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Environmental phenols and parabens in adipose tissue from hospitalized adults in Southern Spain

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ABSTRACT

Urinary concentrations of non-persistent environmental pollutants (npEPs) are widely assessed in biomonitoring studies under the assumption that they are metabolised and eliminated in urine. However, some of these chemicals are moderately lipophilic, and their presence in other biological matrices should also be evaluated to estimate mid/long-term exposure to npEPs and its impact on human health. The present study aims to explore concentrations and potential determinants of npEPs in adipose tissue from a hospital-based adult cohort (GraMo cohort, Southern Spain).

Concentrations of bisphenol-A (BPA), benzophenone-3 (BP-3), triclosan (TCS), three chlorophenols (2,4-DCP, 2,5-DCP and 2,4,5-TCP) and two phenylphenols (2-PP and 4-PP), triclocarban (TCCB) and parabens [methyl- (MeP), ethyl- (EtP), propyl- (n-PrP and i-PrP), butyl- (n-BuP and i-BuP) and benzyl-paraben (BzP)] were analysed in adipose tissue samples from a subcohort of 144 participants. Spearman correlation tests were performed, followed by stepwise multivariable linear regression analyses to assess determinants of the exposure.

Detection frequencies and median concentrations were: BPA (86.8%, 0.54 ng/g tissue), BP-3 (79.2%, 0.60 ng/g tissue), TCS (45.8%, < LOD), 2-PP (18.8%, < LOD), MeP (100.0%, 0.40 ng/g tissue), EtP (20.1%, < LOD) and n-PrP (54.2%, 0.06 ng/g tissue). The remaining npEPs were detected in < 10% of the samples. BPA, MeP, EtP and n-PrP levels were significantly and positively correlated, while BP-3 showed a positive correlation with TCS and 2-PP. Older participants showed higher concentrations of TCS and MeP, while BMI was inversely associated with most of the analysed compounds and perceived recent weight loss was inversely associated with 2-PP. Female participants and residents of rural areas had increased BP-3 concentrations. npEP concentrations were positively associated with the consumption of fatty food but negatively associated with the consumption of vegetables and fruit.

This study reveals the widespread presence of numerous npEPs in adipose tissue from adults in southern Spain, suggesting a generalized distribution of these environmental compounds in fatty tissues. In these adults, many of the determinants of npEP concentrations in adipose tissue were similar to those of more lipophilic and persistent compounds.

1. Introduction

A substantial group of widely-used chemicals are suspected to exert potential adverse effects on humans, including compounds to which humans are daily exposed. In this context, increased attention has been paid to non-persistent environmental pollutants (npEPs) such as

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phenols and parabens, given their generalized presence as industrial pollutants or pesticide residues in food and other consumer items, including personal care products (Guo and Kannan, 2013; Liao et al., 2013a). The production of bisphenol-A (BPA) is one of the highest of any synthetic chemical worldwide, with an estimated volume of 5.5 million tons in 2011 (QYResearch Group, 2015). This organic and moderately lipophilic chemical is employed in the production of polycarbonate plastics and epoxy resins for water bottles, plastic containers, dental sealants or cans for food and beverages, among many other products (Geens et al., 2012a). Triclosan (TCS) is an antimicrobial agent extensively used in toothpaste, disinfectants, detergents and soaps, among numerous personal care products, meaning that human exposure to TCS is highly frequent (CDC, 2017; Frederiksen et al., 2014; Kim et al., 2011a). Benzophenone-3 (BP-3) belongs to a wide family of UV-filters included in sunscreen formulations and in plastics and food-packaging materials and also serves as a fragrance and flavour enhancer (Calafat et al., 2008). Polychlorophenols [dichlorophenols (2,4-DCP and 2,5-DCP), 2,4,5-trichlorophenol (2,4,5-TCP) and phenylphenols (2-PP and 4-PP)] are pesticides and fungicides used primarily as a preservative in citrus fruits after harvesting but also for vegetable preservation. They are also applied to disinfect hospital and veterinary equipment and are included in household products. 2-PP is also added to food packaging and may migrate into the food (Coelhan et al., 2006). Parabens [methylparaben (MeP), ethylparaben (EtP), *n*-propyl and isopropylparaben (n-PrP and i-PrP), *n*-butyl and isobutylparaben (n-BuP and i-BuP) and benzylparaben (BzP)] are alkyl esters of *p*-hydroxybenzoic acid. They are widely used as antimicrobial preservatives, especially against mould and yeast, in cosmetics, pharmaceuticals, food and beverages (Darbre and Harvey, 2008; Liao et al., 2013b; Ursino et al., 2011).

Several in vitro and in vivo studies have reported estrogenic (Boberg et al., 2010; Li et al., 2012; Perez et al., 1998) and/or antiandrogenic activity of the above-mentioned xenobiotics (Bonefeld-Jorgensen et al., 2007). However, complementary mechanisms of action have been described for many of these compounds such as BPA, parabens or BP-3 (Asimakopoulos et al., 2016; Huang et al., 2017), including oxidative status imbalance (Macczak et al., 2017; Tiwari and Vanage, 2017) and induction of an inflammatory response (Elswefy et al., 2016). In addition, exposure in some population groups has been related to behavioural and reproductive abnormalities (Braun et al., 2011; Fernández et al., 2016; Lassen et al., 2014; Perez-Lobato et al., 2016) and chronic diseases (Kim and Park, 2013; Lakind et al., 2014; Lang et al., 2008).

Exposure of the general population to these compounds can occur via dermal, inhalational, or oral routes, including the intake of treated or contaminated food (El Hussein et al., 2007; Hayden et al., 2005; Vandenberg et al., 2007). Exposure to BPA or phenylphenols is considered to be largely dietary, whereas exposure to TCS, BP-3 and parabens is mainly dermal (Søeborg et al., 2014). Orally ingested compounds are usually converted into more hydrophilic derivatives, whereas exposure via the skin may avoid this first-pass metabolism, so that the parent compounds directly enter the bloodstream, increasing their distribution to other tissues (Søeborg et al., 2014). Thus, it has traditionally been assumed that environmental phenols and parabens are excreted via urine within the first 24 h, but pharmacodynamic studies suggest that a small fraction of administered chemicals may remain within the organism (Stahlhut et al., 2009). Moreover, the log of the octanol–water partition coefficient (K_{ow}) of phenols and parabens typically ranges from 1 to 5; therefore, they should be considered at least partially lipophilic compounds that would potentially be distributed in adipose tissues, as reported in several studies (Artacho-Cordón et al., 2017; Barr et al., 2012; Darbre et al., 2004; Fernandez et al., 2007; Geens et al., 2012b; Wang et al., 2015). Urine and serum concentrations are widely used in biomonitoring studies to estimate exposure to these pollutants (Koch et al., 2012), but they have been described as highly dependent on very recent exposure (within past few hours), and multiple samples must often be taken at different time points to correctly classify exposure to certain npEPs e.g. for BPA because of its low interclass correlation (Braun et al., 2012). Hence, there are limitations in the utilisation of urine and serum concentrations to estimate mid/long-term exposure to some of these compounds, a crucial issue for the evaluation of associated health effects (Aylward et al., 2017). In this regard, our group previously reported very low correlation coefficients between concentrations of these compounds in adipose tissue and those in spot urine or serum samples (Artacho-Cordón et al., 2017). The present investigation was prompted by recent evidence obtained in the GraMo cohort suggesting a role for certain xenobiotics stored in adipose tissue in the development of chronic diseases such as diabetes (Arrebola et al., 2013b), hypertension (Arrebola et al., 2015), obesity (Arrebola et al., 2014b), metabolic syndrome (Mustieles et al., 2017) and cancer (Arrebola et al., 2014a). Accordingly, the objective of this study was to explore the distribution of a selection of non-persistent environmental chemicals in adipose tissue samples from an adult hospital-based cohort recruited in Southern Spain and to evaluate potential determinants of the exposure.

2. Material and methods

2.1. Study population

The study population is a subsample of the GraMo cohort (Arrebola et al., 2013a; Arrebola et al., 2010; Arrebola et al., 2009; Artacho-Cordon et al., 2016). Participants were recruited between July 2003 and June 2004 among patients undergoing non-cancer-related surgery at two public hospitals in Southern Spain separated by 70 km (San Cecilio University hospital in Granada and Santa Ana Hospital in Motril). The economy of the city of Granada (urban, 240,000 inhabitants) is mainly based on services (university and tourism), while Motril (semi-rural, 50,000 inhabitants) is a small town on the Mediterranean coast surrounded by extensive areas of intensive greenhouse agriculture. Study inclusion criteria were age over 16 years, absence of diagnosed hormone-related disease or cancer and residence in one of the two study areas for ≥10 years. Out of the 409 individuals initially recruited, 387 agreed to participate in the study. npEP concentrations were measured in a subgroup of participants with an adequate adipose tissue sample available (n = 144, 37.2%). No statistically significant differences in baseline characteristics were found between the initial (n = 387) and final (n = 144) study populations except for the higher percentage of participants who were urban residents in the latter (77.8% vs. 48.1% in the initial cohort) (Supplementary Table 1).

All participants signed their informed consent to participate in the study, which was approved by the Ethics Committee of Granada (*Comité de Ética de la Investigación Biomédica de la Provincia de Granada*).

2.2. Sample preparation and chemical analysis

Adipose tissue samples were intraoperatively collected and immediately coded and stored in aliquots at −80 °C until analysis. Main tissue sources were pelvic waist (46.5%), front abdominal wall (44.4%), and limbs (9.0%). Environmental phenols and parabens were isolated from approximately 100 mg of adipose tissue samples following a previously validated methodology (Artacho-Cordón et al., 2017). Briefly, samples were spiked with 25 μL of internal standard stock solution (200 ng/mL, $^{13}C_{12}$-TCS, $^{13}C_6$-TCCB, $^{13}C_6$-2,4,5-TCP, $^{13}C_6$-2-PP, $^{13}C_6$-n-BuP, $^{13}C_6$-BP-3, D_4-EtP, D_4-n-PrP, D_8-BPA, D_3-2,4-DCP, and D_4-MeP) and then centrifuged and left to equilibrate at room temperature for 30 min. Sample extraction was performed in two steps. First, spiked samples were immersed in acetone, mechanically homogenized with a mixer, and then sonicated in an ultrasound bath for 10 min after the addition of 2 mL methanol, followed by reduction of the total extract volume to 2 mL by evaporation under a gentle nitrogen stream at room temperature. In a second step, lipids were removed by transferring the remaining extract to a 2 mL Eppendorf tube, which was kept at −15 °C

for 15 min and then centrifuged at 18213 ×*g* (at 4 °C) for 10 min. Next, the organic phase was transferred to a new glass tube, resuspended in 200 μL methanol/20% HCOOH (1:1) + 300 μL of 1 M ammonium acetate buffer (pH 5.5) and centrifuged twice more to remove the remaining lipids. Finally, the supernatant was transferred to an HPLC vial for liquid chromatography-tandem mass spectrometry (LC-MS/MS) analyses. Nine npEPs and seven parabens (sum of unconjugated, deglucuronidated and desulfated compounds) were determined by TurboFlow liquid chromatography-tandem mass spectrometry (LC-MS/MS), as described elsewhere (Frederiksen et al., 2013a; Frederiksen et al., 2011). For phenol analyses, the LC-MS/MS system was equipped with an atmospheric pressure chemical ionization source (APCI) running in negative mode and the injection volume was 100 μL, while an electrospray ion source (ESI) running in negative mode and injection volume of 20 μL were used for analyses of parabens. Further data on the flow rate, solvent programming and optimized instrument settings were previously described in detail for both phenol and paraben methods (Frederiksen et al., 2013a; Frederiksen et al., 2011).

Adipose samples were analysed in four batches over a period of six weeks. Each batch included standards for calibration curves (injected at beginning and end of each batch), 30–40 unknown samples, two blanks, two adipose tissue pool controls and two adipose tissue pool controls spiked at low and high levels, as previously described (Artacho-Cordón et al., 2017). In the absence of synthetic adipose tissue, adipose tissue pool samples were used as blanks, in addition to blanks without tissue. All selected npEPs were below the LOD in pool samples except for MeP and BP-3, whose concentrations were therefore subtracted to calculate concentrations in real samples. The inter-day variation, expressed as the relative standard deviation, was < 10%, while the recovery of spiked samples ranged from 85.4 to 107.6% for all analytes at low and high spike levels. Analyses were also repeated twice in a subset of samples to identify and track unintended contamination with the target analytes during handling in the laboratory, as reported elsewhere (Ye et al., 2013). Reagents and standards used were shipped as previously described (Artacho-Cordón et al., 2017). All reagents and solvents were of analytical, HPLC or MS grade, and all chemicals and laboratory equipment were tested for contamination before utilisation.

2.3. Sociodemographic and dietary information

Face-to-face interviews were conducted by trained interviewers during the hospital stay, gathering data on sociodemographic characteristics, lifestyle and dietary habits. Questionnaires and research procedures were standardised and validated in a pilot study with 50 subjects. Body mass index (BMI) was expressed as weight/height squared (kg/m^2). Distances from industry and agriculture and the number of years working in these sectors were gathered. Self-reported weight loss during the previous year was recorded as a dichotomous variable. A subject was considered a smoker (past or present) with any level of daily tobacco consumption (≥1 cig/day).

In a dietary section to assess food habits and eating behaviour, subjects indicated the frequency of their consumption of the following food groups: meat, fats (oil, butter and/or margarine), fish, eggs, cheese, bread, pasta, pulses, vegetables and fruit. The frequency of food consumption was gathered in four categories (< 1 portion/week, 1 portion/week, 2–6 portions/week, or > 7 portions/week) that were then recoded when a trend was detected to minimise the number of parameters in models.

2.4. Statistical analysis

In the descriptive analysis, npEP concentrations (ng/g tissue) were expressed as means with standard deviation (SD); 25th, 50th, 75th and 95th percentiles; and minimum and maximum concentrations. Adipose tissue concentrations of npEP below the LOD were assigned a value of LOD/√2. Spearman's test was used to evaluate monotonic correlations between different npEPs in adipose tissue.

All npEP concentrations were log-transformed to minimise the influence of extreme values; therefore, β coefficients are also presented as exp(β). Potential predictors of adipose tissue concentrations of environmental phenols and parabens were assessed by multivariable linear regression analysis using a combination of backward and forward stepwise multiple linear regression. Determinants of npEPs for which < 75% of samples were above the LOD were considered as dichotomous variables (< LOD/ > LOD), and predictors were evaluated using multivariable logistic regression models. Age, gender, BMI, study area and educational level were always kept in the analyses, regardless of their statistical significance, given published evidence of their potential association. In addition, the final models were further adjusted for adipose tissue source. The significance level was set at $p = 0.05$, and all tests were two-tailed. R statistical computing environment 3.0 (http://www.r-project.org/) and SPSS Statistics 22.0 (IBM, Chicago, IL) were used for data analyses.

3. Results and discussion

3.1. Baseline characteristics of study participants

Table 1 summarises the main characteristics of the study population. The mean (± SD) age of the 144 participants was 52.3 (± 17.8) years. There was a slightly higher proportion of males than females (61.1 vs. 38.9%) and of overweight/obese than normal weight individuals (66.7 vs. 33.3%). Almost half of the population (44.4%) reported weight loss during the previous year; 32 participants (22.2%) lived in the semirural area, 102 (70.8%) had at least primary studies and 55 (38.2%) were smokers at recruitment.

> 70% of participants declared the consumption at least twice weekly of fish, vegetables and fruit, while 66 (45.8%), 90 (62.5%), 71 (49.3%) and 43 (29.9%) participants reported the consumption at least twice weekly of cheese, meat, pulses and eggs, respectively. Nearly 10% and 90% of participants reported the daily consumption of fats and bread, respectively, while three-quarters of the population consumed pasta once per week or less.

3.2. Concentrations of environmental phenols and parabens in adipose tissue

All analysed samples were positive for ≥1 of the selected xenobiotics, and the majority of samples ($n = 87$, 60.4%) were positive for ≥4 compounds (Fig. 1). Frequencies of detection and adipose tissue npEP concentrations are reported in Table 2. With regard to environmental phenols, detectable levels of BPA, BP-3, TCS and 2-PP were found in 86.8%, 79.2%, 45.8% and 18.8% of the analysed samples, respectively, with median concentrations of 0.54 ng/g tissue for BPA and 0.36 ng/g tissue for BP-3. Detectable levels of 2,4-DCP were observed in 9 out of 144 samples and TCCB in 2. Regarding parabens, MeP was detected in all adipose tissue samples, while n-PrP and EtP were detected in 54.2% and 20.1%, respectively, with a median concentration of 0.40 ng/g tissue for MeP and 0.06 ng/g tissue for n-PrP. n-BuP and i-BuP concentrations above LOD were recorded in 8 and 3 of the 144 samples, respectively. Thus, these results confirm the presence of these xenobiotics in the adipose tissue of a relatively large population sample. A wide variability in exposure levels was found among participants, with some samples showing 10 to 50-fold higher levels than the median level in the population. 2,5-DCP, 2,4,5-TCP, 4-PP, iPrP and BzP were not detected in any of the samples.

This research also represents the largest study to date on the adipose tissue burden of environmental phenols and parabens in a human cohort. Only a few studies (summarised in Supplementary Table 2) previously analysed adipose tissue concentrations of these npEPs, and their sample sizes were relatively small (Artacho-Cordón et al., 2017; Barr et al., 2012; Darbre et al., 2004; Fernandez et al., 2007; Geens et al.,

Table 1
Characteristics of the study population (n = 144).

	n (%)
Age (years)[a]	52.3 ± 17.8
Gender	
Female	56 (38.9)
Male	88 (61.1)
Body mass index (Kg/m^2)	
Normalweight (18–25 kg/m^2)	48 (33.3)
Overweight/obese (> 25 kg/m^2)	96 (66.7)
Study area	
Semi-rural	32 (22.2)
Urban	112 (77.8)
Educational level	
Without studies	42 (29.2)
Primary studies	69 (47.9)
Secondary school/university	33 (22.9)
Recent weight loss	64 (44.4)
Menopausal status (only women)	
Premenopausal	22 (39.3)
Postmenopausal	34 (60.7)
Accumulated lactation time (years)[a]	
(only women)	0.5 ± 0.4
Current smoker	55 (38.2)
Residential distance to industry	
< 100 m	27 (18.8)
≥100 m	117 (81.3)
Working in industry in the last 10 years	
Yes	22 (15.3)
No	122 (84.7)
Residential distance to agriculture	
< 100 m	68 (47.2)
≥100 m	76 (52.8)
Working in agriculture (accumulated years)[a]	0.2 ± 1.2
Fish consumption	
< 2 portions/week	42 (29.2)
≥2 portions/week	102 (70.8)
Oily fish consumer	103 (71.5)
White fish consumer	106 (73.6)
Cheese consumption	
≤2 portions/week	76 (52.8)
> 2 portions/week	66 (45.8)
Meat consumption	
≤2 portions/week	51 (35.4)
> 2 portions/week	90 (62.5)
Fat consumption	
> 1 portion/day	127 (88.2)
Everyday	14 (9.7)
Pulse consumption	
≤2 portions/week	73 (50.7)
> 2 portions/week	71 (49.3)
Egg consumption	
≤2 portions/week	101 (70.1)
> 2 portions/week	43 (29.9)
Bread consumption	
> 1 portion/day	9 (6.3)
Everyday	135 (93.8)
Pasta consumption	
≤1 portion/week	108 (75.0)
> 1 portion/week	36 (25.0)
Vegetable consumption	
≤1 portion/week	41 (28.5)
2 portions/week	43 (29.9)
> 2 portions/week	60 (41.7)
Fruit consumption	
≤2 portions/week	26 (18.1)
> 2 portions/week	118 (81.9)
Organic food consumer	52 (36.1)

[a] Mean ± standard deviation.

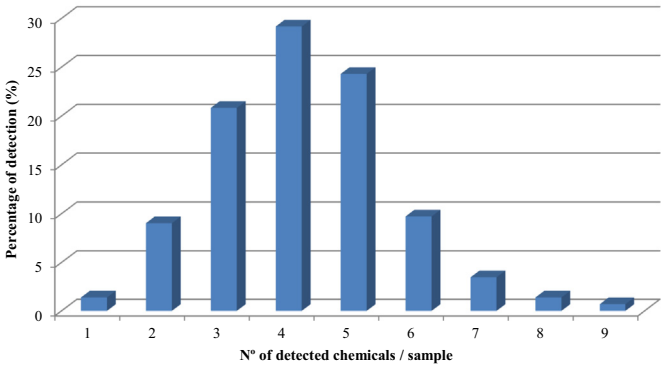

Fig. 1. Frequency of detection of npEPs in adipose tissue from the study population.

2012b; Wang et al., 2015). The detection frequency of BPA in our series was similar to that observed by Geens et al. (2012b) (100%) and Wang et al. (2015) (90.0%), but the median adipose tissue BPA concentration (0.54 ng/g tissue) was lower than reported in populations from the USA, Belgium and Spain (Fernandez et al., 2007; Geens et al., 2012b; Wang et al., 2015). BP-3 was detected in the large majority of adipose tissue samples (79.2%), as in our pilot study (Artacho-Cordón et al., 2017) and in agreement with Wang et al. (2015), but the geometric mean concentration was markedly lower than in the latter study (0.36 vs. 43.4 ng/g tissue, respectively). The detection frequency (45.8%) of TCS in our population were similar to those reported by Geens et al. (2012b), who detected TCS in nearly half of their samples. By contrast, Wang et al. (2015) found detectable TCS levels in all samples from adults in the USA (n = 20). The lack of similar studies hampers comparisons with other populations in the detection frequencies and concentrations of the remaining environmental phenols (2-PP, 2.5-DCP, 2.4.5-TCP, 4-PP and 2.4-DCP).

In relation to parabens, MeP was detected in all the samples from our cohort, similar to the finding by Barr et al. (2012) in a UK population but much higher than the frequency of 25% reported by Wang et al. in the USA (Wang et al., 2015). Median MeP adipose tissue concentrations (0.40 ng/g tissue) were markedly lower than those observed by Barr et al. (2012). n-PrP, EtP and BuP were detected in > 50%, > 20% and > 5% of adipose tissue samples, respectively, in agreement with most studies that assessed exposure to parabens in other biological matrices (de Renzy-Martin et al., 2014; Frederiksen et al., 2013b; Jiménez-Díaz et al., 2016; Philippat et al., 2012). However, Barr et al. (2012), Darbre et al. (2004) and Wang et al. (2015) reported higher detection frequencies for EtP than for n-PrP in adipose tissue. The median adipose tissue n-PrP concentration in the present adult cohort was lower than was observed by Barr et al. (2012) in 40 breast adipose tissue samples from women with primary breast cancer. These findings might be partially explained by sociodemographic, dietary and lifestyle differences in the target populations, although further research is needed to clarify this issue.

Higher levels of BPA, BP-3 and MeP were found in the adipose tissue from limbs, although only 13 individuals were in this category (Supplementary Table 3). Additionally, samples collected from the pelvic waist showed 2-fold higher n-PrP concentrations in comparison to the other sources.

Table 3 displays the results of correlation tests between pairs of xenobiotics in the adipose tissue samples, showing positive correlations between pairs of parabens (Spearman ρ ranging from 0.273 to 0.595, $p < 0.001$). Similar results were found in previous studies of urine samples, indicating that these parabens might share sources of exposure (Jiménez-Díaz et al., 2016; Larsson et al., 2014). BPA was positively associated with both MeP and n-PrP (ρ = 0.384 and 0.201, respectively) in the present study, and these correlations have not been found in other biological matrices (Frederiksen et al., 2013b; Jiménez-Díaz et al., 2016). Finally, positive correlations were found among BP-3, TCS and 2-PP (ρ ranging from 0.188 to 0.419, $p < 0.05$). Our group previously found no correlation between adipose tissue concentrations of selected npEPs and their urine or serum concentrations, with the exception of BP-3 (Artacho-Cordón et al., 2017), suggesting that adipose tissue concentrations of npEPs might offer different information from

Table 2
Levels of npEPs in adipose tissue (n = 144) (ng/g tissue).

		LOD	% > LOD	Percentiles				Max
				25th	50th	75th	95th	
Phenols	BPA	0.143	86.8	0.23	0.54	0.95	3.25	7.88
	TCS	0.732	45.8	< LOD	< LOD	1.23	2.32	14.34
	TCCB	0.918	1.4	< LOD	< LOD	< LOD	< LOD	1.31
	BP-3	0.178	79.2	0.21	0.36	0.60	3.31	40.05
	2.4-DCP	0.102	6.3	< LOD	< LOD	< LOD	0.13	0.31
	2.5-DCP	1.830	0.0	< LOD	< LOD	< LOD	< LOD	< LOD
	2.4.5-TCP	0.486	0.0	< LOD	< LOD	< LOD	< LOD	< LOD
	2-PP	0.100	18.8	< LOD	< LOD	< LOD	0.28	0.46
	4-PP	1.310	0.0	< LOD	< LOD	< LOD	< LOD	< LOD
Parabens	MeP	0.057	100.0	0.22	0.40	0.71	1.32	4.45
	EtP	0.060	20.1	< LOD	< LOD	< LOD	0.13	2.99
	i-PrP	0.049	0.0	< LOD	< LOD	< LOD	< LOD	< LOD
	n-PrP	0.046	54.2	< LOD	0.06	0.14	0.38	15.19
	i-BuP	0.060	2.1	< LOD	< LOD	< LOD	< LOD	1.36
	n-BuP	0.075	5.6	< LOD	< LOD	< LOD	0.12	2.74
	BzP	0.052	0.0	< LOD	< LOD	< LOD	< LOD	< LOD

LOD: limit of detection: SD: standard deviation.

Table 3
Spearman correlation coefficients between pairs of npEPs in adipose tissue.

	BPA	TCS	BP-3	2-PP	MeP	EtP	n-PrP
BPA	–						
TCS	0.094	–					
BP-3	0.045	0.419**	–				
2-PP	0.063	0.188*	0.272**	–			
MeP	0.384**	0.097	−0.062	0.089	–		
EtP	0.132	0.146	−0.009	0.128	0.273**	–	
n-PrP	0.201*	0.075	−0.015	0.072	0.314**	0.595**	–

* $p < 0.05$.
** $p < 0.01$.

those measured in urine or serum. Further research is warranted to explore this possibility.

Comparison with other studies should be made with care, given their limited sample sizes and the reduced number of compounds assessed, which also hampers the elucidation of the real internal npEP levels of the general population. Discrepancies among studies may also be explained, at least in part, by differences in sociodemographic variables and dietary habits, among others.

3.3. Determinants of the concentrations of environmental phenols and parabens in adipose tissue

Sociodemographic and dietary determinants of the adipose tissue npEP concentrations are shown in Table 4 (linear regression models for BPA, BP-3 and MeP) and Table 5 (logistic regression models for TCS, 2-PP, EtP and n-PrP).

A quadratic relationship was observed between age and adipose tissue TCS concentrations and a positive association close to statistical significance between age and adipose tissue MeP concentrations (p-value = 0.060). Numerous publications in the past decade have reported higher urinary levels of BPA and parabens in younger adults, likely reflecting their greater use of personal care products (Engel et al., 2014; Jiménez-Díaz et al., 2016; Larsson et al., 2014). Conversely, increased adipose tissue MeP and TCS concentrations were observed in the older participants, similar to various observations on several persistent organic pollutants (Agudo et al., 2009; Artacho-Cordón et al., 2015a; Artacho-Cordón et al., 2015b; Ibarluzea et al., 2011). Indeed, previous studies suggested that npEPs may not be completely excreted, with part of them being retained in certain body compartments (Aubert et al., 2012; Doerge et al., 2012; Stahlhut et al., 2009). In this regard, a pharmacokinetic study revealed that unconjugated BPA levels remained up to 20 h in the adipose tissue, whereas serum concentrations are rapidly converted (< 5 h) into the nonestrogenic BPA monoglucuronide isoform (Doerge et al., 2012). Regarding parabens, pharmacokinetic studies in rats also revealed that 2% of total parabens was retained in several body tissues after subcutaneous administration (Aubert et al., 2012). Nevertheless, the positive association with age might be also a consequence of a lower metabolic activity in older individuals, which may delay the metabolism and clearance of these chemicals.

The higher adipose tissue BP-3 concentrations in the females than in the males are consistent with most studies on human exposure to environmental phenols and parabens (Engel et al., 2014; Smith et al., 2012), attributable to the presence of this compound in cosmetics and other personal care products that are more frequently used by women. More studies accounting for the use of specific personal care products are warranted in order to confirm this hypothesis.

It has been postulated that BPA and other npEP compounds are obesogens, i.e., promote adipogenesis (Grün and Blumberg, 2007), implying that the BMI of individuals would be higher with greater exposure. In the present study, 2-PP concentrations were lower in individuals perceiving significant weight loss over the previous 12 months than in those who did not, (Artacho-Cordón et al., 2015b). Although the mechanisms involved are not fully understood, the negative influence of weight loss on adipose tissue concentrations of xenobiotics may be related to their release during fat mobilisation, enhancing their exchange between different body compartments and therefore facilitating their elimination (De Roos et al., 2012; Kim et al., 2011b).

In addition, an inverse relationship close to statistical significance was observed between BMI and adipose tissue concentrations of BPA, TCS and 2-PP (0.060 < p-value < 0.090). It has been reported that severe obesity has a potential dilution effect on lipophilic chemicals, whose concentration may be decreased with increased adipose tissue volume (La Merrill and Birnbaum, 2011). This may lead to the detection of a negative association between exposure to obesogens and BMI when exposure estimates are based on adipose tissue concentrations (Smith et al., 2012).

Adipose tissue BPA and MeP concentrations were higher in the participants with more schooling, while residents of the semi-rural coastal area showed higher BP-3 and n-PrP concentrations that approached statistical significance (p-value = 0.095 and p-value = 0.050, respectively). Detectable adipose tissue concentrations of 2-PP were found in samples from participants who had worked in agriculture for a longer time, while elevated EtP concentrations were found in samples from those living at a distance from agricultural land and in those who

Table 4
Determinants of npEP adipose tissue concentrations. Multivariate linear regression analyses.

	BPA						BP-3						MeP					
	β	exp(β)	95%CI		*p*-value		β	exp(β)	95%CI		*p*-value		β	exp(β)	95%CI		*p*-value	
Age	0.00	1.00	0.99	1.02	0.467		0.00	1.00	0.99	1.01	0.671		0.01	1.01	1.00	1.02	0.060	*
Gender = male[a]	0.00	1.00	0.70	1.43	0.994		−0.46	0.63	0.43	0.93	0.021	**	0.00	1.00	0.76	1.32	0.998	
BMI (kg/m^2)	−0.03	0.97	0.94	1.00	0.086	*	−0.02	0.98	0.95	1.02	0.284		−0.02	0.98	0.95	1.01	0.110	
Study area = semi-rural[b]	0.23	1.26	0.83	1.92	0.282		0.36	1.44	0.94	2.20	0.095	*	0.17	1.18	0.85	1.64	0.322	
Educational level = up to primary[c]	0.27	1.31	0.84	2.05	0.238		−0.06	0.94	0.60	1.47	0.791		0.21	1.23	0.88	1.72	0.218	
Oily fish consumption = yes[d]	0.34	1.40	0.94	2.08	0.096	*	–	–	–	–	–		–	–	–	–	–	
White fish consumption = yes[e]	–	–	–	–	–		0.43	1.54	1.01	2.33	0.045	**	–	–	–	–	–	
Fat consumption (oil, butter, margarine) = daily[f]	0.62	1.85	1.04	3.30	0.036	**	–	–	–	–	–		–	–	–	–	–	
Current/former smoker = yes[g]	–	–	–	–	–		0.40	1.49	1.02	2.18	0.039	**	–	–	–	–	–	
Cheese consumption = daily[h]	–	–	–	–	–		0.40	1.49	0.96	2.31	0.074	*	–	–	–	–	–	
Bread consumption > 1 portion/week[i]	–	–	–	–	–		–	–	–	–	–		0.65	1.92	1.00	3.70	0.050	**

CI: confidence intervals.
Only statistically significant variables were included in the models, with the exception of age, gender, BMI, study area and educational level.
[a] Reference category = female.
[b] Reference category = Granada.
[c] Reference category = without studies.
[d,e] Reference category = No.
[f] Reference category = ≤2 portions/week.
[g] Reference category = ≤1 portion/week.
[h] Reference category ≤1 portion/day.
[i] Reference category ≤1 portion/week.
* $p < 0.10$.
** $p < 0.05$.

had been worked in industry for longer. Given that 2-PP is a fungicide used in fruit and vegetable faming (Blasco et al., 2002), these results may indicate occupational exposure to 2-PP. We highlight the elevated intensive greenhouse agricultural activity in Southern Spain, in which pesticides are concentrated in an enclosed space under high humidity and temperature conditions with inadequate or no air renewal (Olea-Serrano et al., 1999), facilitating contamination of the workers and the foods.

Regarding dietary habits, the consumption of fatty food proved to be a relevant predictor of adipose tissue concentrations of npEPs in this cohort. Specifically, the daily consumption of butter and/or margarine was positively associated with adipose tissue TCS, BPA and EtP concentrations, while the consumption of fish was positively associated with adipose tissue 2-PP concentrations. Adipose tissue BPA concentrations were higher in participants declaring the consumption of oily fish, although the association did not reach statistical significance (*p*-value = 0.096), while BP-3 concentrations were higher in those declaring the consumption of white fish. A positive trend approaching statistical significance (*p*-value = 0.074) was also detected for BP-3 concentrations among cheese consumers. In this context, higher urinary BPA and parabens concentrations have been associated with an elevated consumption of fat, fish, or poultry (Larsson et al., 2014; Mervish et al., 2014). These studies did not distinguish between chemicals derived from the food itself (these foodstuffs are rich in fat) or leached from the plastic containers in which the food is usually bought. The few studies that have examined npEP levels in dietary items have published some evidence pointing to fish as a relevant source of BPA exposure (Corrales et al., 2015; Lindholst et al., 2001; Yang et al., 2014). Additionally, a global presence of parabens has been reported in several foodstuffs examined in the USA and China (Liao et al., 2013a; Liao et al., 2013b). MeP concentrations were increased in participants declaring the weekly consumption of bread, while a close to significant relationship was found between 2-PP concentrations and higher pasta consumption (*p*-value = 0.066). Higher urinary concentrations of npEPs were previously reported in consumers of grains and flour (Mervish et al., 2014), while it has also been hypothesised that these chemicals may migrate into these foods from their antibacterial plastic packaging (Jiménez-Díaz et al., 2016; Lu et al., 2014).

Finally, the consumption of vegetables was associated with lower adipose tissue 2-PP concentrations, while the consumption of fruit was associated with lower EtP concentrations, similar to previous findings in urine samples (Mervish et al., 2014). Interestingly, the participants reporting a diet based on organic food also showed lower concentrations of 2-PP (*p*-value = 0.064), which may reflect the reduced use of chemicals in organic agriculture.

Regarding the adipose tissue source, it was only related to n-PrP concentrations, although the association was only close to statistical significance (*p*-value = 0.063). This is of interest given previous findings of no significant differences in paraben adipose tissue concentrations among four different regions of the breast, with the exception of higher levels of n-PrP in axilla versus mid or medial regions (Barr et al., 2012).

Our study has several limitations. It is a hospital-based study and therefore not entirely representative of the general adult population in the study area. In addition, although our research is the largest investigation of adipose tissue npEPs to date, the limited sample size may preclude detection of a wider range of associations. Furthermore, given the cross-sectional study design and the lack of information on the contribution of recent exposures to the adipose tissue burden of npEPs, we cannot rule out reverse-causality in some associations. Data were not available on the use of cosmetics and personal care products by our cohort, although information gathered on other variables (e.g., occupation, social class and schooling) may help to identify population subgroups characterized by the use of these products.

To the best of our knowledge, this study is among the very first to contribute evidence on the distribution and predictors of environmental phenols and parabens in adipose tissue from an adult cohort, showing the widespread presence of certain npEPs in the fat compartment. We consider these results of special interest to public health, given the increasing importance of adipose tissue as a biologically-active matrix, highly relevant in the development of chronic diseases (Kershaw and Flier, 2004). Further research on the potential health implications of our findings is currently being addressed in the GraMo cohort.

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Table 5
Determinants of npEP adipose tissue concentrations. Multivariate logistic regresion analyses.

	TCS				2-PP				EtP				n-PrP			
	OR	95%CI		p-value	OR	95%CI		p-value	OR	95%CI		p-value	OR	95%CI		p-value
Age	1.21	1.06	1.41	0.009 ***	0.98	0.95	1.02	0.343	0.99	0.95	1.02	0.557	1.01	0.99	1.04	0.312
(Age)^2	1.00	1.00	1.00	0.005 ***	–	–	–	–	–	–	–	–	–	–	–	–
Gender = male[a]	1.15	0.53	2.55	0.723	0.53	0.17	1.57	0.252	0.61	0.21	1.77	0.363	1.77	0.79	4.10	0.173
BMI (kg/m^2)	0.92	0.84	1.00	0.060 *	0.90	0.78	1.01	0.099 *	1.02	0.92	1.14	0.654	0.98	0.91	1.06	0.650
Study area = semi-rural[b]	1.02	0.40	2.55	0.972	0.45	0.10	1.70	0.266	1.93	0.61	5.96	0.251	2.58	1.03	7.02	0.050 *
Educational level = up to primary[c]	0.72	0.27	1.90	0.515	0.78	0.18	3.50	0.732	0.68	0.19	2.48	0.557	1.99	0.81	5.09	0.140
Perceived weight loss = yes[d]	–	–	–	–	0.23	0.06	0.71	0.015 **	–	–	–	–	–	–	–	–
Distance to industry ≥ 100 m[e]	0.25	0.09	0.65	0.006 ***	–	–	–	–	–	–	–	–	–	–	–	–
Working at industry in the last 10 years = yes[f]	–	–	–	–	–	–	–	–	6.85	2.05	24.41	0.002 ***	–	–	–	–
Distance to agriculture ≥ 100 m[g]	–	–	–	–	–	–	–	–	3.80	1.24	13.89	0.028 **	–	–	–	–
Work in agriculture (years)	–	–	–	–	1.04	1.01	1.09	0.027 **	–	–	–	–	–	–	–	–
Fat consumption (oil, butter, margarine) = daily[h]	4.42	1.24	18.56	0.028 **	–	–	–	–	5.81	1.41	24.26	0.014 **	–	–	–	–
Fish consumption ≥ 2 portions/week[j]	–	–	–	–	3.43	1.02	14.56	0.064 *	–	–	–	–	–	–	–	–
Pasta consumption > 1 portion/week[k]	–	–	–	–	3.52	0.92	14.06	0.066 *	–	–	–	–	–	–	–	–
Vegetable consumption > 2 portion/week[l]	–	–	–	–	0.28	0.07	0.94	0.051 *	–	–	–	–	–	–	–	–
Fruit consumption > 2 portions/week[m]	–	–	–	–	–	–	–	–	0.32	0.09	1.05	0.059 *	–	–	–	–
Organic food consumption = yes[n]	–	–	–	–	0.31	0.08	1.01	0.067 *	–	–	–	–	–	–	–	–
Adipose tissue source = pelvic waist[o]	–	–	–	–	–	–	–	–	–	–	–	–	0.46	0.20	1.03	0.063 *
Adipose tissue source = limbs[o]	–	–	–	–	–	–	–	–	–	–	–	–	0.34	0.08	1.28	0.117

CI: confidence intervals.
Only statistically significant variables were included in the models, with the exception of age, gender, BMI, study area and educational level. In TCS multivariable model, a quadratic term was included for age

[a] Reference category = female.
[b] Reference category = Granada.
[c] Reference category = without studies.
[d] Reference category = No.
[e] Reference category ≤100 m.
[f] Reference category = No.
[g] Reference category ≤100 m.
[h] Reference category ≤1 portion/week.
[j] Reference category ≤2 portions/week.
[k] Reference category = ≤1 portion/week.
[l,m] Reference category = ≤2 portions/week.
[n] Reference category = No.
[o] Reference category = front abdominal wall.
* $p < 0.10$.
** $p < 0.05$.
*** $p < 0.001$.

Conflict of interest

The authors declare no conflicts of interest to disclose.

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