,3,6,7,8- HCDD TEQ	0.296	<.001
1,2,3,7,8,9- HCDD TEQ	0.248	<.001
1,2,3,4,6,7,8- HCDD TEQ	0.090	0.105
OCDD TEQ	0.145	0.008
2,3,4,7,8- PCDF TEQ	0.231	<.001
1,2,3,4,7,8- HCDF TEQ	0.232	<.001
1,2,3,6,7,8- HCDF TEQ	0.257	<.001
2,3,4,6,7,8- HCDF TEQ	-0.014	0.795
1,2,3,4,6,7,8- HCDF TEQ	-0.067	0.228
PCB105 TEQ	0.133	0.015
PCB114 TEQ	0.160	0.005
PCB118 TEQ	0.157	0.004
PCB123 TEQ	0.093	0.116
PCB126 TEQ	0.146	0.008
PCB156 TEQ	0.157	0.004
PCB157 TEQ	0.144	0.008
PCB167 TEQ	0.158	0.004
PCB169 TEQ	0.175	0.001
PCB189 TEQ	0.105	0.057
Covariate of Interest		
Sex	0.026	0.631
Self-reported diabetes	-0.326	<0.001
BMI	0.045	0.415
Consumption of local dairy	0.106	0.056
Age	0.220	0.001
Race	-0.161	0.003

TEQ index or Covariate	Pearson or point-biserial correlation	p-value
Use of antimicrobial medications	0.028	0.613
Self-reported kidney disease	-0.174	0.002
Glucose (log)	0.192	<0.001
Insulin (log)	0.016	0.765
Self-reported stroke	-0.121	0.028
Triglycerides (log)	0.121	0.027
VLDL cholesterol (log)	0.120	0.029

PCB=Polychlorinated biphenyl; PCDD=Polychlorinated dibenzo-dioxin;

PCDF=Polychlorinated dibenzo-furan; HCDD=Hexachloro dibenzo-dioxin;

HCDF=Hexachloro dibenzo-furan; TCDD=Tetrachloro dibenzo-dioxin.

All TEQ values are calculated from lipid adjusted pollutant concentrations.

All TMAO and TEQ values have been transformed by the function $f(x) = \log(1+x)$.

Race was coded 1=white, 2=black.

Bold p-values are compatible with false discovery rate < 0.05 after modified Benjamini-Hochberg correction; italicized p-values are compatible with false discovery rate < 0.10. **For all self-reported variables, a negative bold correlation corresponds to a significant positive association (because questions were coded as 1=yes, 2= no).**

Table 3A

Multivariate model investigating adjusted associations of total dioxin TEQ with TMAO (main effects)

Covariate or interaction	Estimated regression coefficient	Standard Error	p-value
Intercept	3.187	0.366	<.001
Total dioxin TEQ (log) (E)	0.148	0.044	.001
Sex (S)	−0.041	0.073	.572
Diabetes (D)	−0.423	0.078	<.001
BMI	0.001	0.004	.794
Race (R)	−0.284	0.065	<.001
Kidney disease (K)	−0.340	0.124	.007

Table 3B

Multivariate model of adjusted associations of total dioxin TEQ with TMAO with interactions

Covariate or interaction	Estimated regression coefficient	Standard Error	p-value
Intercept	3.239	0.798	<.0001
Total dioxin TEQ (log) (E)	0.111	0.252	0.664
Sex (S)	−0.817	0.314	0.0097
Exposure/sex interaction	0.270	0.111	0.0151
Diabetes (D)	−0.437	0.077	<.0001
BMI	0.043	0.015	0.0052
Exposure/BMI interaction	−0.014	0.005	0.0053
Race (R)	−0.274	0.064	<.0001
Kidney disease (K)	−0.373	0.122	0.0025

The estimates above define a formula for the prediction of log-transformed TMAO. For example, in Table 3B, One begins with 3.239, adds 0.110 per unit of log-transformed total dioxin TEQ, subtracts 0.817 for males or twice 0.817 for females, adds 0.270 per unit of log-transformed total dioxin TEQ for males or twice 0.270 per unit of log-transformed total dioxin TEQ for females, etc. Predictive equation = $3.239 + E \{0.110 + 0.270 S - 0.014 \text{ BMI}\} - 0.817 S - 0.437 D + 0.043 \text{ BMI} - 0.274 R - 0.373 K$. For variable “Sex”, 1=male, 2=female; For variable “Race”, 1=white, 2=black; For variables “Diabetes” and “Kidney disease”, 1=yes, 2=no (n=320). $R^2 = 0.2292$

Table 4

Estimates of adjusted dioxin total TEQ/TMAO associations in various strata, as determined by multivariate regression modeling with interactions.

Strata	Estimate of adjusted dioxin Total TEQ/TMAO association	Standard error	p-value
BMI=20, Male	.109	.117	.353
BMI=30, Male	-.026	.102	.796
BMI=40, Male	-.162	.109	.137
BMI=20, Female	.379	.083	<.001
BMI=30, Female	.244	.051	<.001
BMI=40, Female	<i>.108</i>	<i>.055</i>	<i>.051</i>

The multivariate regression model – which also adjusted for diabetes, race, and kidney disease explained 22.3% of the variation in the outcome. The estimates above are amounts by which the predicted value of log-transformed TMAO changes if log-transformed dioxin total TEQ increases by one unit, with everything else in the model held constant. Because there are interactions of dioxin with BMI and gender, for any combination of BMI and gender, there is a possibly different association of dioxin with TMAO. For example, the association of dioxin with TMAO is significant ($p < .001$) among females with Normal BMI (i.e., BMI=20) but not among males with BMI=20 ($p = .353$) and not quite among morbidly obese females (i.e., BMI=40; $p = .051$). The “obese” strata represents an individual with BMI=30. Please see Table 1 for BMI strata sample sizes. Moreover, the numbers in the second column, including .379 and .244, are obtained by the formula $0.110 + 0.270 S - 0.014 \text{ BMI}$ which can be derived from Table 3. Bold p-values denote $p < 0.05$; italicized p-values denote $0.05 < p < 0.10$.