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Effects of exposure to water disinfection by-products in a swimming pool: A metabolome-wide association study

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ABSTRACT

Background: Exposure to disinfection by-products (DBPs) in drinking water and chlorinated swimming pools are associated with adverse health outcomes, but biological mechanisms remain poorly understood.
Objectives: Evaluate short-term changes in metabolic profiles in response to DBP exposure while swimming in a chlorinated pool.
Materials and methods: The PISCINA-II study (EXPOsOMICS project) includes 60 volunteers swimming 40 min in an indoor pool. Levels of most common DBPs were measured in water and in exhaled breath before and after swimming. Blood samples, collected before and 2 h after swimming, were used for metabolic profiling by liquid-chromatography coupled to high-resolution mass-spectrometry. Metabolome-wide association between DBP exposures and each metabolic feature was evaluated using multivariate normal (MVN) models. Sensitivity analyses and compound annotation were conducted.
Results: Exposure levels of all DBPs in exhaled breath were higher after the experiment. A total of 6,471 metabolic features were detected and 293 features were associated with at least one DBP in exhaled breath following Bonferroni correction. A total of 333 metabolic features were associated to at least one DBP measured in water or urine. Uptake of DBPs and physical activity were strongly correlated and mutual adjustment reduced the number of statistically significant associations. From the 293 features, 20 could be identified corresponding to 13 metabolites including compounds in the tryptophan metabolism pathway.
Conclusion: Our study identified numerous molecular changes following a swim in a chlorinated pool. While we could not explicitly evaluate which experiment-related factors induced these associations, molecular characterization highlighted metabolic features associated with exposure changes during swimming.

1. Introduction

Physical exercise, including swimming, is highly recommended because of its positive effects on general health. However, the health of swimmers might be at risk due to disinfection methods used in swimming pools, which lead to the formation of disinfection by-products (DBPs). DBPs are present in swimming pool water, and residential sources such as drinking water, shower and bath water. They result

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from the disinfectants (such as chlorine) reacting with natural organic matter (such as saliva, hair, or perspiration in the case of swimming pools). During swimming, showering and bathing, inhalation and dermal absorption are the main exposure routes, which lead to high concentrations of skin permeable and volatile DBPs in the blood, such as trihalomethanes (THMs).(Villanueva and Font-Ribera, 2012) Exposure from ingestion of water also occurs during swimming. More than 600 DBPs have been identified, including THMs and haloacetic acids (HAAs), and the International Agency for Research on Cancer (IARC) has classified several of them as possibly carcinogenic to humans (group 2B). (World Health Organization, 2004b)

Several epidemiological studies have investigated health effects of long-term exposure to residential DBPs and all identified an association with increased risk of bladder cancer. (Costet et al., 2011; Villanueva et al., 2004; Villanueva et al., 2015) Short-term effects of DBP exposure in swimming pools have been suggested to comprise increased lung epithelium permeability, in both adults(Font-Ribera et al., 2010) and children(Bernard et al., 2003). Asthma development and other respiratory complications have been related to long-term exposure in swimming pools.(Levesque et al., 2006; Villanueva et al., 2015) This has most consistently been observed among those who are occupationally exposed, such as swimming pool workers and professional swimmers.(Goodman and Hays, 2008; Thickett et al., 2002) In addition, some epidemiological studies have suggested potential adverse reproductive and developmental effects(Villanueva et al., 2015), but these findings were not validated in a recent large European study. (Kogevinas et al., 2016)

Several studies have suggested the genotoxic and mutagenic potential of some DBPs.(Beddowes et al., 2003; Du et al., 2013; Honer et al., 1980; Khallef et al., 2015; Kogevinas et al., 2010; Pals et al., 2013; Pals et al., 2011; Richardson et al., 2010; Stayner et al., 2014; Wang et al., 2014; Yuan et al., 2005) Specifically, higher levels of biomarkers of genotoxicity such as changes in micronuclei (MN) and DNA damage (comet assay) in peripheral blood lymphocytes have been reported in relation to brominated THM concentrations (excluding chloroform) in exhaled breath.(Kogevinas et al., 2010) Increased levels of markers of genotoxicity in maternal binucleated lymphocytes were also identified during the first and second trimester of pregnancy in relation to THM exposure from residential water (Khallef et al., 2015; Stayner et al., 2014) and swimming pool water(Honer et al., 1980; Richardson et al., 2010).

Experimental studies in cell lines (Beddowes et al., 2003; Yuan et al., 2005) and in blood biosamples (Du et al., 2013; Pals et al., 2013; Pals et al., 2011; Wang et al., 2014) have identified a link between genotoxicity/mutagenicity and oxidative stress, notably through the production of reactive oxygen species following exposure to various forms of DBPs.

The World Health Organization (WHO), the US Environmental Protection Agency (EPA) and other agencies have set drinking water guideline values or regulations for various THMs. (The National Primary Drinking Water Regulations, 2015; World Health Organization, 2011) In the US, levels of haloacetic acids (HAA) are also regulated, while this presently is not the case in Europe.(Union, 2015; United States Environmental Protection Agency) For swimming pool water, far fewer regulations are in place, although mutagenic levels in swimming pool water were found to be similar to those of drinking water. (Richardson et al., 2010)

The simultaneous acquisition of information of hundreds or thousands of metabolites in biospecimens from human subjects exposed to environmental toxicity has been used to successfully identify new biomarkers of exposure or effect, and to generate new hypotheses on possible mechanisms linking exposures to diseases (Bonvallot et al., 2013; Yuan et al., 2016; Zhang et al., 2016). In the present study, we adopt a metabolome-wide association study (MWAS) approach to identify possible changes in metabolic profiles after swimming in a chlorinated swimming pool. Our study was implemented in an experimental setup and features repeated measurements (before and after the swim) of both exposures and metabolic profiles for each participant. To accommodate this multiple measurement design, we used a flexible multivariate normal (MVN) model to regress levels of each metabolic feature against the measured exposure levels. This approach, coupled with an extensive effort to identify associated metabolic features, has the potential to help unravel molecular pathways affected by exposure to DBPs and ultimately inform on the mechanisms explaining the underlying toxicity. Therefore, the overall aim of our study is to investigate the short-term effects of DBP exposure on the metabolome and more specifically, the possible involvement of metabolic pathways linking DBP exposure and adverse health outcomes.

2. Materials and methods

2.1. Participants and samples

The participants for this study were part of the PISCINA II study, an experimental study performed in a 25 m long indoor chlorinated pool in Barcelona, Spain, between June and December 2013. It included 116 volunteers, aged 18–40 years, non-smoking and non-professional swimmers, who swam for 40 min at a leisurely pace in the swimming pool, resting at their own initiative. Four participants were evaluated per day, between 9 am and 2 pm (before having lunch).

From these 116 volunteers, 60 were selected for subsequent metabolomic profiling. Selection criteria were defined to ensure that: i) complete exposure measurements in the swimming pool were available, ii) biological samples were available; iii) there was an even proportion of men and women; iv) data on physical activity, lifestyle, and all possible adjustments covariates (see below) were available. For each of these 60 participants, blood samples collected by venepuncture using BD Vacutainer R Push Button Blood Collection Set with Pre-Attached Holder in a room detached from the swimming pool, in two occasions, before and 2 h after swimming. For this analysis, blood was collected in serum tubes. Samples were kept at 4 °C and were sent to the laboratory to be processed. After 1 h of free coagulation, samples were centrifuged at 4 °C and 0,25 mL of serum was stored in Screw Cap Micro Tubes at − 80 °C.

All participants were requested not to visit any swimming pools one week before the experiment and not to shower on the morning of the experiment. Information about water consumption and use, activities, transport, diet and medication was obtained from the participants through questionnaires before the start of the experiment. Height and weight were measured and body mass index (BMI) was calculated by dividing the weight in kg by the height squared in meters. During the experiment, swimming distance and swimming duration were measured. Energy expenditure (kcal) was estimated using the speed of swimming and the participant's weight, assuming 8.3 METs (metabolic equivalent tasks: kcal per kg per hour) of expenditure for swimming at 46 m/min according to the following equation based on work form Ainsworth et al. (2011):

$$Kcal = weight\ (kg) \times \frac{distance\ swam\ (m)}{minute} \times swimming\ duration\ (hr). \times \left(\frac{8.3\left(\frac{kcal/kg}{hr}\right)}{46\ (m/\mathrm{min})} \right)$$

Heart rate (beats/min) was recorded at every second using a Polar RCX5 heart rate monitor.

Informed consent was provided by each participant before commencement of the experiment. The study was approved by the ethics committee of the research centre according to national and international regulations.

2.2. Exposure variables

Two main types of exposures to DBPs were collected during the