Environmental Research 156 (2017) 120–127

Contents lists available at ScienceDirect

Environmental Research

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

Assumed non-persistent environmental chemicals in human adipose tissue; matrix stability and correlation with levels measured in urine and serum

F. Artacho-Cordón[a,*], JP. Arrebola[a,b,c], O. Nielsen[d], P. Hernández[e], NE. Skakkebaek[d,f], MF. Fernández[a,b], AM. Andersson[d,f], N. Olea[a,b], H. Frederiksen[d,f]

[a] Univ. Granada, Radiology and Physical Medicine Dept./ibs.GRANADA, Granada, Spain
[b] CIBER Epidemiology and Public Health (CIBERESP), Spain
[c] Radiation Oncology Department, Complejo Hospitalario Universitario de Granada, Spain
[d] Department of Growth and Reproduction, Rigshospitalet, University of Copenhagen, Denmark
[e] Surgical Department, Complejo Hospitalario Universitario de Granada, Spain
[f] International center for research and research training in Endocrine Disruption of Male Reproduction and Child health (EDMaRC), Rigshospitalet, University of Copenhagen, Denmark

ABSTRACT

The aim of this study was to (1) optimize a method for the measurement of parabens and phenols in adipose tissue, (2) evaluate the stability of chemical residues in adipose tissue samples, and (3) study correlations of these compounds in urine, serum, and adipose tissue.

Samples were obtained from adults undergoing trauma surgery. Nine phenols and seven parabens were determined by isotope diluted TurboFlow-LC-MS/MS. The analytical method showed good accuracy and precision. Limits of detection (LOD) for parabens and phenols ranged from 0.05 to 1.83 ng/g tissue. Good recovery rates were found, even when biological samples remained defrosted up to 24 h. Benzophenone-3 (BP-3; range of values: < LOD-1.48 ng/g tissue) and methylparaben (MeP; < LOD-1.78 ng/g tissue) were detected in > 70% of adipose tissue samples, while bisphenol-A (BPA; < LOD-3.28 ng/g tissue) and 2-phenylphenol (2-PP; < LOD-0.78 ng/g tissue) were detected in > 40% of adipose tissue samples. In general, levels were similar between adipose tissue and serum, while a correlation between adipose tissue and urine was only found for BP-3.

In conclusion, adipose tissue samples in this study were found to contain environmental chemicals considered to be non-persistent, whose levels were weakly or not at all correlated with the urine burden. Therefore, adipose tissue may potentially provide additional information to that obtained from other biological matrices. Further investigations are warranted to explore whether adipose tissue might be a suitable matrix for assessment of the consequences for human health of mid/long-term exposure to these chemicals.

1. Introduction

Over the past century, humans have been exposed to several synthetic substances, some of which have been identified as endocrine disrupting chemicals (EDCs). Several persistent EDCs, such as dichlorodiphenyl-trichloroethylene (DDT), hexachlorocyclohexane (HCH), and polychlorinated biphenyls, bioaccumulate and biomagnify in the food chain and were therefore banned some decades ago. In many countries, however, there are still no restrictions on the production and use of numerous substances that also exert suspected endocrine disrupting activity, but have lower liposolubility and hence no tendency to bioaccumulate and biomagnify in the food chain, the so-called non-persistent EDCs (npEDCs). These include bisphenol A (BPA), the main component of polycarbonate and epoxy resin (EU, 2011), and some parabens (SCCS, 2013), widely employed as antimicrobial preservatives in cosmetic products and pharmaceuticals. Other confirmed or suspected npEDCs are benzophenone-3 (BP-3), commonly used as UV filter and absorber; triclosan (TCS) and triclocarban (TCCB), antiseptic agents; polychlorophenols (2,4-DCP, 2,5-DCP, and 2,4,5-TCP), pesticides; and phenylphenols (2-PP and 4-PP), used as fungicides. Recent human biomonitoring studies have demonstrated a wide exposure to several of these substances among the general population (Casas et al., 2011; CDC, 2015; Frederiksen et al., 2014).

Human exposure to parabens, BP-3, BPA, and various other phenols (*e.g.*, 2-PP) usually occurs through ingestion, inhalation, or dermal absorption (El Hussein et al., 2007; Hayden et al., 2005; Vandenberg

* Correspondence to: Univ. Granada, Radiology and Physical Medicine Department, 18071 Granada, Spain.
E-mail address: fartacho@ugr.es (F. Artacho-Cordón).

http://dx.doi.org/10.1016/j.envres.2017.03.030
Received 30 November 2016; Received in revised form 9 February 2017; Accepted 18 March 2017

et al., 2007), and serum and urine levels have been described as highly dependent on current rather than chronic exposure (Engel et al., 2014; Heffernan et al., 2014; Meeker et al., 2013). These compounds are mainly excreted through the urine as free BPA (9.5%) or in a conjugated form as glucuronide (69.5%) or sulfate metabolites (21.0%), reducing their bioactivity and facilitating their excretion (Ye et al., 2005). However, some researchers have described these npEDCs as "pseudo-persistent" compounds (Flint et al., 2012) to which constant daily exposure can be compared to long-term chronic exposure (Fernández and Olea, 2014). Moreover, the log of the octanol–water partition coefficient (K_{ow}) of phenols and parabens typically ranges from 1 to 5, with the K_{ow} of BPA being estimated at 2.2–3.82 (Shelby, 2008); therefore, BPA should be considered a partially lipophilic compound that can potentially be distributed in adipose tissue tissues (Fernandez et al., 2007). In this regard, the release of BPA *via* urine was found to be lower than expected in adults in the NHANES study (National Health and Nutrition Examination Survey) (Stahlhut et al., 2009). This suggests that, after repeated exposures, BPA might at least partially be present in certain body compartments such as adipose tissue, from where it would be slowly released (Stahlhut et al., 2009). A similar dynamic may also affect other npEDCs with comparable physicochemical properties (Wang et al., 2015), *e.g.* BP-3, TCS, and 2-PP with log K_{ow} = 3.18, 4.76, and 2.84, respectively.

Urine has been the preferred biological matrix to assess recent human exposure to npEDCs, given its availability and the ease of its collection (Ye et al., 2006). Thus, urine has traditionally been considered the matrix of choice in epidemiological studies, and thousands of samples from the general population are monitored year after year. However, measurements may not accurately reflect the overall average exposure dose, because of the low intra-individual concordance, the changeable nature of personal lifestyles and dietary habits, and the influence of the timing of urine sampling on the measurement of npEDC concentrations (Braun et al., 2011). Urine and serum levels of some npEDCs, *e.g.* parabens, have been shown to be highly correlated, reflecting similar time-dependent fluctuations (Frederiksen et al., 2011). Therefore, measurements of npEDCs in single urine and serum samples might not be the most appropriate biomarker for the evaluation of long-term exposure to these compounds. Despite some attempts (Frederiksen et al., 2011), a reliable biomarker of chronic exposure to npEDCs has not yet been identified.

Encompassed in a wider project on the relevance of the adipose tissue burden of npEDCs and its role in human disease, we present here the results of a pilot study that aimed: (1) to optimize a methodology to identify and quantify seven parabens and nine phenols in adipose tissue; (2) to evaluate the stability of these chemicals in adipose tissue samples, and (3) to examine the correlation among urine, serum, and adipose tissue levels of npEDCs in samples from adults.

2. Materials and methods

2.1. Study subjects and sample collection

This pilot study included urine, serum, and adipose tissue samples from 14 adults recruited between May and July 2015 from among patients undergoing trauma surgery at the public University Hospital Complex of Granada, Southern Spain. Inclusion criteria were: age over 16 years, absence of hormone-related disease or cancer, and no receipt of hormone therapy. Baseline characteristics of the study population, including sex, age, and surgical site are summarized in Supplementary Table 1. All patients signed informed consent to participate in the study, which was approved by the Biomedical Research Ethics Committee of Granada Province.

Fasting urine and blood samples were collected by nursing staff before surgery, and adipose tissue samples were obtained during the intervention. Urine and adipose tissue samples were directly collected in BPA-free glass tubes, while blood samples was extracted in collecting tubes for serum extraction (previously tested for BPA contamination), and serum samples were placed in BPA-free glass tubes. All three matrices were stored at −20 °C until analysis.

2.2. Control material

Adipose tissue needed for validation of the proposed methodology was kindly supplied by Dr. Niels Kroman from the Department of Breast Surgery, Rigshospitalet, University of Copenhagen, Denmark. Adipose tissue samples (~20 g) came from patients undergoing breast cancer surgery. After surgery, the samples were immediately placed in a glass tube and sent to the laboratory, where they were homogenized using a mixer (Pro Scientific INC, USA). The homogenates were pooled before storage in aliquots at −20 °C, obtaining a single anonymized adipose tissue pool.

2.3. Reagents and standards

Bisphenol A (BPA), triclosan (TCS), triclocarban (TCCB), benzophenone-3 (BP-3), 2,4-dichlorophenol (2,4-DCP), 2,5-dichlorophenol (2,5-DCP), 2,4,5-trichlorophenol (2,4,5-TCP), 2-phenylphenol (2-PP), 4-phenylphenol (4-PP), methylparaben (MeP), ethylparaben (EtP), n-propylparaben (n-PrP), *n*-butylparaben (n-BuP), and benzylparaben (BzP) were purchased from Sigma–Aldrich (Brøndby, Denmark), while isopropylparaben (i-PrP) and isobutylparaben (i-BuP) were supplied by Alfa Aesar (Karlsruhe, Germany). Carbon-labeled internal standards ($^{13}C_{12}$-TCS, $^{13}C_6$-TCCB, $^{13}C_6$-2,4,5-TCP, $^{13}C_6$-2-PP, $^{13}C_6$-n-BuP and $^{13}C_6$-BP-3) were purchased from Cambridge Isotope laboratories (Andover, MA; distributed by VWR international, Herlev, Denmark). Deuterium-labeled internal standards D_4-EtP and D_4-n-PrP were obtained from the Institut für Dünnschicht technologie und Mikrosensorike. V. (Teltow, Germany), D_8-BPA and D_3-2,4-DCP from Sigma Aldrich (Brøndby, Denmark), and D_4-MeP from CDN Isotopes (Quebec, Canada). Stock solutions of native standards were prepared containing 5, 500, and 2500 ng/mL of each selected paraben and phenol compound in 50% methanol, while stock solutions of internal standards were prepared at a concentration of 200 ng/mL, diluting labeled standards in 50% methanol as previously published (Frederiksen et al., 2013, 2011) All other reagents and solvents were of analytical, HPLC, or MS grade, and all chemicals and labware were tested for contamination before use.

2.4. Sample preparation and chemical analyses

Adipose tissue samples and the control pool were extracted as follows. A total of 100 mg of adipose tissue sample was accurately weighted in glass tubes and spiked with 25 µL of internal standard stock solution (200 ng/mL). Samples were centrifuged and equilibrated at room temperature for 30 min. One milliliter of acetone was added and homogenized with a mixer. One extra milliliter of fresh acetone was immediately used for cleaning the mixer and finally transferred to the glass containing the homogenized sample. Two milliliters of methanol were then added for the total homogenization and penetration of the internal standard into the matrix, and the extract was sonicated in an ultrasound bath for 10 min. Next, the amount of total extract volume was reduced to less than 2 mL by evaporation of the organic solvent under a gentle nitrogen stream at room temperature. The remaining extract was transferred to a 2 mL Eppendorf tube and stored at −20 °C for 15 min followed by centrifugation at 18213 × *g* (at 4 °C) for 10 min. The organic phase was then transferred to a new glass tube and evaporated to dryness under a gentle nitrogen stream. For removal of the remaining lipids, the residue was resuspended in 200 µL of methanol/20% HCOOH (1:1), followed by the addition of 300 µL of 1 M ammonium acetate buffer (pH5.5); this solution was placed in an Eppendorf tube and then centrifuged twice at 18213 × *g* for 10 min at 4 °C. Finally, the supernatant was transferred to an HPLC vial.

Urine and serum samples were extracted and analyzed following