Levels and determinants of adipose tissue cadmium concentrations in an adult cohort from Southern Spain

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Abstract

This study was conceived as a first step to evaluate the suitability of adipose tissue Cadmium (Cd) concentrations as a biomarker for the assessment of long-term exposure. Specifically, the aim of this work was to explore the socio-demographic, dietary, and lifestyle determinants of adipose tissue Cd concentrations.

The study population is a subsample of GraMo cohort. Adipose tissue samples were intraoperatively collected from 226 adult volunteers recruited in two public hospitals from Granada, Spain. Cd Concentrations in adipose tissue were analyzed by High-Resolution Inductively Coupled Plasma Mass Spectrometry (HR-ICP-MS). Data on socio-demographic characteristics, lifestyle, diet and health status were collected by face-to-face interviews. Predictors of Cd concentrations were assessed by multivariable linear regression with a stepwise variable selection.

We found detectable levels of Cd in the adipose tissue of all the study participants, with a mean concentration (±standard deviation) of 12.66±18.91 µg/Kg. Smoking habit at recruitment was associated with increased adipose tissue Cd concentrations (β for smokers=0.669 $p<0.001$; β for former smokers=0.502, $p<0.001$; reference=non-smokers). Age was positively associated with Cd concentrations ($\beta=0.014$, $p<0.001$), and men showed lower concentrations than women ($\beta=-0.424$, $p<0.001$). Obesity, measured as Body Mass Index (BMI), showed an inverse association with Cd concentrations ($\beta=-0.038$, $p<0.001$). Egg consumption ≥2 portions/week ($\beta=0.241$, $p=0.025$) was positively associated with Cd concentrations. Perceived exposure to paints was also positively associated with Cd concentrations. The observed associations with age, smoking habit, BMI and egg and meat consumption did not substantially change after sex/gender stratification. Our results are consistent with currently-known Cd sources and suggest other potential pathways, which might be population-specific.

As a whole, our findings underline the potential relevance of adipose tissue as a biological matrix for exposure characterization to Cd, as well as for the assessment of long-term clinical implications of the exposure, particularly in obesity-related diseases.

Keywords: Cadmium; adipose tissue; exposure predictors; sociodemographic characteristics; diet; lifestyle.

1. Introduction

Cadmium (Cd) is a metallic element included in the group 12 of the periodic table, with atomic number 48. Cd has been widely used in industrial processes, such as certain paints, Cd-Nickel batteries, as an estabiliser in thermoplastics (e.g. polyvinyl chloride), as well as in photography, lithography, tyres and photoelectric cells in solar panels (Herron, 2003). Cd can also be found as an impurity in zinc, lead and copper ores and alloys, iron, steel, fossil fuels, cement and some fertilizers (IARC, 2012). Phosphorous fertilizers are frequently used in greenhouses (Rodríguez-Martín et al., 2013), which can be a source of Cd contamination of soils, water, and, consequently, the trophic chain (Pan et al., 2010). Other antropogenic sources include recycling, mining and smelting of zinc-bearing ores, the incineration of waste, the combustion of fossil fuels, and the releases of landfills, among others. The global Cd emmission in the mid-90s was estimated in 3000 Tm, decreasing by a half in Europe in the period 1990-2003 (UNEP, 2008). Natural sources of Cd can be found in the Earth's crust and oceans with an average abundance of 0.1-0.2 mg/kg.

Cd is considered a relevant persistent pollutant which is slowly degraded in the environment and living organisms (Järup, 2003). Consequently, some of the abovementioned uses have been restricted under the REACH regulation in the European Union in recent years (European Parliament and Council Directive 2013/56/EU, European Commission Regulation 494/2011). However, the majority of the general population still show detectable levels of Cd in their blood and urine (López-Herranz et al., 2016; Pirard et al., 2018).

Food ingestion and smoking are considered the main sources of Cd exposure in the general population. Ambient air, drinking-water, contaminated soils and dust can also contribute to the exposure, although to a lesser extent, but they could be more relevant for children (Schwartz, 2004). High concentrations of cadmium are commonly found in leafy vegetables, starchy roots, cereals/grains, nuts and pulses, and also in specific animal products, e.g. kidney, liver and certain shellfish (IARC, 2012). The mean dietary exposure for the European general population is estimated in 2.3 µg/kg b.w. per week (EFSA, 2009), which is in a similar range than the Spanish population (AESAN, 2011). Vegetarians frequently have an increased dietary exposure (up to 5.4 µg/kg b.w. per week), as well as regular consumers of bivalve molluscs and wild mushrooms, that also show increased exposure levels (4.6 and 4.3 µg/kg b.w. per week respectively) (IARC, 2012). Smoking is considered a relevant source of Cd exposure because of the relatively high concentrations in tobacco leaves. In 1988 the Joint FAO/WHO Expert Committee on Food Additives established a health based guidance value for cadmium of 7 µg/kg b.w. per week. In 2009 the CONTAM Panel of the European Food Safety Authority (EFSA) established a tolerable weekly intake of 2.5 µg/kg b.w., which can be easily exceeded in vegetarians, smokers, children and people living in contaminated areas (EFSA, 2009).

Although the health effects of the chronic exposure to low doses of Cd (such as those occuring in the general population) remain unclear, there are certain evidences of renal dysfunction and urinary stone disease, hypertension, lung and prostate cancer, osteoporosis, low birth weight in the offspring, spontaneus miscarriage, obesity, and diabetes (Prozialeck and Edwards, 2012; IARC, 2012; Tinkov et al., 2017). Indeed, Cd is classificated in the category 1 (carcinogenic to humans with a sufficient evidence in humans) by the International Agency for Research on Cancer (IARC, 2012).

Biomonitoring studies are frequently used to assess human exposure to environmental pollutants and their health implications, since internal levels of a pollutant typically account for the overall exposure from different sources and exposure pathways (Needham et al., 2007). The most common human biological matrices used to assess internal levels of Cd are blood and urine, although some research has been performed on hair, nails and saliva. The half-life of Cd strongly differs among biological compartments, e.g. from up to 60 years in the kidneys (Ramírez, 2002) to 3-4 months in blood (Talio et al., 2010). However, there is scant research on Cd concentrations in the adipose tissue, which is considered the main reservoir of lipophilic pollutants as well as an important biological matrix in the development of chronic non-infectious diseases, e.g. cancer and metabolic syndrome. Indeed, adipose tissue concentrations are considered the most precise and stable estimator for the evaluation of accumulated exposure to other moderately lipophilic pollutants, such as persistent organic pollutants (Kohlmeier and Kohlmeier, 1995; Artacho-Cordón et al., 2015). This might be extended to Cd considering its lipophilic characteristics, e.g. log K_{ow}=3.86 (Sakultantimetha et al., 2009). Additionally, adipose tissue is a target for essential trace elements, as an organ in which they can perform their biological effects. Particularly, *in vitro* experiments with adipocytes have shown that chromium enhances GLUT 4 translocation, which can turn out in increased glucose transport in fat cells (Tinkov et al., 2015; Wiernsperger and Rapin, 2010). Additionally, vanadium has shown an insulin-mimetic potential, although its role as an essential trace element in humans remains unclear (Wiernsperger and Rapin, 2010). Moreover, there are experimental evidences that some trace elements in adipose tissue can induce insuline resistance and hypertriglyceridemia in *in vivo* models, suggesting a potential metabolic disrupting effect that should be confirmed in further epidemiologic studies (Hubler et al., 2015; Tinkov et al., 2015). Nevertheless, research on the adipose tissue concentrations of toxic and essential trace elements, as well as their biological implications, is still very scarce. Thus, our study aims to shed some light in these aspects.

Considering the abovementioned statements, the present study was conceived as a first step to evaluate the suitability of adipose tissue Cd concentrations as a biomarker for the assessment of long-term exposure. Specifically, the aim of this work was to explore the socio-demographic, dietary, and lifestyle determinants of adipose tissue Cd concentrations.

2. Materials and methods

2.1. Study area, design and characteristics of participants

This study is part of a wider research which aims to analyse and identify environmental factors affecting the development of chronic diseases in GraMo, an adult cohort in Southern Spain. Study subjects were recruited in two public hospitals from Granada province: San Cecilio University Hospital in the city of Granada (240,000 inhabitants, urban area), and Santa Ana Hospital in the town of Motril (50,000 inhabitants, semi-rural area). The recruitment of the population has been extensively described elsewhere (Arrebola, 2009). The cohort was recruited in 2003-2004, from patients undergoing non-cancer-related surgery [hernias (41%), gallbladder diseases (21%), varicose veins (12%) and other conditions (26%)]. All subjects signed the informed consent forms and the study was approved by the Ethics Committee of Granada (Comité de Ética de la Investigación Biomédica de la Provincia de Granada).

Out of the 409 individuals contacted, 387 (95%) agreed to participate and were included in the initial cohort. Adequate adipose tissue samples for TE analyses were obtained from 226 (58%), that were used for cross-sectional analyses in the present study. Adipose tissue was collected from pelvic waist (50%), front abdominal wall (40%) and limbs (10%). There were no statistically significant differences in sex/gender or age distributions between participants and non-participants. The main characteristics of the participants are shown in Table 1.

2.2. Independent variables

Socio-demographic characteristics, life-style, diet and health status data were gathered in face-to-face interviews, conducted by trained personnel at the recruitment during their hospital stay. Research procedures were standarised and validated in a pilot study with 50 subjects. The questionnaire was designed and validated in a previous investigation (Buckland et al., 2009; González and Riboli, 2010).

Body mass index (BMI) was expressed as weight/height squared (kg/m^2). Participants were considered smokers or alcohol consumers at any level of consumption. Residence in the city of Granada at the time of the surgery was considered “urban” and residence in the area of Motril was considered “semi-rural”. The dietary section comprised a food frequency questionnaire that included the following food groups: meat, cold meats, fats, fish, eggs, milk, cheese, vegetables, legumes, fruit, bread, and pasta.

2.3. Sampling and Cd analyses

Samples of 5–10 g of adipose tissue were intra-operatively collected and immediately coded and stored at −80 °C until chemical analysis. The adipose tissue samples were freeze-dried in a liophilizator for a minimum of 72 hours, until they reached a plateau weight, at the Slovenian National Building and Civil Engineering Institute (ZAG) in Ljubljana (Slovenia). Then, they were kept at -80ºC until their analysis. Subsamples of 0.1 g of adipose tissue underwent total digestion with a mixture of 7 mL of HNO_3 and 0.1 mL of HF in a microwave oven (Multiwave 3000, Anton Paar, Graz, Austria). The internal standard added to the digested samples was 1 µg/L of Indium. The multielement analyses of the samples were performed in 2015 at the Laboratory for Inorganic Environmental Geochemistry and Chemodynamics of Nanoparticles, Ruđer Bošković Institute, Zagreb (Croatia), by High-Resolution Inductively Coupled Plasma Mass Spectrometry (HR-ICP-MS) using an Element 2 instrument (Thermo, Bremen, Germany). Protocols, conditions and parameters have been described elsewhere (Rodríguez-Pérez et al., 2018).

2.4. Statistical analyses

First, the shapes of the relationships between potential continuous predictors and Cd concentrations were visually evaluated through locally weighted scatterplot smoothing (LOWESS) and Generalized Additive Models (GAM). Next, predictors of Cd concentrations were examined in multivariable linear regression models, using a combination of backward and forward stepwise variable selection techniques. In order to assess sex/gender-specific predictors, models were stratified by sex/gender (male/female), and women-specific predictors (i.e., accumulated lactation time, gravidity, and menopausal status), were entered in the model for females. In pursuance to assess the potential influence of the different sources of adipose tissue (waist, abdomen and limbs) in the associations found, multivariable models were adjusted for this variable, but no relevant modifications in the coefficients were observed (data not shown in tables). The level of statistical significance was set at p=0.050 (p=0.100 as borderline significant). In the multivariable models, Cd concentrations were entered as a log-transformed variable.

Generalized standard-error inflation factors were used to verify the absence of collinearity between independent variables, while the homoscedasticity was tested by plotting residual against fitted values. The linearity of quantitative independent variables was checked with partial regression plots, and the normality of errors was verified using normal QQ plots with 95% confidence intervals. Data analyses were performed using R statistical computing environment v3.0 (R Core Team, 2013) and SPSS Statistics 22.0 (IBM Corp., 2013).

3. Results and discussion

3.1. Adipose tissue Cd concentrations in the study population

The adipose tissue concentrations of Cd and the characteristics of the study population are summarized in Table 1. We detected Cd in all (100%) the analyzed samples, which are depicted in Figure 1.

TABLE 1. Study population characteristics and Cd concentrations

			N	%
SOCIODEMOGRAPHIC	Sex/gender	Women	99	43.8
		Men	127	56.2
	Residence	Urban	109	48.2
		Rural/semi-rural	117	51.8
	Education	Up to primary studies	71	31.4
		Secondary/university	155	68.6
LIFESTYLE AND DIET	Smoking	Non-smoker	90	40.0
		Current smoker	68	30.0
		Former smoker	68	30.0
	Alcohol consumer		114	50.4
	Cheese consumption	<2 portions/week	107	47.1
		2-6 portions/week	70	31.1
		>6 portions/week	49	21.8
	Egg consumption	<2 portions/week	86	37.5
		=2 portions/week	73	32.6
		>2 portions/week	67	29.9
	Meat consumption	≤2 portions/week	85	37.4

		>2 portions/week	141	61.2
	Processed meat consumer		201	88.9
	Chicken consumer		175	77.4
	Vegetables consumption	≤1 portion/week	60	26.9
		=2 portions/week	61	27.4
		>2 portions/week	105	45.7
	Legumes consumption	<1 portions/week	13	5.8
		1-2 portions/week	120	52.7
		>2 portions/week	93	41.5
	Fish consumption	≤1 portions/week	69	30.7
		=2 portions/week	84	36.9
		>2 portions/week	73	32.4
	Fish type	Fatty fish	51	22.5
		Lean fish	70	31.0
		Both	105	46.5
	Self-perceived exposure to paints	Yes	43	19.0
WOMEN SPECIFIC	Post-menopausal		45	45.5
	Breastfeeding	Yes	77	89.5

	Mean	**Standard Deviation**	**Percentiles**	
			25th	**75th**
Age (yrs)	53.6	11.8	47.0	62.0
BMI (kg/m^2)	26.7	4.4	23.4	29.3
Water consumption (glasses/day)	5.8	4.2	3.0	8.0
Cd concentration in adipose tissue (ng/kg)	12.7	18.9	5.0	14.0
WOMEN SPECIFIC	**Mean**	**Median**	**25th**	**75th**
Number of pregnancies	2.8	3.0	2.0	4.0
Gravidity (number of	2.5	2.0	2.0	3.0

children)				
Accumulated lactation time (months)	6.4	4.5	2.0	8.0

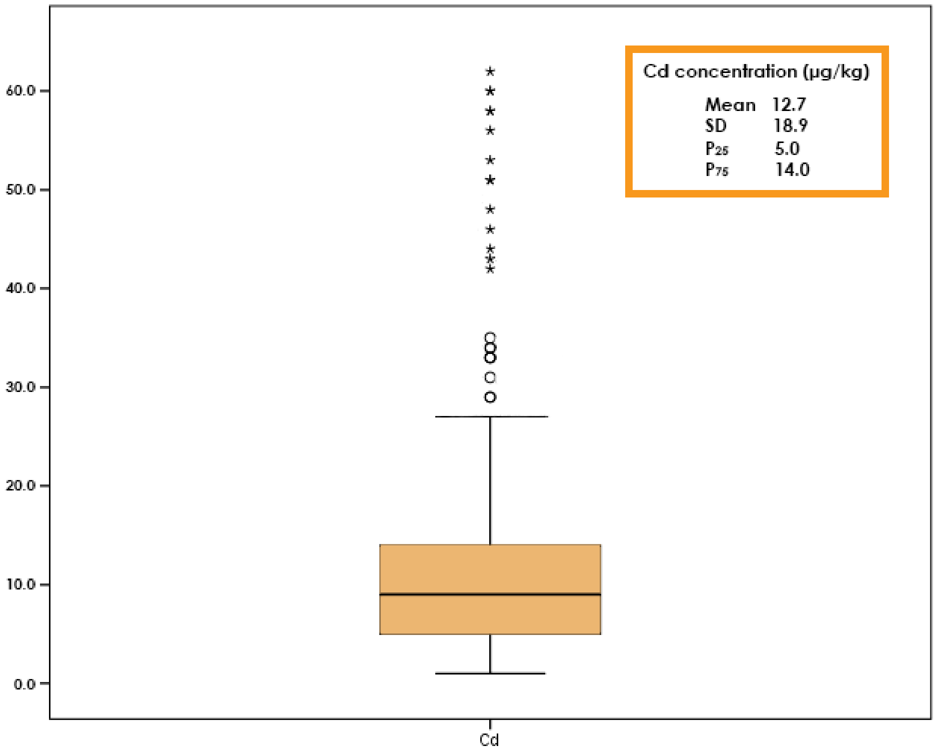

Figure 1. Adipose tissue Cd concentrations in the study population (µg/kg)

To the best of our knowledge, this is one of the very first epidemiogical studies exploring Cd concentrations in human adipose tissue. Qin et al. (2010) observed mean adipose tissue Cd concentrations of 0.47 µg/kg in patients with uterine leiomyoma and of 0.38 µg/kg in a control group. The concentrations in our population are higher, possibly due to the strong differences between the populations under study in terms of clinical background but also in the study regions, diet and lifestyle of Southern Spain vs. Hong Kong, where the study of Qin et al. (2010) was conducted.

Our results of detectable levels of Cd in all the study participants are in agreement with previous studies in other biological matrices. Previous researches with healthy volunteers found Cd 5th-95th percentiles of 0.15-2.04 µg/L in blood, 0.01-0.05 µg/L in plasma, 0.06-0.79 µg/L in urine, 0.004-

0.17 ng/mg in hair (Goullé, 2005). Other authors report reference levels (95th percentile) of 0.55 µg/L in blood, 0.32 µg/L in urine, 0.41 ng/mg in hair, 0.018 ng/mg in nails and 0.43 µg/L in saliva (Tirado et al., 2015). Our results in adipose tissue (12.66 µg/kg) are extremely difficult to compare to those obtained in other matrices. Differences are expected according to the matrix composition (with different affinities of Cd to certain molecules), sample accessibility and available amount, etc.

Cd is considered a persistent and bioaccumulable element. After ingestion and absorption, Cd is mainly transported bound to certain low-molecular-weight thiols, such as metallothionein and glutathione (Zalups and Ahmad, 2003). Around one third of Cd intake is transported to the kidneys, where Cd-MT complex is filtrated in the glomerulus and is reabsorbed in the proximal tubule, remaining in the tubulus cells for years (Cucu et al., 2011; Saboliç et al., 2010). Indeed, only 0,007% of the body burden is excreted via urine and 0.009% via feces (Kjellström and Nordberg, 1978). Cd is also partly secreted into the biliary tract in the form of Cd-Glutathione but, after enzimatic degradation to Cd-Cysteine complexes, Cd is reabsorbed in the small intestines. Liver and kidneys accumulate an estimated 50% of Cd body burden, while the rest is distributed widely in other organs and tissues (Hammond and Beliles, 1980). Cd can also form large aggregates with lipids (Kerek et al., 2017).

3.2. Predictors of adipose tissue Cd concentrations

Table 2 shows the results from the multivariable analysis of the potential predictors of adipose tissue Cd concentrations.

Table 2. Predictors of log-transformed adipose tissue Cd concentrations (µg/kg) in GraMo cohort (men and women). Multivariable linear regression analysis.

	Beta	Standard Error	p-value
Sex/gender=male	-0.424	0.106	<0.001
Age (years)	0.014	0.003	<0.001
BMI (Kg/m^2)	-0.038	0.009	<0.001

Smoking habit[1]			
Former smoker	0.502	0.124	<0.001
Current smoker	0.669	0.127	<0.001
Egg consumption[2]≥2 portions/week	0.241	0.107	0.025
Meat consumption[3]>2 portions/week	-0.210	0.105	0.046
Fish consumption[4]≥2 portions/week	0.160	0.108	0.140
Self-perceived exposure to paints	0.175	0.123	0.156

Multiple R^2=0.2596; adjusted R^2= 0.2284; p =<0.0001

[1] Ref. category: non-smoker

[2] Ref. category: egg consumption < 2 portions/week

[3] Ref. category: meat consumption ≤2 portions/week

[4] Ref. category: fish consumption <2 portions/week

Smoking habit emerged as a substantial predictor of Cd adipose tissue concentrations, in an apparently dose-response manner, with the current smokers showing the highest exposure levels (2-fold higher levels than non-smokers), followed by former smokers (1.7-fold higher levels). This finding is in agreement with previous investigations in other biological matrices, e.g. in blood, where smokers have been reported to exert even 4-5-fold higher Cd concentrations than non-smokers (Järup et al., 1998). Actually, each cigarette is estimated to contain 1.7 µg of Cd accumulated in tobacco leaves, and the smoker inhales up to 10% of this quantity (Morrow, 2001). The relatively strong positive association between smoking habit and adipose tissue Cd concentracions is depicted in Figure 2, with an increasing number of smokers/former smokers in the upper deciles of Cd concentrations. Our results support the consideration of smoking as the most relevant determinant of Cd concentrations in the body, as previously suggested by other researchers (Marano et al., 2012; Satarug and Moore, 2004). In fact, Ratelle et al. have recently identifed smoking as the main predictor of Cd concentrations in urine and blood in Northwestern Canadian communities population, even more important than a diet rich in animal organs, e.g. liver and kidney (Ratelle et al., 2018).

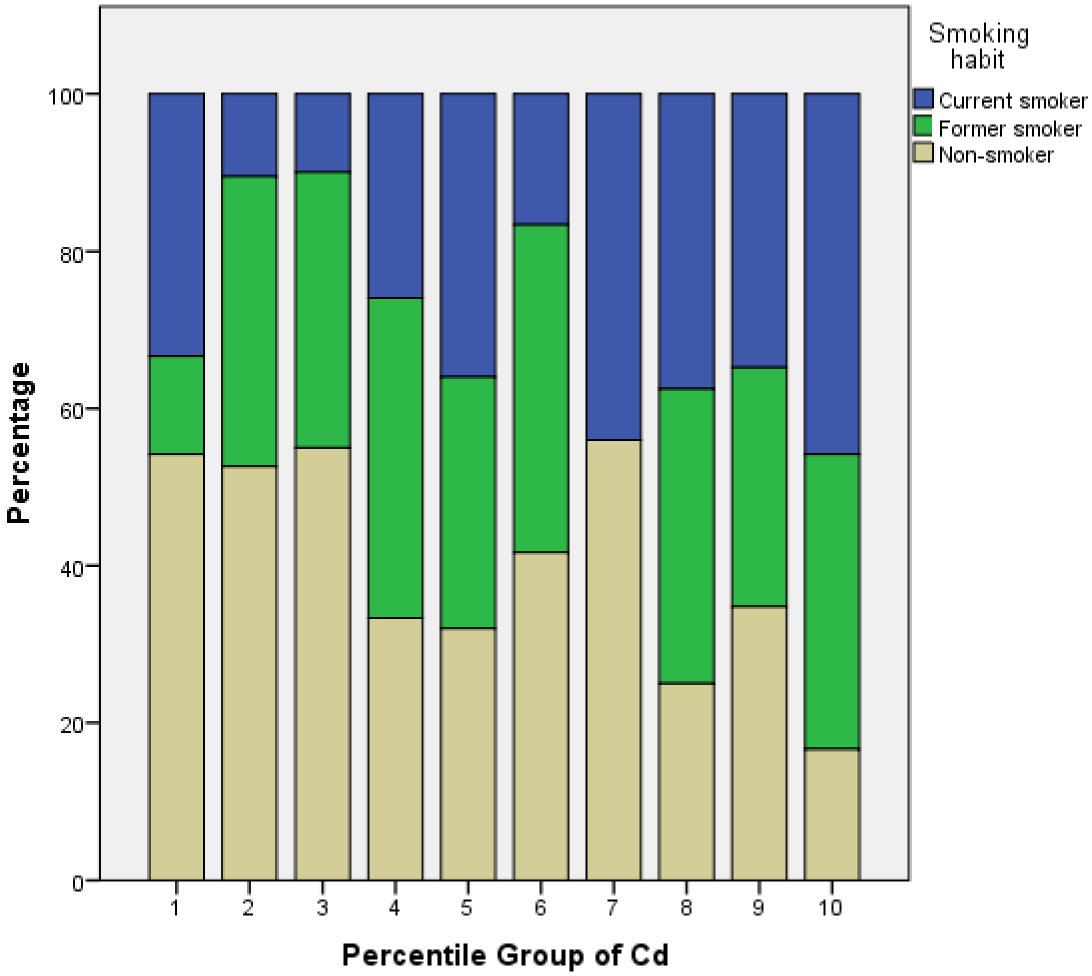

Figure 2. Smoking habit distribution according to deciles of adipose tissue Cd concentrations.

In the present study, we evidenced a positive association between age and Cd concentrations in adipose tissue. Despite the very limited previous research on adipose tissue, this finding is consistent with the bioaccumulative characteristics of this metal (Kjellström and Nordberg, 1978). Previous mathematical models in adult women predicted a lifespan monotonic curvilinear increase of urinary Cd with age until it leveled off at the age of 60-70 (Amzal et al., 2009). A similar plateau, around the age of 50, was found by Satarug et al., consistent with the degeneration of the kidney reabsortion function (Satarug et al., 2002). Results showing the increase of urine Cd with age have been reported in the United States, Canada, China, Korea and Spain (USCDC, 2009; Health Canada, 2010; Sun et al., 2016; Lee et al., 2012; Huang et al., 2013; López-Herranz et al., 2016).

In our opinion, the abovementioned findings in the multivariable models point to a certain degree of potential Cd bioaccumulation in adipose tissue, i.e. higher levels in the older participants as well as increased concentrations in former smoker *vs* non-smokers. Further research is warranted to confirm the existance and the extent of the potential bioaccumulation of Cd in adipose tissue, as well as to characterize if there is any degree of speciation and interaction with other metals, that could affect the biological implications of Cd concentrations (Yokel et al., 2006). For example, Cd can displace Zn already binded to MT to form Cd-MT (Funk et al., 1987), which could influence Zn bioaccumulation (Smidt et al., 2007). Furthermore, the complex Cu-Glutathione has the potential to remove both Zn and Cd from their MT-binded forms (Ferreira et al., 1993). In addition, co-exposure to Cd and Cr, or Cd and Pb, has been reported to reduce the glomerular filtration rate even more than when the exposure is limited to the individual TEs (Tsai et al., 2017).

Obesity was negatively associated Cd concentrations, being consistent with the inverse associations found with Cd concentrations in urine (Padilla et al. 2010) and in blood (Garner and Levallois, 2016). A dilution effect might partially account for this observation, so that Cd concentrations in the adipose tissue would be diluted at very high levels of obesity, resulting in negative associations between BMI and the concentration of certain pollutants in adipose tissue (Arrebola et al, 2014; Wolff et al., 2000).

Another feasible explanation of the negative association with BMI involves alterations found in the regulation of MT gene expression in obese and type 2 diabetes patients, and their influence on Zn concentrations (Do et al., 2002; Haynes et al., 2013; Szrok et al., 2016). Although results on this issue are not totally clear yet, similar mechanisms could alter Cd concentrations in adipose tissue, as Cd can easily displace Zn binded to MT. Indeed, the median BMI in GraMo cohort was 26.70, which is in the range of overweight as defined by the WHO (WHO, 2018). However, Cd has also been acknowledged as a potential obesogen and, therefore, capable of inducing obesity and obesity-related diseases (Green et al., 2018). Future research should include other obesity measures, such as Waist Circumference, Waist-to-Hip Ratio, or Bioelectric Impedance, which could shed more light on this issue.

In our study we also evidenced some dietary predictors of Cd concentrations, although their influence was less evident in comparison to the rest of variables. We found a positive association of Cd adipose tissue concentrations with egg and meat consumption, but no statistically-significant association was found with the intake of vegetables and legumes. Previous studies have reported a major contribution of grains (26.9%) to dietary Cd intake in Europe, followed by vegetables (16.0%) and meat (7.7%) (EFSA, 2012).

Meat consumption is considered a relevant source of Cd exposure based on the sorption and retention of Cd^{2+} by amine groups in meat proteins (Lopes et al., 2007). Indeed, a previous study performed in Tenerife Island (Spain), González-Weller et al. (2006) identified meat as a relevant exposure source. In contrast, we found a negative association in our population that needs to be further investigated, considering the specific types of meat products as well as the potential residual confounding effect of unmeasured variables.

Furthermore, our findings of a positive association between egg consumption and Cd concentrations was somehow unexpected. The grain-based diet of hens and the content of fat and albumin (which exerts high affinity to Cd) might explain this finding. The absorption of persistent pollutants by chickens and its excretion through eggs has been a matter of concern for decades (Van Eijkeren et al., 2006; Lovett et al., 1998). Further research is needed on this issue. In fact, metal levels in bird eggs have been previously used as a marker of environmental contamination (Tsipoura et al., 2011; Gochfeld, 1997).

Despite the recent increasing concern regarding fish as a potential source of metal exposure (Perera et al., 2015), we found a positive but non significant association of Cd adipose tissue concentrations with the frequency of fish consumption. This might be related to the fact that Cd concentrations in saltwater fish are lower than in fresh water fish. This is because Cd combines with chlorides to form $CdCl_2$, with less bioavailability for fish (the best form for direct uptake through fish gills is the free ion Cd^{2+}, which is more abundant in low salinity water) (Perera et al., 2015).

Although we did not gather information on the fish origin, our study population is based on the Mediterranean coast, so that it is expected that saltwater fish is predominant in their diets. Noteworthy, because of its geological history, the Mediterranean Sea has an increased salinity in comparison to the Atlantic and other oceans in the world (Iorga and Lozier, 1999). Furthermore, large variability in the content of persistent pollutants in fish can be detected among fishing sites due to the differences in the exposure of sea fauna (Nicklisch et al., 2017), which might induce increased variability and, therefore, a certain degree of exposure misclassification. On the other side, previous studies have reported a predominant consumption of white fish (49.5% women, 61.2% men) in inhabitants of Granada province, and a relatively low consumption of crustacea (18.0% woman, 12.1% men) or fatty fish (32.5% women, 26.5% men) (Welch et al., 2002), the latter traditionally considered to have a higher metal content (Olmedo et al., 2013). Indeed, our study population declared a higher consumption of white than blue fish (Table 1).

In the multivariable models, males showed lower Cd concentrations than females. This is in agreement with previous studies of Cd concentrations in blood, urine and kidneys (Vahter et al., 2007; López-Herranz et al., 2016). These differences might be related to sex- (physiological) and/or gender-specific aspects (sociological). Interestingly, women at fertile age frequently show relatively decreased iron stores, which has been reported to induce gastrointestinal absorption of Cd (Åkesson et al., 2002). In order to elucidate sex/gender-related differences in the predictors of the exposure, as well as the influence of women-specific characteristics, we further stratified the multivariable analyses by sex (Table 3), and tested women-specific variables in the models for females. We did not evidence any significant association of the number of pregnancies, number of children, months of breastfeeding, or menopausal status with Cd concentrations.

Interestingly, those males that declared a frequent direct exposure to paints (including occupational and non-occupational exposure) showed increased Cd concentrations, although the association was only marginally significant. Other authors observed increased urinary Cd concentrations in individuals working with paints (Awodele et al., 2014), but the exposure can also be domestic since Cd is a common component in a number of pigments on the market (Faulkner and Schwartz, 2009). We believe that this finding also relates to socio-occupational aspects in the study region. In our study population, 29 men (23%) and only 14 women (14%) declared a frequent exposure to paints, which might hamper the finding of significant associations in women. However, the model coefficient in females was very far from the one in males, which also points to differences in the perception of the exposure among genders. Indeed, from those declaring frequent exposure, 7 (24%) men and only 1 (7%) woman were involved in occupations implying a daily handling of paints (professional painters). This could explain an increased occupational exposure in the male population.

Table 3. Predictors of log-transformed adipose tissue Cd concentrations (µg/kg) in GraMo cohort stratificated by sex/gender. Multivariable linear regression analysis.

	Men			**Women**		
	Beta	**Standard error**	**p-value**	**Beta**	**Standard error**	**p-value**
Age (yrs)	0.017	0.004	<0.001	0.010	0.005	0.065
BMI (kg/m^2)	-0.029	0.013	0.021	-0.041	0.015	0.008
Smoking habit[1]:						
Former smoker	0.491	0.181	0.008	0.440	0.202	0.032
Current smoker	0.642	0.179	<0.001	0.640	0.195	0.001
Egg consumption[2]≥2 portions/week	0.210	0.151	0.167	0.197	0.161	0.226
Meat consumption[3]>2 portions/week	-0.162	0.149	0.280	-0.282	0.150	0.063
Fish consumption[4]≥2 portions/week	0.237	0.149	0.114	0.056	0.165	0.735
Self perceived	0.287	0.165	0.085	-0.009	0.204	0.966

exposure to paints		

Men: multiple $R^2=0.3011$; adjusted $R^2=0.2529$; $p<0.001$. Women: multiple $R^2=0.1981$; adjusted $R^2=0.1260$; $p=0.009$

[1] Ref. category: non-smoker

[2] Ref. category: egg consumption < 2 portions/week

[3] Ref. category: meat consumption<=2 portions/week

[4] Ref. category: fish consumption<2 portions/week

Our findings reveal a generalized exposure to Cd in the study population, and highlight the potential relevance of adipose tissue Cd concentrations as a biomarker for exposure assessment. Despite our hospital-based cohort may not be be entirely representative of the general population, as well as the cross-sectional design, which is susceptible of reversed-causality, most of the reported associations seem robust and consistent with previous research. Although this matrix might pose limitations in comparison to other biological compartments in terms of accessibility, we believe that adipose tissue Cd concentrations can provide relevant results. In this regard, the analysis of adipose tissue can shed light on the potential health effects of Cd, and can complement previous results in relevant matrices, such as urine or blood, which might have different biological meanings. Indeed, previous studies have suggested potential associations of long-term Cd exposure with the induction of oxidative stress (Liu et al., 2009), inflammation (Riemschneider et al., 2015), and endocrine disruption (Takiguchi and Yoshihara, 2006), as well as further development of certain obesity-related pathologies, such as metabolic syndrome (Tinkov et al., 2017) and cancer (Johnson et al., 2003), in which adipose tissue is believed to play an important (though not fully understood) role.

Further research is warranted on the clinical relevance of the adipose tissue Cd concentrations, as well as on specific aspects of their use as an exposure biomarker, such as the potential speciation in the matrix and concentrations in other populations from different regions. Considering previous studies, potential interactions between Cd and other trace elements should be taken into account in the assessment of possible health effects (Tsai et al., 2017).

4. Conclusions

We detected Cd in all the adipose tissue samples from the study population, and identified certain predictors of the exposure, such as age, sex/gender, BMI, smoking habit, eggs and meat consumption and exposure to paints (the latter only in men). This points to a potential relevance of adipose tissue Cd concentrations for exposure characterization as well as for the assessment of long-term effects of chronic exposure to low levels of Cd, which are currently being studied in GraMo cohort.

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Conflict of interest

The authors declare no conflict of interest.

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