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Is H. pylori an endogenous source of diethyl phthalate in humans?

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Abstract

Monoethyl phthalate (MEP) is a metabolite used to assess exposure to diethyl phthalate (DEP). *Helicobacter Pylori* (*HP*) has been shown to produce DEP in laboratory studies. We used NHANES 1999-2000 data for 1623 adults to investigate whether *HP* seropositivity was associated with MEP levels. MEP levels were higher in individuals with *HP* seropositivity (p=0.0237), however the association differed by race. These results suggest that *HP* may be an endogenous source of DEP in some populations.

Keywords

Diethyl phthalate; Monoethyl phthalate; Helicobacter pylori; NHANES; microbiome

Phthalates are commonly used chemicals that result in widespread human exposure. Diethyl phthalate (DEP) exposure is known to occur through use of consumer personal care products, such as deodorants, perfumes, lotion, and several types of cosmetics (CDC, 2013). The highest content of DEP is in fragrances, with DEP concentration as high as 25-50% by volume of the fragrance (Registry, 1995). While DEP exposures are generally regarded as starting outside the body, there is the potential for endogenous sources of exposure. Laboratory scientists have demonstrated that *Helicobacter Pylori* (*HP*) produces DEP as a chemotactic factor (Keire et al., 2001). The authors postulated that DEP produced by *HP* may contribute to higher levels of DEP metabolites in human urine. *HP* is a common bacterium found in the stomach with a prevalence of approximately 30% in the United States adult population (Epplein et al., 2011). Given that *HP* is common bacterial infection, we investigated whether individuals with *HP* seropositivity had higher levels of MEP, the primary metabolite of DEP.

We used data from the National Health and Nutrition Examination Survey (NHANES) 1999-2000 to evaluate the association between *HP* seropositivity and MEP concentration. The NHANES is a population-based national sample containing extensive demographic, medical, and laboratory data for a statistical random sample of the non-institutionalized United States population (CDC, 2014a). We limited the study to adults (age ≥ 18 years)

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because the seroprevalence of HP infection is relatively consistent over all age groups. Only participants with complete information of MEP and *HP* were analyzed.

All biological samples were collected the day of the clinic visit. MEP levels were measured in spot urine samples by reversed-phase high-performance liquid chromatography (HPLC) and atmospheric-pressure chemical ionization tandem mass spectrometry (APCI-MS/MS). Creatinine was measured by the Jaffé rate reaction (CDC, 2014b). *HP* status was determined in serum through the use of an enzyme linked immunosorbent assay (ELISA). The ELISA was used to determine the presence of *HP* in human serum by exposing the serum to a specific antigen, strain ATCC 43504. The concentration of the antibody in the serum binding the antigen was then detected spectrophotometrically and reported as optical density (CDC, 2008). We used a previously defined cut-point to establish HP seropositivity; positive *HP* status was considered > 0.90, while negative status was ≤ 0.90 (Chen and Blaser, 2012).

We used linear regression to evaluate association between MEP levels and *HP* status. MEP concentration was creatinine-adjusted to correct for urinary dilution and log transformed as the distribution was right skewed. In addition to the crude model, we also adjusted the model for potential confounders, including age (18-39, 40-59, > 60 years), gender, body mass index (BMI, < 25 or ≥25 kg/m^2), race/ethnicity (Mexican American, Non-Hispanic White, Non-Hispanic Black, and Other ethnicity), education status (less than high school, high school diploma/GED, more than high school), and urinary cotinine levels (current smoker > 10 ng/mL, non-smoker ≤ 10 ng/mL). Factors not significantly associated with MEP levels or that did not change the association with *HP* were not included in the final model. Our final model included gender, BMI, race/ethnicity, as well as an interaction term between race/ethnicity and *HP*. We used least square mean values of MEP to account for the impact of model covariates. All statistical analysis was done using SAS JMP Pro 10 (Cary, NC).

A total of 1623 individuals had complete data on *HP* and MEP. They ranged in age from 18 to 85 years (mean = 46, SD = 20) with 41% being *HP* seropositive (Table 1). Creatinine-adjusted MEP levels spanned four orders of magnitude, with a median of 114μg/g (25^{th} percentile = 50 μg/g, 75^{th} percentile = 297 μg/g). Women, individuals with higher BMI, and non-Hispanic Black and Other race/ethnic groups all had higher levels of MEP. In the unadjusted model, individuals who were *HP* seropositive had higher levels of MEP than those who were *HP* seronegative (21 ug/g higher, p-value =0.0196). Association with *HP* status remained significant (p = 0.0237) when gender and BMI were included. To account for potential differences in the association between *HP* status and MEP levels by race/ethnicity we used a four-level interaction term between race/ethnicity and *HP* status (Figure 1). Results of this analysis suggested that among Non-Hispanic Whites and Mexican Americans, there was a positive association between *HP* seropositivity and MEP levels; in contrast, the Non-Hispanic Blacks and Other race/ethnicity groups showed an inverse association with HP seropositivity (interaction p = 0.0954).

These results, although limited, suggest that HP infection may contribute to urinary MEP levels in humans. Most surprising is our different results by race/ethnicity. Even though non-Hispanic Blacks had higher levels of MEP, as well as higher prevalence of HP seropositivity, we observed an inverse association between MEP and HP seropositivity in this racial/ethnic

group, while for non-Hispanic Whites and Mexican Americans we saw the positive association that we hypothesized. Given the cross-sectional nature of this analysis and the lack of information regarding HP treatment and DEP sources, it is difficult to establish whether there is a difference in response by race/ethnicity, differential ELISA sensitivity by race/ethnicity, or rather a different pattern of DEP exposure by race/ethnicity groups. *HP* seropositivity was determined by the ELISA strain ATCC 43504 antigen, while the demonstration of DEP as a chemotactic factor was performed on *HP* strain ATCC 43579 (Keire et al., 2001). Both ATCC 43504 and ATCC 43579 are virulent *HP* strains that possess the cytotoxin associated gene (*cag*) pathogenicity island that further translates to CagA protein in host cells and responds to the ELISA assay used in this analysis (Keates et al., 2008; Vaucher et al., 2000). While CagA is a well-studied marker for *HP* infection status, it is only present in about 60% of *HP* strains in the United States. CagA prevalence varies by race in the United States: Whites (50%), Blacks (79%), and Hispanic (64%) (Epplein et al., 2011; Parsonnet et al., 1997); we could not find information on the prevalence of the different *HP* strains by race. According to the World Gastroenterology Organization Global Guidelines, variation in virulence is associated with different strains of *HP*, which yields variance in expression by age, ethnicity, gender, geography, and socioeconomic status ((Organization, 2010), page 3);. Additionally, infection treatment history was not reported at the time of data collection. Thus, we may have some exposure misclassification if those with active disease are categorized along with those with some small grade infection. Other investigators have shown that ethnic groups use varying amounts/types of cosmetics, which may influence urinary phthalate levels (Romero-Franco et al., 2011). We used a large population-based study with good ascertainment of MEP concentration and *HP* status as well as detailed information on important covariates. Future studies should focus on individuals with active disease and measurements during and following the course of treatment to better understand the potential impact of HP infection on MEP levels. As MEP is the phthalate metabolite measured at highest concentrations in all populations, it is important to understand all potential sources, including those arising from an individual's microbiome.

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Abbreviations

APCI-MS/MS	Atmospheric-pressure chemical ionization tandem mass spectrometry
BMI	Body Mass Index
cag	Cytotoxin-associated gene
CagA	Cytotoxin-associated gene A product
CDC	Centers for Disease Control and Prevention
DEP	Diethyl Phthalate
ELISA	Enzyme Linked Immunosorbent Assay
HP	*Helicobacter Pylori*
HPLC	High-performance liquid chromatography
MEP	Monoethyl Phthalate
NHANES	National Health and Nutrition Examination Survey
SD	Standard Deviation

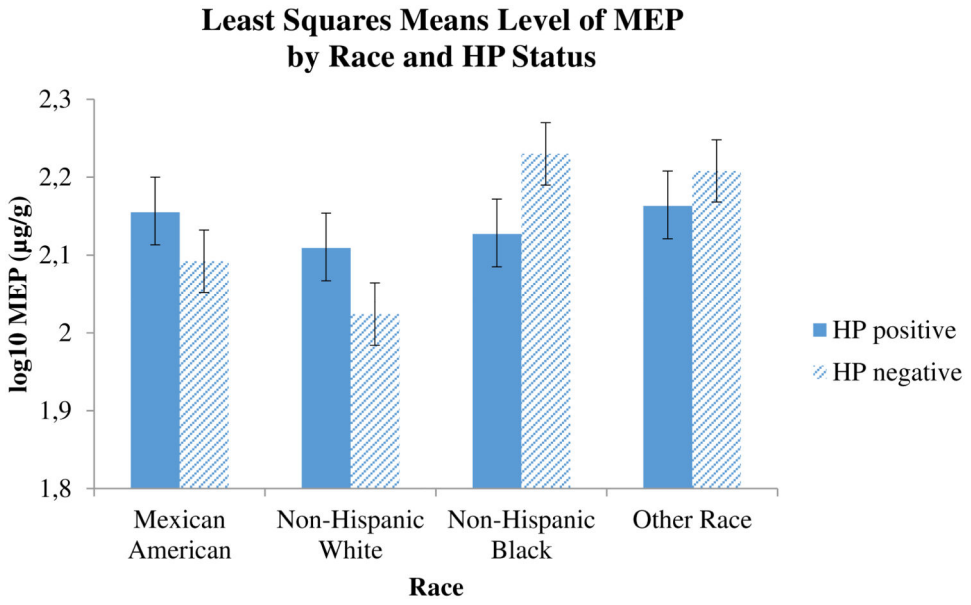

Figure 1.

Comparison of varying levels of MEP by race and HP status. $R^2 = 0.014$, interaction p-value = 0.095, race p-value = 0.027*.[1]

[1] **Parameter Estimates:**

Log_{10} MEP = 2.14 - 0.015 β_1 + 0.047 β_2 + 0.040 β_3 - 0.072 β_4 + 0.031 β_1 **HP* - 0.022 β_2 **HP* - 0.052 β_3 **HP*+ 0.043 β_4 **HP*

β_1 = Mexican American, β_2 = Other race/ethnicity, β_3 = Non-Hispanic Black,β_4= Non-Hispanic White. Non-Hispanic White was used as a control group.

Table 1

Demographic and lifestyle factors and creatinine-adjusted mono-ethyl phthalate (MEP) concentrations for 1623 adult NHANES 1999-2000 participants.

Demographic and Lifestyle Factors	Number of Subjects (N = 1623)	HP^+ (%)	Mean MEP level (μg/g), SD
HP			
Positive	663 (41%)		138 (1.05)
Negative	960 (59%)		117 (1.02)
Age			
18-39	709 (44%)	31	123 (1.05)
40-59	413 (25%)	46	135 (1.05)
≥ 60	501 (31%)	50	123 (1.05)
Gender			
Female	862 (53%)	39	141 (1.05)
Male	761 (47%)	43	112 (1.05)
Body mass index			
< 25	557 (35%)	36	110 (1.05)
≥ 25	1066 (65%)	43	138 (1.05)
Race/ethnicity			
Mexican American	467 (29%)	59	135 (1.05)
Non-Hispanic Black	309 (19%)	54	148 (1.07)
Non-Hispanic White	690 (42%)	21	110 (1.07)
Other	157 (10%)	47	151 (1.10)
Education status			
Less than high school	636 (39%)	60	138 (1.05)
High school diploma (including GED)	381 (24%)	32	123 (1.07)
More than high school	601 (37%)	26	117 (1.05)
Missing data	5 (0.30%)		
Cotinine			
Current smoker (> 10 ng/mL)	383 (24%)	44	129 (1.02)
Non-smoker (≤ 10 ng/mL)	1240 (76%)	40	123 (1.07)