Occupational Diseases and Environmental Medicine, 2020, 8, 111-122
https://www.scirp.org/journal/odem
ISSN Online: 2333-357X
ISSN Print: 2333-3561

Arsenic Exposure and Association with Hepatitis E IgG Antibodies

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How to cite this paper: Rahman, H.H., Niemann, D. and Singh, D. (2020) Arsenic Exposure and Association with Hepatitis E IgG Antibodies. *Occupational Diseases and Environmental Medicine*, **8**, 111-122.
https://doi.org/10.4236/odem.2020.83009

Received: July 24, 2020
Accepted: August 22, 2020
Published: August 25, 2020

Abstract

Arsenic is a known toxic chemical, has immuno-modulatory properties, and can change the susceptibility of infection in humans. Acute hepatitis E is an infectious disease; it can be self-limiting, but in severe cases, can cause acute-on-chronic liver failure. The presence of IgG anti-HEV (hepatitis E IgG antibody) in blood represents a past hepatitis E infection in an individual. Very few studies have investigated the association between arsenic levels and hepatitis E seroconversion in humans. The primary objective of this study was to assess the relationship between total urinary arsenic and speciated urinary arsenic (urinary arsenous acid, urinary arsenic acid, urinary arsenobetaine, urinary arsenocholine, urinary dimethylarsinic acid, urinary monomethylarsonic acid) and the presence of IgG anti-HEV. The 2011-2012, 2013-2014, and 2015-2016 National Health and Nutrition Examination Survey (NHANES) III data sets were analyzed, with participants aged 20 years and older (n = 7061). We used weighted logistic regression to determine the association between total and speciated urinary arsenic concentrations and IgG anti-HEV. For each analyte considered, a separate weighted logistic regression model was fitted. Each of these models regressed log-transformed analyte levels on the log-odds of the presence of IgG Anti-HEV. To evaluate the relationships between the urinary arsenic measurements, pairwise Pearson correlation coefficients were determined for each of the urinary arsenic measurements. Of the human subjects included, 6628 (93.9%) were negative for IgG Anti-HEV while 433 (6.1%) were positive for IgG anti-HEV. Total urinary arsenic was associated with 1.161 times greater odds of IgG anti-HEV (95% CI: [1.035, 1.303]) for each unit increase in concentration on a log-scale. For speciated urinary arsenic measurements, the odds ratios and 95% CI's were: arsenobetanine 1.168 [1.075, 1.270], arsenocholine 1.201 [0.944, 1.529], dimethylarsinic acid 1.183 [1.009, 1.386], monomethylacrsonic acid 1.095

[0.915, 1.310], aresnous acid 0.907 [0.762, 1.079], and arsenic acid 1.951 [0.905, 4.209]. Our analysis indicates that total urinary arsenic, arsenobetanine, and dimethylarsinic acid are significantly associated with the odds of the presence of IgG anti-HEV. Future prospective studies are required to evaluate the association between hepatitis E infection and arsenic exposures.

Keywords

Total Arsenic, Speciated Urinary Arsenic, Hepatitis E, IgG Anti-HEV, NHANES

1. Introduction

Arsenic (As) is a naturally occurring element and known carcinogen linked to bladder, skin, and lung cancers in addition to ischemic heart disease, skin lesions, and depression [1] [2]. It is estimated that 4.8% of domestic well users or 2.1 million people have well arsenic levels greater than the US Environmental Protecting Agency (EPA) regulation (>10 μg/L) [3]. In populations who do not rely on well water, dietary arsenic exposure is the primary concern, particularly from rice and apple juice. Workers have also been linked to increased rates of lung and bladder cancer due to a co-exposure of arsenic and wood dust, asbestos, and silica [1].

Hepatitis E is a non-enveloped positive strand RNA virus that often causes an acute, self-limiting disease. Hepatitis E is spread fecal-orally such as through contaminated water [4]. In developed countries, it can cause chronic infection particularly in organ transplant recipients, patients with HIV, and those requiring chemotherapy for hematological malignancies. Beyond liver cirrhosis, hepatitis E can cause neurological disorders, kidney injury, pancreatitis, and hematological disorders [5]. Hepatitis E can be asymptomatic or cause acute fulminant hepatitis; Hepatitis E Virus (HEV) is the leading global cause of acute viral hepatitis [4].

This study taking place in the United States (US) will focus on hepatitis E in developed countries. IgG anti-HEV is used to test for HEV in human serum or plasma [6]. Both IgG and IgM antibodies can be used to detect different stages in the course of an acute hepatitis E infection. IgG antibodies, used in this study, increase during the recovery phase. During this period of viral clearance, levels of IgM antibodies decrease and become undetectable. IgG anti-HEV persists long term for years, and possibly for life [7]. Anti-HEV IgG is useful for assessing the prevalence of HEV in a population as it develops early on after clinical onset and is long lasting to determine past exposure to HEV [8].

The National Health and Nutrition Examination Survey (NHANES) found a 6.6% seroprevalence of HEV in the general US population between 2009 and 2012 [9]. Risk factors in the US include increasing age, Hispanic population, meat consumption, and birth outside the US. Previously, HEV was considered uncommon in developed countries, with occasional cases attributed to travel.

However, now it is understood that cases are acquired locally through contact or exposure to animals or areas contaminated with runoff from pig farms [10]. Although HEV in developing countries is known to cause millions of cases per year, the epidemiology of HEV in developed countries is relatively unknown [11].

Knowledge Gap

Heaney *et al.* [4] reported a connection between arsenic exposure during pregnancy and an enhanced susceptibility to HEV in rural Bangladesh. Several studies have been conducted surrounding HEV in pregnant women, but few have studied the general population. In the US, in utero environmental arsenic exposure was shown to alter the fetal immune system and cause immune dysregulation through altered lymphocyte profiles in newborns [12]. In the general population, arsenic exposure has been linked to higher rates of other types of hepatitis including hepatitis A and B [13] [14].

Chronic exposure to arsenic is associated with impaired immune responses, increasing the risk for infections, such as HEV. Arsenic affects both the adaptive and innate immune defenses, causing the host to become immunocompromised [15]. Although arsenic has been linked to an increased risk of infectious disease, there is limited literature linking arsenic and HEV infection. The relationship between HEV and arsenic in the US is currently unknown.

The primary objective of the current study was to determine the relationship between total urinary arsenic and IgG anti-HEV in the US population. As secondary analyses, the relationships between specific types of urinary arsenic (speciated arsenic) and the presence of IgG anti-HEV were evaluated.

2. Methods

2.1 Study Population

Participants in the study were selected as part of the National Health and Nutrition Examination Survey dataset. The National Health and Nutrition Examination Survey is a national study in the US to determine the health and nutritional status of children and adults. The sample includes non-institutionalized US civilians in all 50 states and Washington D.C., taking place through interviews and physical exams [16]. Three survey cycles: 2011-2012 (G), 2013-2014 (H), 2015-2016 (I) were combined in our data analysis, involving 7061 human subjects. Data from the following National Health and Nutrition Examination Survey files were used in the analysis: "Speciated Arsenics - Urine - Special Sample": UASS_H and UASS_I; "Arsenic - Total - Urine - Special Sample": UTASS_H and UTASS_I; and "Hepatitis E: IgG & IgM Antibodies": HEPE_G, HEPE_H, and HEPE_I. In the 2011-2012 (G) dataset, both total and speciated urinary arsenic samples were included in UASS_G ("Arsenics - Total & Speciated - Urine - Special Sample").

Urinary arsenic samples were collected from participants 18 years and older in the H and I datasets, and participants 20 years and older in the G dataset, who

met the one-third subsample selection criteria. Participants 18 years and older in H and I, and 20 years and older in G, who were not included in the one-third subsample, but who smoked at least 100 cigarettes in their lifetime and now smoke every day were included [17] [18] [19] [20] [21]. Hepatitis E samples were collected from examined participants ages 6 and older [22]. Demographic data for each data set was reported as: "Demographic Variables & Sample Weights": DEMO_G, DEMO_H, and DEMO_I [23] [24] [25]. The Research Ethics Review Board of the National Center for Health Statistics approved the National Health and Nutrition Examination Survey study [26].

2.2. Urinary Arsenic Assessment

Laboratory methods used by the National Health and Nutrition Examination Survey are listed in the "Arsenics - Speciated - Urine Laboratory Procedure Manual" [27]. Urine samples were collected from participants at Mobile Examination Centers (MECs) and shipped to the Division of Laboratory Sciences, National Center for Environmental Health at the CDC for analysis. Analytes with variable names ending in "LC" determined those that were below the limit of detection. "0" meant the result was at or above the limit of detection and "1" meant the result was below the limit of detection. The lower limit of detection (LLOD) for speciated arsenic was: 0.12 μg/L for urinary arsenous acid, 0.79 μg/L for urinary arsenic acid, 1.16 μg/L for urinary arsenobetaine, 0.11 μg/L for urinary arsenocholine, 1.91 μg/L for urinary dimethylarsinic acid, and 0.20 μg/L for urinary monomethylarsonic acid [18] [20] [21]. The LLOD for urinary total arsenic was 0.26 μg/L [17] [19] [21].

2.3. Hepatitis E Assessment

Detailed laboratory methods used for the sampling of IgG anti-HEV are listed in the "IgG Hepatitis E Antibody Laboratory Procedure Manual" [28]. The DSI DS-EIA-ANTI-HEV-G enzyme immunoassay kit was used to determine the presence of the IgG antibody to HEV in human serum. After collecting samples at MECs, specimens were sent to the Division of Viral Hepatitis, National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention at the CDC for analysis [22].

2.4. Statistical Analysis

Three survey cycles of National Health and Nutrition Examination Survey data (2011-2012, 2013-2014, 2015-2016) were combined in the analysis. Survey weights were adjusted according to the National Health and Nutrition Examination Survey documented methodology. The weights utilized were for the total urinary arsenic and speciated urinary arsenic subsamples. Utilizing the combined survey cycles and considering the subsampling that was conducted for urinary arsenic measurements, 7061 human subjects were included in the analysis.

The proportion of human subjects with measured analyte levels (e.g. urinary

arsenic acid) below the lower limit of detection was computed. For each analyte considered, a separate weighted logistic regression model was fitted. Each of these models regressed log-transformed analyte levels on the log-odds of the presence of IgG Anti-HEV. These models were fit utilizing the survey package in R, version 3.6.2, which allows for estimating survey-weighted generalized linear models [29]. All pairwise Pearson correlation coefficients were computed for each of the urinary arsenic (total and speciated) measurements.

2.5. Covariates

Covariates included sociodemographic factors such as sex (male, female), age (≥20 years), ethnicity (Mexican American, other Hispanic, Non-Hispanic White, Non-Hispanic White), body mass index (BMI), alcohol consumption (doesn't consume, currently consume), Poverty Index Ratio (PIR) (<1.3, 1.3 - 3.5, >3.5) and smoking status (every day, someday, not at all).

3. Results

Of the included human subjects, 6628 (93.9%) were negative for IgG Anti-HEV, while 433 (6.1%) had a positive test for IgG Anti-HEV. Significant differences in urinary total arsenic were observed by sex, age, ethnicity, smoking, and PIR status.

We observed significantly different levels of total urinary arsenic between males and females. **Table 1** shows that arsenic levels were significantly (p-value < 0.0001) lower in females (GM: 5.830, 95% CI: [5.419, 6.272]) compared to males (GM: 6.938, 95% CI: [6.469, 7.441]) which is about 16% lower. The level of total urinary arsenic significantly (p-value < 0.0001) increases with age. Each one-year increase in age was associated with a 1.004 times greater concentration of urinary arsenic with a 95% CI of [1.003, 1.006]. Compared to Mexican Americans, Non-Hispanic Blacks and Non-Hispanic Asians were observed to have significantly (p-values < 0.0001) higher arsenic levels. Among them, the Non-Hispanic Asian (GM: 14.218, 95% CI [12.932, 15.633]) had the highest level of total arsenic which was more than double (RR: 2.319, 95% CI [2.032, 2.647]) that of the Mexican Americans (GM: 6.130, 95% CI [5.630, 6.675]). The total arsenic level of Non-Hispanic Blacks (GM: 8.432, 95% CI [7.616, 9.335]) was about 38% higher compared to Mexican Americans. Based on the available data, an association between total arsenic level and BMI, alcohol consumption, and PIR status was not detected (p-value > 0.05 for all these analytes).

In **Figure 1**, the distribution of total urinary arsenic is shown by whether study participants were positive or negative for IgG Anti-HEV. This figure shows that the first quartile, median, and third quartile levels of total urinary arsenic were higher in study participants who were positive for IgG Anti-HEV compared to those who were negative.

The individual weighted logistic regression models for total and speciated arsenic are presented in **Table 2**. This table also presents the percentage of study participants for which measured values were below the limit of detection. For

Table 1. The relative ratio of urinary total arsenic concentration along with geometric means for categorical levels.

Characteristic	Relative Ratio (95% CI)	Geometric Mean (95% CI)	p-value
Sex			**<0.0001**
Male	1.000	6.938 (6.469, 7.441)	
Female	0.840 (0.785, 0.900)	5.830 (5.419, 6.272)	
Age	1.004 (1.003, 1.006)		**<0.0001**
Ethnicity			
Mexican American	1.000	6.130 (5.630, 6.675)	
Other Hispanic	1.238 (1.091, 1.405)	7.589 (6.820, 8.444)	0.002
Non-Hispanic White	0.908 (0.827, 0.997)	5.567 (5.162, 6.003)	0.05
Non-Hispanic Black	1.375 (1.217, 1.554)	**8.432 (7.616, 9.335)**	**<0.0001**
Non-Hispanic Asian	2.319 (2.032, 2.647)	**14.218 (12.932, 15.633)**	**<0.0001**
Other Race—Including Multi-Racial	0.934 (0.794, 1.100)	5.729 (4.983, 6.586)	0.42
BMI	1.004 (1.000, 1.008)		0.054
Alcohol			0.22
Doesn't consume	1.000	6.501 (6.069, 6.964)	
Currently consumes	1.060 (0.966, 1.164)	6.131 (5.568, 6.751)	
Current Smoking			
Every day	1.000	5.523 (4.976, 6.129)	
Some days	1.337 (1.117, 1.602)	**7.387 (6.146, 8.878)**	**0.003**
Not at all	1.235 (1.103, 1.382)	**6.820 (6.252, 7.439)**	**0.0006**
PIR Status			
<1.3	1.000	6.310 (5.962, 6.677)	
1.3 - 3.5	0.938 (0.873, 1.008)	5.918 (5.493, 6.376)	0.09
≥3.5	1.040 (0.952, 1.135)	6.560 (5.987, 7.188)	0.39

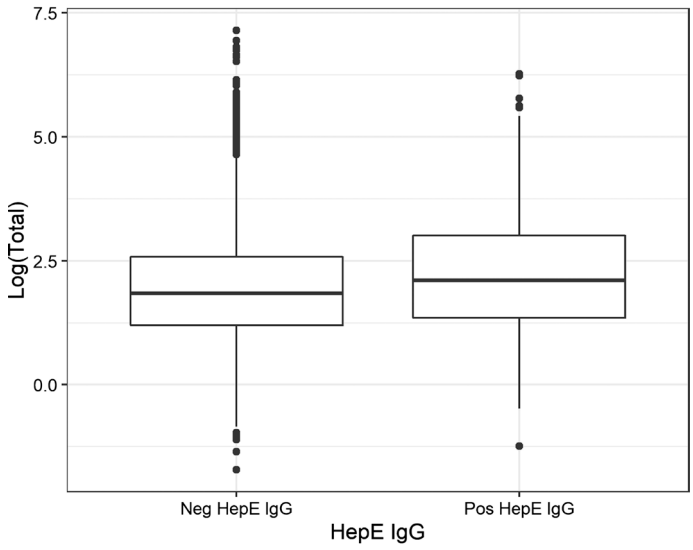

Figure 1. Boxplot showing the distribution of total urinary arsenic by whether study participants were positive or negative for IgG anti-HEV.

these participants, the lower limit of detection was imputed in the regression model. Total urinary arsenic, arsenobetanine, and dimethylarsinic acid were observed to be significantly associated with the odds of the presence of IgG anti-HEV. For example, when increasing by a log-unit in total urinary arsenic, the odds of the presence of IgG anti-HEV increased by a factor of 1.161 with a 95% confidence interval of [1.035, 1.303]. Similarly, a log-unit increase in arsenobetanine and dimethylarsinic acid, the IgG anti-HEV increased by the factors of 1.168 (95% CI [1.075, 1.270]) and 1.183 (95% CI [1.009, 1.386]), respectively. For other speciated arsenic, arsenous acid, arsenic acid, arsenocholine, and monomethylacrsonic we did not observe evidence of a significant association with presence of IgG anti-HEV antibodies.

Tables 3-5 show the 50th, 75th, and 90th percentile values of the measured analytes categorized by positive or negative for IgG anti-HEV antibodies. 90th percentile values were higher in the IgG anti-HEV positive individuals for total

Table 2. The individual weighted logistic regression models for total and speciated arsenic.

Individual model*	Below lower detection limit	Estimate	Odds Ratio (95% CI)	p-value
Total Arsenic Acid (μg/L)	0.0%	0.150	**1.161 (1.035, 1.303)**	**0.01**
Arsenous Acid (μg/L)	26.5%	−0.098	0.907 (0.762, 1.079)	0.28
Arsenic Acid (μg/L	67.8%	0.668	1.951 (0.905, 4.209)	0.10
Arsenobetaine (μg/L)	40.4%	0.156	**1.168 (1.075, 1.270)**	**0.0006**
Arsenocholine (μg/L)	57.9%	0.183	1.201 (0.944, 1.529)	0.14
Dimethylarsinic acid (μg/L)	5.9%	0.168	**1.183 (1.009, 1.386)**	**0.04**
Monomethylacrsonic acid (μg/L)	26.1%	0.091	1.095 (0.915, 1.310)	0.33

*Each analyte was log-transformed prior to model fitting.

Table 3. 50th percentile levels of each of the total and speciated arsenic.

IgG Anti-HEV	Total	Arsenous acid	Arsenic acid	Arsenobetaine	Arsenocholine	Dimethylarsinic acid	Monomethylacrsonic acid
Negative	6.31	0.34	0.56	0.84	0.08	3.42	0.63
Positive	8.21	0.34	0.56	1.55	0.08	3.815	0.63

Table 4. 75th percentile levels of each of the total or speciated arsenic.

IgG Anti-HEV	Total	Arsenous acid	Arsenic acid	Arsenobetaine	Arsenocholine	Dimethylarsinic acid	Monomethylacrsonic acid
Negative	13.14	0.67	0.62	4.29	0.20	5.84	0.80
Positive	20.27	0.64	0.62	8.12	0.20	7.20	0.90

Table 5. 90th percentile levels of each of the total or speciated arsenic.

IgG Anti-HEV	Total	Arsenous acid	Arsenic acid	Arsenobetaine	Arsenocholine	Dimethylarsinic acid	Monomethylacrsonic acid
Negative	30.16	0.99	0.62	17.41	0.20	10.20	1.28
Positive	49.25	1.03	0.62	28.75	0.31	13.78	1.52

arsenic and all arsenic species except for arsenic acid. While Table 2 and Table 3 show that median arsenic acid and arsenocholine cannot be compared between positive and negative IgG anti-HEV due to a high proportion of measurements reported as below the LOD; Table 4 and Table 5 allow for a comparison of percentiles that are above the LOD.

After adjusting for age, evidence of a relationship between total arsenic and IgG anti-HEV was substantially moderated. Specifically, while the unadjusted odds ratio for positive IgG anti-HEV was 1.161 (95% CI [1.035, 1.303]), for each unit increase in total arsenic on a log-scale after adjusting for age the odds ratio was 1.077 (95% CI [0.947, 1.225]). After adjusting for sex, BMI, and ethnicity, evidence of an association between total arsenic and IgG anti-HEV was further moderated, with an adjusted odds ratio of 1.018 (95% CI [0.890, 1.167]).

4. Discussion

This study observed an association between urinary arsenic and higher presence of hepatitis E IgG antibodies in the body. This association was observed for total arsenic, arsenobetaine, and dimethylarsinic acid levels in the body. Total arsenic in the urine can be used as an indicator of the amount of absorbed arsenic in an individual because the primary route of arsenic elimination is through the renal system [30]. Exposure to arsenic can occur through both organic and inorganic forms. Inorganic arsenic is of concern to human health as it is toxic and carcinogenic; organic arsenic has low toxicity to humans [31]. High urinary arsenic levels indicate exposure to toxic inorganic arsenic. The primary organ for arsenic metabolism is the liver, which metabolizes arsenic to form mono-methylated and dimethylated arsenic species [32].

Our study demonstrated that along with total arsenic, speciated arsenic (arsenobetaine and dimethylarsinic acid) are associated with higher IgG anti-HEV antibodies. In the US, arsenobetaine and dimethylarsinic acid are the major contributors to total urinary arsenic levels. Organic arsenic, particularly arsenobetaine, can be used as marker to estimate total arsenic levels [33]. Out of these two, dimethylarsinic acid levels are more significant since dimethylarsinic acid is more toxic to humans. Dimethylarsinic acid is formed after exposure to inorganic arsenic and has been shown to play a role in carcinogens in animal studies [34]. On the other hand, arsenobetaine, a speciated arsenic that occurs naturally in the marine environment such as seaweeds, is relatively non-toxic [35].

Hepatitis E is a commonly under-diagnosed disease that occurs worldwide [5]. HEV is commonly divided by genotypes based on developing and developed countries. Our study found total arsenic levels to be highest in non-Hispanic Asians and non-Hispanic Blacks as compared to non-Hispanic Whites. This may be due to higher chances of being exposed to arsenic, partly due to higher exposure and inefficient public health access to study populations born in foreign countries. Higher arsenic exposures in developing countries, especially in pregnant women, have been associated with increased susceptibility to HEV infec-

tions. In developing countries HEV is commonly due to poor sanitation and contaminated water, whereas in developed countries HEV is commonly transmitted zoonotically through infected pig meat. Although the disease is usually self-limiting, those with poor prognosis in developed countries include individuals who are immunocompromised or who have underlying chronic liver disease [5].

Acharya *et al.* [36] determined that the mortality of patients with cirrhosis who then contracted HEV was 70%. Chronic HEV has been linked with neurological manifestations including Guillain-Barré syndrome, Bell's palsy, neuralgic amyotrophy, acute transverse myelitis, and acute meningoencephalitis. HEV has also been linked to glomerular disease, causing membrano-proliferative and membranous glomerulonephritis in the kidneys. Furthermore, in acute HEV, aplastic anemia and thrombocytopenia have been reported [5]. One study [37] showed that chronic arsenic exposure increased the risk of hepatic and cardiovascular diseases in certain populations. According to another study [38], lag in T cell proliferation is one of the processes through which arsenic may inhibit adaptive T-cell immunity which later may result in tolerance to T-cell response following exposure to arsenic. This is particularly important when the body is exposed to infectious agents, including infection due to HEV, due to delayed T-cell proliferation that combined with inadequate innate immune response may result in higher susceptibility to HEV infection [38]. Our study signifies the importance of arsenic exposures in immunocompromised populations and the development of opportunistic infections.

The findings presented in this study are the first to find a link between arsenic and HEV in the general population and in a developed country (the US). Previously HEV was often associated with pregnant women or in developing countries, spread in contaminated water or due to poor sanitation. Our findings show that all individuals can be at risk for HEV, especially with exposure to arsenic. Arsenic exposure in the US is commonly through consumption of specific foods and arsenic contaminated water [1]. This association brings the issue of arsenic exposure to the forefront as a public health issue and is a risk to people in developed countries.

There are limitations to the current study. A primary limitation is that many of the study participants have speciated arsenic levels that are below the lower limit of detection. Due to the cross-sectional nature of this study, it cannot be ascertained that an increase in arsenic in the body would result in higher seroconversion rates of IgG antibodies. Another is the lower prevalence of hepatitis E infection rates in the US population. It is most prevalent in older populations that may incur other chronic infections that may lead to immunosuppression, varying the effects of arsenic exposure. However, abundance of studies showing arsenic effects on immune functions may support causation. Our study is supportive of an association between higher arsenic levels in the body and IgG antibodies. Studies that measure immune responses could be performed to under-

stand the significance of the immune response to arsenic exposure. Thus, future studies are required that measure the casualty between arsenic exposure and HEV infection.

5. Conclusion

Urinary arsenic acid may be related to hepatitis E infection in the US population. Previous literature suggests arsenic has a negative effect on the immune system, which likely increases the susceptibility of hepatitis E in humans. Total urinary arsenic as well as both organic and inorganic arsenic species were linked to higher odds of hepatitis E. Further studies on arsenic toxicity and hepatitis E infection should be considered.

Acknowledgements

Thank you to Dr. Patrick Trainor for his work on the data analysis of this paper.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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