Environmental Research 149 (2016) 206–215

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Environmental and personal determinants of the uptake of disinfection by-products during swimming

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ARTICLE INFO

Article history:
Received 22 December 2015
Received in revised form
20 April 2016
Accepted 9 May 2016

Keywords:
Biomarkers
Disinfection by-products
DBPs
Haloacetic acids
Internal dose
Swimming pools
Trihalomethanes
THMs

ABSTRACT

Background: Trihalomethanes (THMs) in exhaled breath and trichloroacetic acid (TCAA) in urine are internal dose biomarkers of exposure to disinfection by-products (DBPs) in swimming pools.
Objective: We assessed how these biomarkers reflect the levels of a battery of DBPs in pool water and trichloramine in air, and evaluated personal determinants.
Methods: A total of 116 adults swam during 40 min in a chlorinated indoor pool. We measured chloroform, bromodichloromethane, dibromochloromethane and bromoform in exhaled breath and TCAA in urine before and after swimming, trichloramine in air and several DBPs in water. Personal determinants included sex, age, body mass index (BMI), distance swum, energy expenditure, heart rate and 12 polymorphisms in *GSTT1*, *GSTZ1* and *CYP2E1* genes.
Results: Median level of exhaled total THMs and creatinine adjusted urine TCAA increased from 0.5 to 14.4 μg/m^3 and from 2.5 to 5.8 μmol/mol after swimming, respectively. The increase in exhaled brominated THMs was correlated with brominated THMs, haloacetic acids, haloacetonitriles, haloketones, chloramines, total organic carbon and total organic halogen in water and trichloramine in air. Such correlations were not detected for exhaled chloroform, total THMs or urine TCAA. Exhaled THM increased more in men, urine TCAA increased more in women, and both were affected by exercise intensity. Genetic variants were associated with differential increases in exposure biomarkers.
Conclusion: Our findings suggest that, although affected by sex, physical activity and polymorphisms in key metabolizing enzymes, brominated THMs in exhaled breath could be used as a non-invasive DBP exposure biomarker in swimming pools with bromide-containing source waters. This warrants confirmation with new studies.

Abbreviations: BMI, Body mass index; BrClAA, Bromochloroacetic acid; BrDClM, Bromodichloromethane; BrHAAs, Brominated haloacetic acids; BrTHMs, Brominated THMs; $CHBr_2CN$, Dibromoacetonitrile; $CHBr_3$, Bromoform; $CHCl_3$, Chloroform; $C_3H_3Cl_3O$, 1,1,1-Trichloropropanone; $CHCl_2CN$, Dichloroacetonitrile; CHBrClCN, Bromochloroacetonitrile; *CYP2E1*, Cytochrome P450 2E1 gene; DBrAA, Dibromoacetic acid; DBrClM, Dibromochloromethane; DBP, Disinfection by-product; DClAA, Dichloroacetic acid; DClBrAA, Dichlorobromoacetic acid; DMNA, Dimethylnitramine; FCl, Free chlorine; *GSTT1*, Glutathione S-transferase theta 1 gene; *GSTZ1*, Glutathione S-transferase zeta 1 gene; HAA, Haloacetic acid; HAN, Haloacetonitrile; HK, Haloketone; IQR, Interquartile range; METs, Metabolic equivalent tasks; MHR, Maximum heart rate; NCl_3, Trichloramine; NDMA, Nitrosodimethylamine; NH_2Cl, Monochloramine; $NHCl_2$, Dichloramine; NPOC, Non-purgeable organic carbon; SNP, Single-nucleotide polymorphism; TCAA, Trichloroacetic acid; THAAs, Total haloacetic acids; THM, Trihalomethane; TTHMs, Total trihalomethanes; TOBr, Total organic bromine; TOC, Total organic carbon; TOCl, Total organic chlorine; TOI, Total organic iodine; TOX, Total organic halogen

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http://dx.doi.org/10.1016/j.envres.2016.05.013
0013-9351/

1. Introduction

Swimming in pools is a popular and healthy activity that involves high exposure to disinfection by-products (DBPs). Mixtures of chemicals produced during disinfection process with different toxicities are present in treated drinking water. An increased bladder cancer risk has been consistently associated with long-term exposure to residential trihalomethanes (THMs) (Villanueva et al., 2004, 2015) and an association with pool attendance has also been suggested (Villanueva et al., 2007). Polymorphisms in key metabolizing enzymes, including glutathione S-transferase (*GSTT1*, *GSTZ1*) and cytochrome P450 (*CYP2E1*), have been suggested to modify DBP-associated bladder cancer risk (Cantor et al., 2010). Slight increased risks for some reproductive outcomes may also be related to DBP exposure (Villanueva et al., 2015). The exposure to trichloramine, a volatile irritant DBP in indoor pools, has been associated with an increased risk of respiratory effects in highly exposed populations (Villanueva and Font-Ribera, 2012).

Hundreds of DBPs have been identified in swimming pool water (Richardson et al., 2010; Xiao et al., 2012), which has a different composition than tap water. Nitrogenous organic mater from bathers (e.g. sweat, urine) produces nitrogenous DBPs such as chloramines, haloacetonitriles (HANs) or nitrosamines (Chowdhury et al., 2014), which are more cytotoxic and genotoxic than THMs and haloacetic acids (HAAs) (Richardson et al., 2007). The route of exposure depends on the chemical properties of each DBP. The major exposure route for volatile DBPs such as THMs or chloramines is inhalation (Erdinger et al., 2004; Marco et al., 2015), whereas less volatile and skin permeable compounds such as HAAs (Kim and Weisel, 1998) can enter the body through accidental ingestion or inhalation of aerosol (Cardador and Gallego, 2011).

Only THMs and HAAs have been measured in the human body as indicators of internal dose. The majority of studies assessing DBPs exposure in swimmers have measured THMs in exhaled breath (Caro and Gallego, 2008; Aggazzotti et al., 1998; Font-Ribera et al., 2010), blood (Aggazzotti et al., 1998, 1990) or urine (Aprea et al., 2010; Caro and Gallego, 2007, 2008). Only a few studies have measured specific HAAs in urine after swimming (Cardador and Gallego, 2011). The uptake of THMs during swimming has been related to the levels in water (Caro and Gallego, 2007; Font-Ribera et al., 2010; Aggazzotti et al., 1990) and air (Font-Ribera et al., 2010; Caro and Gallego, 2008; Aggazzotti et al., 1990), and the level of trichloroacetic acid (TCAA) in urine was related to the level in water (Cardador and Gallego, 2011). However, the correlations between these exposure biomarkers with other more toxic and rarely measured DBPs in swimming pools are not known. Individual characteristics of swimmers such as age, sex or body mass index (BMI) did not show to affect the uptake of these exposure biomarkers (Font-Ribera et al., 2010; Cardador and Gallego, 2011). However, intensity of physical activity during swimming has been shown to increase the uptake of THMs (Marco et al., 2015).

In this study, we aimed to further characterize exposure to DBPs among swimmers. Specifically, (1) to describe how well two internal dose biomarkers (THMs in exhaled breath and HAAs in urine) reflect the levels of several DBPs in water and trichloramine in air; and (2) to identify the individual characteristics that affect these internal dose biomarkers, including sex, BMI, body surface area, the polymorphisms of specific metabolizing genes and several measures of physical activity. These results will help understanding how exposure to DBPs occurs in swimming pools and will be especially useful to improve methodological aspects in epidemiological studies assessing short-term health effects after swimming in pools.

2. Materials and methods

2.1. Study design

In total, 116 non-smoking non-professional swimmers 18–40 years old swam for 40 min in a single, indoor, 25 m-long chlorinated swimming pool in Barcelona, Spain. Four participants were evaluated individually per day, between 9 am and 2 pm (before lunch) in June and between September and December 2013, with a total of 30 experimental days. Participants were asked to swim during a fixed time (40 min) at a free pace, resting as much as they wanted. Before and after swimming, biological samples and measurements were obtained in a room inside the sports centre separated from the swimming pool area. Participants had not attended swimming pools during the week before, did not take a shower in the morning of the experimental day and DBP-free bottled water was provided during the study period.

2.2. Internal dose biomarkers

Four THMs (chloroform ($CHCl_3$), bromodichloromethane (BrDClM), dibromochloromethane (DBrClM) and bromoform) were measured in exhaled breath before the swimmers entered the swimming pool and right after they left the pool, using the Bio-VOC™ Sampler (Markes International Ltd, UK). After breathing deeply three times, the subjects retained the air for 10 s and then breathed continuously into the disposable cardboard mouthpiece until the end of a quiet breathing to obtain the alveolar air retained by the Bio-VOC™ Sampler. Once 150 mL alveolar air had been collected, a screw-in plunger was used to steadily discharge the sample into a sorbent tube trap. This process was repeated four times resulting in a total exhaled breath volume of 600 mL. After collection, sorbent tubes were capped with brass storage caps fitted with PTFE ferrules and were stored at 4 °C in a solvent-free environment until analyses. THMs were desorbed from sorbent tubes and concentrated in a thermal desorption (TD) unit equipped with a Unity Series 2 Thermal Desorber and an Ultra 50:50 Multi-tube Auto-sampler (Markes International Ltd.). The THMs were transferred to a Gas Chromatograph 7890 (Agilent Technologies) coupled to Mass Spectrometer 5975C Inert XL MSD with a Source in Electronic Impact Mode (Agilent Technologies). Ten mL urine samples were collected before and 30 min after swimming for TCAA and creatinine analyses. Urine samples were stored at −20 °C and shipped on dry ice to the laboratory. TCAA concentrations in the five mL urine samples were measured using solid phase extraction followed by liquid chromatography tandem mass spectrometry (LC-MS-MS). Methods have been described previously (Salas et al., 2014). Creatinine was also measured in order to adjust for dilution, and TCAA concentration was expressed as creatinine adjusted levels (μmol TCAA/mol creatinine).

2.3. Environmental measurements

Free chlorine (FCl), three chloramines, pH, four THMs, nine HAAs, four HANs, three haloketones (HKs), a nitrosamine, a nitramine, total organic carbon (TOC), and total organic halogen (TOX) in water were measured. Water samples were collected at two different locations of the pool while participants were swimming, and the samples were stored at 4 °C until laboratory analyses. FCl, chloramines, THMs, HANs, and HKs were measured for each participant, TOC was measured twice per day, TOX, and HAAs once per day and nitrosamines and nitramines only for selected days. For THM analyses, 3–5 mg $Na_2S_2O_3 \cdot 5\,H_2O$ and 30–50 mg $KHSO_4$ were added to a 20 mL air-tight headspace-free glass vial with crimp cap. For HAA, HAN and HK analyses, 10 mg NH_4Cl were added to 100 mL amber glass vials with Teflon faced screw