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Adipose tissue cadmium concentrations as potential determinants of serum PON1 status in an adult cohort from Southern Spain

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ABSTRACT

Paraoxonase 1 (PON1) is an antiatherogenic enzyme inhibited by environmental stressors such as cadmium (Cd). Thus, this human study aimed to assess (1) the associations between adipose tissue Cd concentrations and serum PON1 status and (2) the influence of the $PON1_{Q192R}$ phenotype on the susceptibility of PON1 status to Cd. As a continuation from previous work, a subsample from the GraMo cohort (Granada, Spain) was studied. Three PON1 activities using non-organophosphorous substrates {PALS (phenyl acetate in the absence of NaCl), PAHS (phenyl acetate in 2 M NaCl) and CMPA [4-(chloromethyl)phenyl acetate]} were assayed in serum (n = 296) to obtain the three functional $PON1_{Q192R}$ phenotypes (QQ, QR and RR). Complementarily, Cd concentrations in adipose tissue (n = 226) and information on socio-demographic characteristics, lifestyle, diet and health status acquired by face-to-face interviews were available. The associations between PON1 activities in serum and Cd concentrations in adipose tissue (subset of n = 138) were explored by using linear regression models, which were adjusted for sex, alcohol consumption and $PON1_{Q192R}$ phenotype. Additionally, benchmark dose (BMD) modelling was performed. Higher adipose tissue Cd concentrations were associated with lower PON1 activities. $PON1_{Q192R}$ specific susceptibility to Cd increased in the order QQ < QR < RR. The Cd BMD ranges for an averaged subject were 0.002–0.016 and 0.046–0.115 $mg_{Cd}/kg_{adipose\text{-}tissue}$ for RR and QR, respectively. For QQ cases, the BMD were higher than the Cd concentrations found in adipose tissue. These results suggest that part of the increased susceptibility of the RR cases to cardiovascular diseases might putatively be mediated by its higher sensitivity to Cd, with adipose tissue Cd levels serving as a biomarker of chronic exposure.

Introduction

The family of paraoxonases (PON) is comprised of three enzymes, i.e. PON1, PON2 and PON3, which are located in the chromosome 7 (7q21.3-q22.1) (Moya and Máñez, 2018; Primo-Parmo et al., 1996). PON1 is mainly expressed and synthesised in the liver and is distributed in the circulation bound to high-density lipoprotein (HDL), as well as PON3 to a lower extent. In turn, PON2 is expressed in cerebrum, liver, testicles and kidney with an exclusively cellular location (Ng et al., 2001). PON1 is the predominant isoform found in human blood.

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Additionally, PON1 is transferred from HDL in the circulation to cell membranes, including those in arterial tissues (Deakin et al., 2011). Although the term paraoxonase is related to the phosphotriesterase activity for the detoxification of organophosphorus compounds (aryldialkylphosphatase) (Aldridge, 1953), such reaction is restricted to PON1. Besides, PON1 showed arylesterase and lactonase activities (Moya and Máñez, 2018).

Interestingly, HDL-bound PON1 hydrolyses and thus detoxifies oxidised low-density lipoprotein (LDL) and homocysteine thiolactone in the circulation, and hence it is considered as a protective factor against cardiovascular diseases (Bhattacharyya, 2008; Durrington et al., 2023; Kunutsor et al., 2016). Substantial research proved that reduced PON1 activities increased the probability of developing atherosclerosis (Durrington et al., 2023). Additionally, PON1 activities have been evaluated for their association with diabetes (Hashemi et al., 2019; Mastorikou et al., 2008; Wu et al., 2018), chronic kidney disease (Watanabe et al., 2023), chronic liver disease (Salari et al., 2022), cancer (Arenas et al., 2018), psoriasis (Bassu et al., 2023) and Alzheimer's disease (Zuin et al., 2023), polycystic ovary syndrome (Bizoń et al., 2021), respiratory diseases including chronic obstructive pulmonary disease (Sarioglu et al., 2020) and asthma (Sarioglu et al., 2015), among others. Importantly, the functional PON1 status, i.e. activity and enzyme levels, is considered to be more important than PON1 genotype itself (Mackness et al., 2001).

Among several polymorphisms, Q192R and L55M are located in the coding region whereas −108 C>T is found in the promoter region (Costa et al., 2003; Dardiotis et al., 2019; Moya and Máñez, 2018). Both Q192R and L55M polymorphisms are involved in the catalytic activity and both L55M and -108C>T are involved in PON1 serum concentrations. Yet, the main determinant of the PON1 status is the $PON1_{Q192R}$ polymorphism (Dardiotis et al., 2019; Moya and Máñez, 2018) and thus a number of methods have been developed to determine different PON1 activities by using different substrates, such as the organophosphorus compounds diazoxon and paraoxon and/or the non-organophosphorus compounds phenyl acetate and 4-(chloromethyl)phenyl acetate, which can be used to establish the functional $PON1_{Q192R}$ status (Eckerson et al., 1983; Richter et al., 2008). Q and R alleles showed different efficacy for the degradation of certain organophosphorus pesticides (Dardiotis et al., 2019; Moya and Máñez, 2018), being higher the degradation of paraoxon by the allele R than by the Q, whereas it was higher that of diazoxon by the Q allele than by the R. Thus, the PON1 status was considered to be a biomarker of susceptibility to organophosphorus pesticides, based on results from *in vitro* and experimental animal studies, as well as limited evidence from human studies (Costa et al., 2013; Dardiotis et al., 2019). Additionally, the Q allele confers higher protection than the R one against cardiovascular diseases (Durrington et al., 2023; Mackness et al., 2001).

Along with life habits, diet and physical activity, the exposure to certain chemical contaminants has been associated with an increased risk of cardiovascular and metabolic diseases (Chowdhury et al., 2018; Planchart et al., 2018; Xu et al., 2025) in which the functional PON1 status might be involved (Moya and Máñez, 2018). One of the environmental contaminants of major public health concern (WHO, 2019) which was found to alter PON1 was cadmium (Cd) (Costa et al., 2017), a heavy metal that is found in the earth's crust. However, anthropogenic sources continue to cause remarkable releases of Cd into the environment, including mining, Ni-Cd battery production and use, metallurgical industry, combustion of fossil fuels and waste incineration (EFSA-EPA, 2012; EFSA, 2009; IARC et al., 2012, 1993; Jin et al., 2020). In fact, Cd atmospheric deposition from both natural and anthropogenic sources and application of phosphate fertilizers increase Cd contents in the soils (Kubier et al., 2019). As broadleaf vegetables uptake Cd efficiently, the general population is mainly exposed to Cd through tobacco smoking and diet (ATSDR-EPA, 2012; EFSA, 2009). The toxicity of Cd is recognized from the 1950s as a result of occupational exposure, targeting the kidneys, lungs and bones (ATSDR-EPA, 2012; Bautista et al., 2024; EFSA, 2009; Jin et al., 2020). Additionally, high Cd exposure due to rapid industrialization in Japan in the last century caused osteomalacia, severe bone pain and renal tubular dysfunction which was named as the *Itai-itai* disease (Morikawa et al., 1992). Furthermore, Cd and Cd compounds are considered carcinogenic to humans (Group 1) according to the International Agency for Research on Cancer (IARC) (IARC et al., (2012), (1993). Overall, decades of research proved that Cd is a risk factor for long-term adverse effects, including mortality, cardiovascular diseases, type-2 diabetes mellitus and metabolic syndrome, among others (Chowdhury et al., 2018; Larsson and Wolk, 2016; Planchart et al., 2018; Tinkov et al., 2018; Ujueta et al., 2019; Xu et al., 2025). However, there are still controversies regarding the exposure thresholds, complete characterization of long-term effects as well as potential interactions with other pollutants. These arise from the inherent limitations of epidemiologic studies assessing long-term effects of low-level Cd exposure in relation to residual confounding —particularly from smoking, a major Cd source— reverse causality, and variability in Cd biomarkers across populations, among others. Moreover, most available data are cross-sectional, restricting causal interpretation and temporal inferences (EFSA, 2011; Eum et al., 2008; Salcedo-Bellido et al., 2021; Xu et al., 2025).

Interestingly, Cd was recently found in adipose tissue samples from GraMo adult cohort, being the smoking habit one of its main determinants. Thus, adipose tissue Cd levels were considered as a potential biomarker of long-term exposure to Cd (Echeverría et al., 2019). In fact, in the same cohort, smokers showed a raised risk of T2DM with increasing levels of Cd in the adipose tissue (Salcedo-Bellido et al., 2021). Although a few human studies found associations between Cd levels in blood and reduced PON1 activities (Hernández et al., 2009; Laird et al., 2015; Pollack et al., 2014), this is the first study reporting associations between Cd levels in adipose tissue and PON1 activity in serum of a human cohort.

Based on the above considerations, the aims of the current work were 1) to assess the associations between adipose tissue Cd concentrations and serum PON1 status, as measured with two different substrates and two salinity concentrations (three PON1 activities) and 2) to evaluate the influence of the functional $PON1_{Q192R}$ phenotype on the susceptibility of the PON1 status to Cd. These associations may help elucidate potential pathways linking Cd exposure to long-term pathologies such as metabolic disorders and atherosclerosis, through a mechanistic approach embedded within an epidemiological study in a well-characterized human cohort.

Materials and methods

GraMo cohort description

This research work is part of a larger, ongoing prospective study (GraMo cohort) that aims to investigate the role of various environmental pollutants on the development of chronic conditions. The details of the GraMo cohort, including methods of recruitment and biological sample collection, have been widely reported elsewhere (Arrebola et al., 2015, 2014, 2013, 2009; Echeverría et al., 2019; Rodríguez-Pérez et al., 2018; Salcedo-Bellido et al., 2021). Briefly, the initial cohort (n = 409) was recruited intraoperatively in 2003–2004 from two public hospitals in the province of Granada, southern Spain: the Clinico San Cecilio University Hospital in the city of Granada and suburbs (inland) and the Santa Ana Hospital in the town of Motril (coastal area). Both adipose tissue and serum samples were immediately coded and stored at −80 ºC until chemical and biochemical analysis. Data on socio-demographic characteristics, lifestyle, diet and health status were collected in face-to-face interviews conducted by trained staff at the time of recruitment during their hospital stay. A pilot study of 50 subjects standardised and validated the research procedures. The questionnaire was previously designed and validated (Buckland et al., 2009; Gonzalez and Riboli, 2010). Out of the 409 participants initially recruited, 387

(94.6 %) consented to participate. Of these, 138 had both adipose tissue Cd concentrations and PON1 activities in serum (Fig. 1). Overall, no relevant differences in their general characteristics were observed between participants included in the current study and the rest of the cohort participants (data not shown).

All participants signed the informed consent forms and the study was approved by the Biomedical Research Ethics Committee of Granada (Comité de Ética de la Investigación Provincial de Granada, 8/2016).

Adipose tissue sampling and Cd analyses

Detailed information was provided previously (Echeverría et al., 2019). Briefly, adipose tissue samples of 5–10 g were freeze-dried in a lyophiliser at the Faculty of Natural Sciences and Engineering, Department of Geology, University of Ljubljana (UL NTF) in Ljubljana (Slovenia) for at least 72 h until a plateau weight was reached and stored at −80 °C until analysis. Subsamples of 0.1 g of adipose tissue were subjected to total digestion with a mixture of 7 mL HNO_3 and 0.1 mL HF in a microwave oven (Multiwave 3000, Anton Paar, Graz, Austria). Then, the samples were acidified with 2 % (v/v) HNO_3 (65 %, supra pur, Fluka, Steinheim, Switzerland) without further dilution, and indium (In, 1 μg / L) was added as an internal standard. 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). Further methodological details were described elsewhere (Rodríguez-Pérez et al., 2018).

Determination of the functional PON1 status in serum

The PON1 activities were assayed according to a method described elsewhere (Richter et al., 2008) by using the non-organophosphorus substrates phenyl acetate both in the absence of NaCl (LS) and in saline conditions [2 M NaCl (HS)] and 4-(chloromethyl)phenyl acetate (with no NaCl present). Consequently, the following three PON1 activities were measured: enzymatic hydrolysis of (1) phenyl acetate in LS (PALS), (2) phenyl acetate in HS (PAHS) and (3) 4-(chloromethyl) phenyl acetate in LS (CMPA) (Richter et al., 2008). Briefly, serum samples were diluted 1:80 for PALS and 1:40 for both PAHS and CMPA in dilution buffer [20 mM tris-(hydroxymethyl)-aminomethane adjusted to pH 8.0 with 1 M HCl, 1 mM $CaCl_2$ (TRIS LS)]. Substrate concentrations were 3.26 mM phenyl acetate in TRIS LS for PALS, 3.26 mM phenyl acetate in saline buffer [2 M NaCl in TRIS LS (TRIS HS)] for PAHS and 3 mM 4-(chloromethyl)phenyl acetate in TRIS LS for CMPA. The reaction started by adding 200 μL of the corresponding substrate to 20 μL of diluted serum. Each serum sample was run in triplicate. Replicates that varied by > 10 % were repeated. The absorbance of the phenyl acetate product was measured at 270 nm and that of 4-(chloromethyl)phenyl acetate at 280 nm in an SPECTROstar® Omega Absorbance plate reader, BMG Labtech (Germany). PALS, PAHS and CMPA activities were calculated by using molar extinction coefficients of 1.31 and 1.3 $mM^{-1} \cdot cm^{-1}$ for phenyl acetate and 4-(chloromethyl)phenyl acetate products, respectively. Then, the three functional $PON1_{Q192R}$ phenotypes QQ, QR and RR were inferred by plotting PAHS versus CMPA according to a procedure described previously (Richter et al., 2008) and the corresponding phenotype frequencies were calculated. Herein, we determined the PON1 status and inferred the functional genotype as PON1 activity is a better predictor of cardiovascular risk than the PON1 genotype (Mackness et al., 2001).

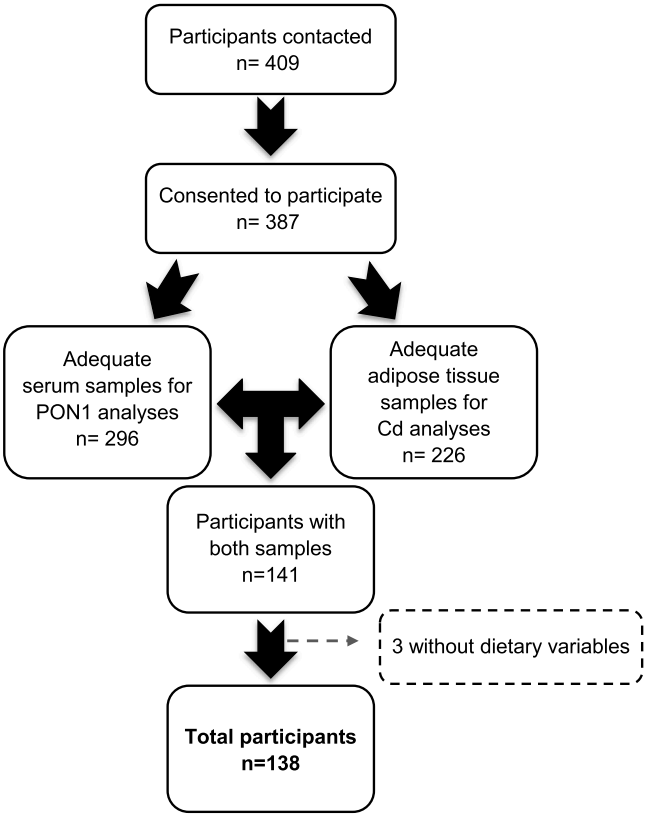

Fig. 1. Flowchart of participants from GraMo cohort selected for this study.

Statistical analysis

PON1 activities (PALS, PAHS and CMPA) were described using medians and 25th and 75th percentiles. Generalised additive models (GAM) were used to visually assess the functional form of the shape of potential associations between Cd levels in adipose tissue and PON1 activities in serum (suppl. Figure S1). Linear relationship was identified for concentrations of Cd throughout most of the range until approximately 0.6 μg/kg. The associations of Cd levels in adipose tissue with PON1 activities in serum was explored by means of linear regression models, using PON1 activities as the dependent variable and log-transformed Cd concentrations as the independent variable. Covariates were selected based on the available literature. The covariates considered in this study were: sex (women, men); age (years, continuous); body mass index (BMI, kg/m^2), calculated from height and weight; occupation (manual, non-manual); and PON1 Q192R functional phenotype (QQ, QR, RR). Dietary variables included main cooking fat (olive oil, other vegetable oils, animal or unspecified vegetable fat); dairy consumption (never, ≤ 2 portions/week, 2–6 portions/week, > 6 portions/week); cheese consumption (never, ≤ 2 portions/week, 2–6 portions/week, > 6 portions/week); meat consumption (≤ 1 portion/week, 2 portions/week, > 2 portions/week); vegetable consumption (never, ≤ 1 portion/week, 1 portion/week, 2 portions/week, > 2 portions/week); and alcohol consumption, defined as ≥ 1 drink/week. We first created the "most saturated" model by entering all the covariates, that were sequentially removed using a backward technique based on their modification in the Bayesian Information Criterion (BIC). Any variable with a p-value below 0.1 was excluded from the model, even if BIC suggested otherwise. All models were adjusted for sex, alcohol consumption and $PON1_{Q192R}$ phenotypes to ensure comparability across multivariable analyses. Additionally, we evaluated the same models but including an interaction term between Cd exposure and $PON1_{Q192R}$ phenotypes. The interaction term was defined as the product of these two variables.

Description and models for PON1 activities were performed by using R software version 4.4.0 (R Core Team, 2025) (R Foundation for Statistical Computing, Vienna, Austria) and *gtsummary* (Sjoberg et al., 2021).

Benchmark dose approach

The benchmark dose (BMD) approach was used to assess the dose-response relationship between serum PON1 activities and adipose tissue Cd levels by using individual data.

The benchmark dose response (BMR) for PON1 activities was 16.6 % on the basis of coronary heart disease events (Mackness et al., 2003). Briefly, PON1 activity towards paraoxon was 16.6 % lower in men who experienced a new coronary event than that in those who did not, and hence it was associated with a protective effect [OR (95 % CI) = 0.87 (0.77–0.98), p-value = 0.039] (Mackness et al., 2003). Model averaging was applied and the BMD confidence intervals (CI) were obtained from the selected models. BMD modelling was performed using *Proast 71.1* package (RIVM, 2025) (RIVM, Bilthoven, The Netherlands). BMD modelling was run in the R software version 4.3.2 (R Core Team, 2025) (R Foundation for Statistical Computing, Vienna, Austria). Additionally, benchmark dose modelling was performed for an averaged subject. A similar approach was performed previously for the dose-response relationship of perfluoroalkyl substances with serum lipids (Knutsen et al., 2018; Steenland et al., 2009). Serum PON1 activities were calculated for each adipose tissue Cd level by using the corresponding model with the interaction term between Cd exposure and $PON1_{Q192R}$ phenotypes (described in the sub-section "Statistical analysis"). The representative covariate profile was entered in the models, a human with non-alcohol consumption. Both sexes were evaluated.

Results

Description of the study population

The study population characteristics and adipose tissue Cd concentrations are available in Table 1. Dietary characteristics of the GraMo cohort are provided as Supplementary material (Suppl. Table S1). No differences were observed in Cd exposure or PON1 activity between participants from Granada (inland) and Motril (coastal) areas (Suppl. Table S2).

Serum PON1 activities and functional $PON1_{Q192R}$ phenotypes in GraMo cohort

The population distribution derived from the rate of hydrolysis of PAHS versus that of CMPA showed three functional $PON1_{Q192R}$ phenotypes (Suppl. Figure S2). Of those cases, 138 had available adipose tissue Cd concentrations, for whom the frequencies of the inferred functional $PON1_{Q192R}$ phenotypes were n = 72 for QQ (52.2 %), n = 42 for QR (30.4 %) and n = 24 for RR (17.4 %) (Table 1).

The median (P25, P75) PALS, PAHS and CMPA PON1 activities were 86.8 (72.5, 105.2), 55.7 (42.1, 66.4) and 14.8 (11.5, 17.0) μmol / min / mL, respectively (Table 2).

Associations between adipose tissue Cd concentration and serum PON1 activities

Increasing adipose tissue Cd concentrations were associated with reduced PALS, PAHS and CMPA PON1 activities, according to multivariable linear regressions (Table 3).

As the functional $PON1_{Q192R}$ phenotype was considered a relevant variable, the models were adjusted accordingly (Model 1 in Table 3). Based on that, the interaction between the functional $PON1_{Q192R}$ phenotype and Cd concentration in adipose tissue was incorporated into the models – expressed as the product term between the variables-, revealing a phenotype-specific susceptibility to Cd (Model 2 in Table 3). The magnitude of the associations with Cd concentrations increased in the order QQ < QR < RR (Table 3).

Table 1
Study population characteristics and adipose tissue Cd concentrations.

	All	QQ	QR	RR
Characteristic	**N = 138**	**N = 72**	**N = 42**	**N = 24**
Sex				
Woman	67.0 (48.6 %)	36.0 (50.0 %)	22.0 (52.4 %)	9.0 (37.5 %)
Man	71.0 (51.4 %)	36.0 (50.0 %)	20.0 (47.6 %)	15.0 (62.5 %)
Cause of intervention				
Hernia	60.0 (43.5 %)	33.0 (45.8 %)	19.0 (45.2 %)	8.0 (33.3 %)
Gallbladder	23.0 (16.7 %)	9.0 (12.5 %)	10.0 (23.8 %)	4.0 (16.7 %)
Benign tumour	18.0 (13.0 %)	8.0 (11.1 %)	6.0 (14.3 %)	4.0 (16.7 %)
Other	37.0 (26.8 %)	22.0 (30.6 %)	7.0 (16.7 %)	8.0 (33.3 %)
Residence				
Urban (Granada)	46.0 (33.3 %)	20.0 (27.8 %)	16.0 (38.1 %)	10.0 (41.7 %)
Rural/Semirural (Motril)	92.0 (66.7 %)	52.0 (72.2 %)	26.0 (61.9 %)	14.0 (58.3 %)
Age (years)	51.0 (36.3, 62.8)	48.0 (35.0, 60.0)	53.0 (36.5, 64.5)	52.5 (39.8, 71.0)
Occupation				
Non-manual workers	34.0 (24.6 %)	19.0 (26.4 %)	7.0 (16.7 %)	8.0 (33.3 %)
Manual worker	104.0 (75.4 %)	53.0 (73.6 %)	35.0 (83.3 %)	16.0 (66.7 %)
Body mass index (Kg/m^2)	27.1 (24.5, 29.9)	27.6 (24.7, 32.3)	25.9 (24.0, 28.5)	28.7 (26.2, 31.1)
Alcohol consumption	74.0 (53.6 %)	37.0 (51.4 %)	23.0 (54.8 %)	14.0 (58.3 %)
Smoking habit				
No smoker	59.0 (42.8 %)	35.0 (48.6 %)	18.0 (42.9 %)	6.0 (25.0 %)
Former smoker	38.0 (27.5 %)	14.0 (19.4 %)	13.0 (31.0 %)	11.0 (45.8 %)
Smoker	41.0 (29.7 %)	23.0 (31.9 %)	11.0 (26.2 %)	7.0 (29.2 %)
Perceived exposure to paints				
Non-exposure to paints	115.0 (83.3 %)	57.0 (79.2 %)	35.0 (83.3 %)	23.0 (95.8 %)
Exposure to paints	23.0 (16.7 %)	15.0 (20.8 %)	7.0 (16.7 %)	1.0 (4.2 %)
	Median (Q1, 3)	Median (Q1, 3)	Median (Q1, 3)	Median (Q1, 3)
Cadmium (μg/kg)	9.0 (5.0, 15.8)	8.0 (5.0, 13.3)	9.5 (5.3, 20.8)	9.0 (5.8, 18.0)

Qualitative data expressed as n (%); Quantitative data expressed as median (percentile 25th, percentile 75th); QQ, QR and RR refers to $PON1_{Q192R}$ functional phenotype.

Benchmark dose approach

Significant benchmark dose-response models were found for PALS, PAHS and CMPA PON1 activities. Particularly, the RR functional phenotype was more sensitive to Cd in terms of inhibition of PON1 activity than the QR and QQ phenotypes (Fig. 2). However, the uncertainty associated to the benchmark dose intervals was higher than the recommended value (More et al., 2022).

Thus, model averaging of the dose-response relationships between adipose tissue Cd concentrations and PALS, PAHS and CMPA PON1 activities of an averaged subject was performed by using the results acquired from the multivariable linear regressions with the interaction between the functional $PON1_{Q192R}$ phenotype and Cd. The RR phenotype was the most sensitive to Cd (Fig. 3).

The resulting BMD CI in mg $_{Cd}$ / kg $_{adipose\ tissue}$ of the RR phenotype were 0.0042–0.0137 for PALS, 0.0061–0.0162 for PAHS and 0.0021–0.0089 for CMPA (Fig. 3).

Table 2
PON1 status in serum of the GraMo cohort.

PON1	All	QQ	QR	RR
Activity	**N = 138**	**N = 72**	**N = 42**	**N = 24**
PALS	86.8 (72.5, 105.2)	96.5 (82.0, 124.6)	83.3 (74.0, 100.6)	65.4 (52.7, 76.8)
PAHS	55.7 (42.1, 66.4)	65.5 (59.4, 76.0)	47.5 (41.7, 55.5)	32.0 (25.3, 37.8)
CMPA	14.8 (11.5, 17.0)	14.1 (10.8, 16.2)	15.4 (12.5, 17.8)	14.9 (11.1, 15.9)

Data expressed as median (percentile 25th, percentile 75th); PALS, PAHS, and CMPA, PON1 activities (in μmol / min / mL) towards phenyl acetate, phenyl acetate in 2 M NaCl and 4-(chloromethyl)phenyl acetate, as substrates, respectively. QQ, QR and RR refers to $PON1_{Q192R}$ functional phenotype.

Table 3
Multivariable linear regression models for the associations between adipose tissue Cd levels and PON1 activities in serum of the GraMo cohort.

	Model 1[a]				Model 2 with interaction[a,b]			
Variables	**β**	**CI 95%**	**10^{β}**	**$10^{CI\ 95\%}$**	**β**	**CI 95%**	**10^{β}**	**$10^{CI\ 95\%}$**
PALS								
Cd (μg/g)	-1.90	(-3.32, -0.47)	0.013	(0, 0.336)	0.35	(-2.53, 3.22)	2.215	(0.003, 1647.1)
Phenotype								
QQ	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
QR	-0.06	(-0.11, -0.01)	0.875	(0.784, 0.978)	-0.04	(-0.11, 0.02)	0.909	(0.784, 1.054)
RR	-0.21	(-0.27, -0.15)	0.618	(0.542, 0.706)	-0.12	(-0.2, -0.04)	0.756	(0.63, 0.908)
Cd * Phenotype								
Cd * QR					-1.79	(-5.2, 1.62)	0.016	(0, 41.829)
Cd * RR					-6.51	(-10.78, -2.23)	0.000	(0, 0.006)
	Model 1[a]				Model 2 with interaction[a,b]			
Variables	**β**	**CI 95%**	**10^{β}**	**$10^{CI\ 95\%}$**	**β**	**CI 95%**	**10^{β}**	**$10^{CI\ 95\%}$**
PAHS								
Cd (μg/g)	-1.70	(-2.84, -0.56)	0.020	(0.001, 0.273)	-0.48	(-2.8, 1.85)	0.332	(0.002, 70.108)
Phenotype								
QQ	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
QR	-0.14	(-0.18, -0.1)	0.727	(0.665, 0.794)	-0.13	(-0.19, -0.08)	0.736	(0.653, 0.829)
RR	-0.34	(-0.38, -0.29)	0.461	(0.414, 0.512)	-0.28	(-0.34, -0.22)	0.525	(0.453, 0.609)
Cd * Phenotype								
Cd * QR					-0.78	(-3.54, 1.99)	0.168	(0, 96.952)
Cd * RR					-4.18	(-7.64, -0.72)	0.000	(0, 0.19)
	Model 1[a]				Model 2 with interaction[a,b]			
Variables	**β**	**CI 95%**	**10^{β}**	**$10^{CI\ 95\%}$**	**β**	**CI 95%**	**10^{β}**	**$10^{CI\ 95\%}$**
CMPA								
Cd (μg/g)	-2.31	(-4.1, -0.52)	0.005	(0, 0.299)	-0.31	(-3.88, 3.25)	0.487	(0, 1798.1)
Phenotype								
QQ	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
QR	0.06	(0, 0.12)	1.137	(0.989, 1.306)	0.06	(-0.02, 0.14)	1.137	(0.945, 1.366)
RR	-0.02	(-0.09, 0.05)	0.950	(0.804, 1.122)	0.09	(-0.01, 0.19)	1.240	(0.989, 1.556)
Cd * Phenotype								
Cd * QR					-0.76	(-5, 3.48)	0.173	(0, 3001.27)
Cd * RR					-8.41	(-13.71, -3.1)	0.000	(0, 0.001)

β, beta coefficient; CI 95 %, confidence interval at 95 %; Ref., reference; PALS, PON1 activity towards phenyl acetate; PAHS, PON1 activity towards phenyl acetate in 2 M NaCl; CMPA, PON1 activity towards 4-(chloromethyl)phenyl acetate. QQ, QR and RR refers to $PON1_{Q192R}$ functional phenotype.
[a] Both models were adjusted for sex and alcohol consumption variables; [b] Cd * Phenotype, interaction between Cd and $PON1_{Q192R}$ functional phenotype. The initial models, adjusted for all the variables, are detailed in the Supplementary material (Suppl. Table S3).
PALS, PAHS and CMPA activities in serum were the dependent variables. The concentration of Cd in adipose tissue was the independent variable in both models 1 and 2, as well as the interaction Cd * Phenotype for model 2.

In comparison, higher BMD CI in mg $_{Cd}$ / kg $_{adipose\ tissue}$ were found for the QR phenotype, i.e., 0.046–0.0853 for PALS, 0.059–0.0889 for PAHS and 0.073–0.115 for CMPA (Fig. 3).

The QQ phenotype was the least responsive to Cd, with BMD CI higher than the Cd concentrations found in adipose tissue (Fig. 3).

Discussion

Metals reduce PON1 activity

To the best of our knowledge, the current work is the first to report associations between increasing adipose tissue Cd concentrations and lower PON1 activities in serum of human populations. Previously, Cd concentrations in blood/serum of humans were associated with reduced PON1 activities (Hernández et al., 2009; Laird et al., 2015; Pollack et al., 2014) (Fig. 4A).

In the GraMo cohort, the smoking status was associated with adipose tissue Cd concentrations (Echeverría et al., 2019), whereas other studies, discussed herein, reported associations with Cd concentrations in blood (Bizoń and Milnerowicz, 2017; Hernández et al., 2009; Laird et al., 2015; Pollack et al., 2014). Furthermore, current smokers had higher levels of Cd and lead (Pb) in blood and lower PON1 activities when compared with non-smokers (Almeida Lopes et al., 2017; Bizoń and Milnerowicz, 2017) (Fig. 4B). Those results suggested that smoking reduced PON1 activities (James et al., 2000) due to higher metal levels (Almeida Lopes et al., 2017; Bizoń and Milnerowicz, 2017) (Fig. 4A).

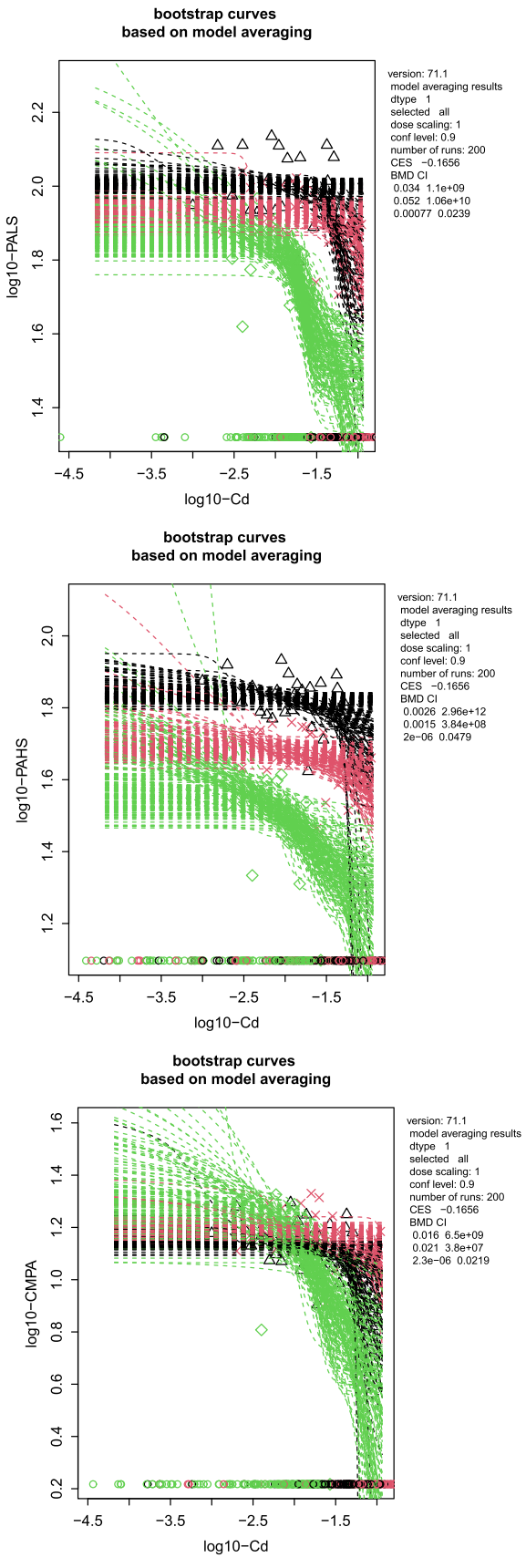

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Fig. 2. Model average estimate of the dose–response relationship between adipose tissue Cd levels and PON1 activities in serum by using the $PON1_{Q192R}$ functional phenotype as a covariate.
The three PON1 activities PALS, PAHS and CMPA were assayed towards phenyl acetate, phenyl acetate in 2 M NaCl and 4-(chloromethyl)phenyl acetate, respectively. $PON1_{Q192R}$ functional phenotypes are represented by dashed lines, using the following symbols and colours △ (QQ, black), X (QR, red) and ⋄ (RR, green).

Complementary to that, additional metals measured in blood which were associated with reduced PON1 activities in humans were Pb (Li et al., 2006; Permpongpaiboon et al., 2011; Pollack et al., 2014) and mercury (Hg) (Ayotte et al., 2011) (Fig. 4A). However, some studies did not find significant associations for Cd, Pb (Almeida Lopes et al., 2017; Rovira et al., 2018) and Hg (Almeida Lopes et al., 2017; Pollack et al., 2014; Rangel-Méndez et al., 2016) levels in blood and PON1 activity. Even, blood levels of Pb (Hernández et al., 2009) and Hg (Hernández et al., 2009; Laird et al., 2015) were associated with increased PON1 activities.

Nevertheless, there were interactions between metals, as for Hg and selenium (Se) (Laird et al., 2015), which might explain some of the discrepancies between studies. Particularly, Se blood levels were associated with increasing PON1 activities (Ayotte et al., 2011; Laird et al., 2015), but not in a case-control study (Rovira et al., 2018). Thus, the above-mentioned associations between increased PON1 activities and Hg concentrations were not significant when corrected for Se (Laird et al., 2015). The increase in PON1 activities mediated by Se is related to its antioxidant properties, as a cofactor for glutathione peroxidase and thioredoxin reductase (Wrobel et al., 2016), which prevents PON1 inactivation by oxidative stress (Aviram et al., 1999, 1998a). Complementarily, a biologically inert Cd-Se complex might also alleviate the Cd-mediated effects (Zwolak, 2020) (Fig. 4C).

Other less studied metals in blood of humans with either non-significant results or associated with (decreased and/or increased) PON1 activities are As, B, Ba, Cu, Mn, Ni, Sr and Zn (Hernández et al., 2009; Laird et al., 2015; Rovira et al., 2018). Inconsistent results among studies might be related to different exposure levels, experimental designs, statistical methods and some study limitations.

Complementarily, both *in vitro* and experimental animal studies support the notion that metals, including Cd, inhibit PON1 activities [reviewed in (Costa et al., 2017)] (Afolabi et al., 2012) (Fig. 4A). In experimental animals, short-term exposure triggered increased PON1 activities which was considered to be an adaptive response to Cd-mediated oxidative stress (Ferramola et al., 2011; Zhou et al., 2019). Nevertheless, PON1 activities decreased after longer periods of exposure to Cd (Ferramola et al., 2011; Hassan et al., 2025) or at higher doses (Afolabi et al., 2012; Costa et al., 2017).

The above-mentioned Cd effects are hypothesized to be mediated by multiple mechanisms of action. One of the mechanisms for the inhibition of PON1 is believed to be related to Cd indirect effects (Fig. 4D). Cd is known to induce the generation of reactive oxygen species (ROS) and hence the consequent oxidative stress (ATSDR-EPA, 2012; Bautista et al., 2024; EFSA, 2009). In this oxidative microenvironment, PON1 inhibition could occur via several pathways (Fig. 4D). In fact, ROS were able to inhibit PON1 (Nguyen et al., 2009; Nguyen and Sok, 2003; Rozenberg and Aviram, 2006). Concurrently, Cd was associated with dyslipidaemia (Lu et al., 2023; Tinkov et al., 2018; Xing et al., 2022; Xu et al., 2025), particularly increased triglyceride and decreased HDL levels. This reduction in HDL might impair PON1 activities as PON1 is associated with HDL (Aviram et al., 1998b; Rosenblat et al., 2006) (Fig. 4D). Furthermore, an increased level of LDL in a Cd-mediated oxidative microenvironment is more susceptible to propagating lipid peroxidation (Tinkov et al., 2018) (Fig. 4D), which was reported to inhibit PON1 (Aviram et al., 1998a; Navab et al., 1997; Tavori et al., 2011). In fact, an oxidative environment by Cu and oxidized LDL caused a progressive inhibition of PON1 (Aviram et al., 1998a; Navab et al.,

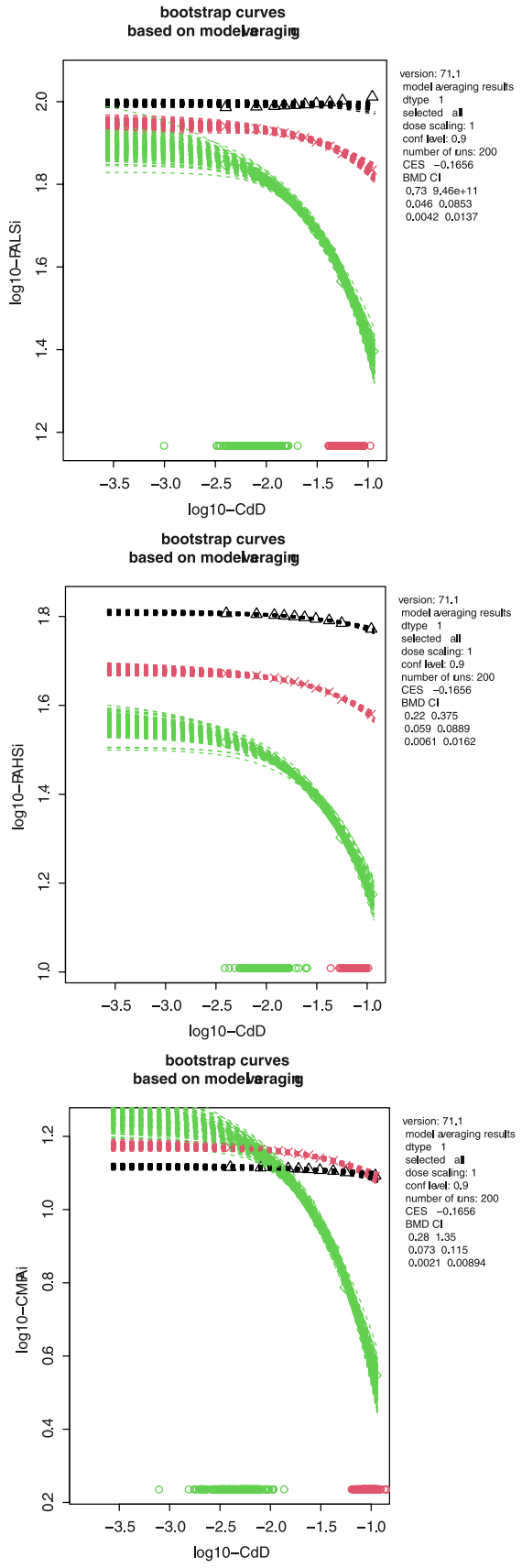

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Fig. 3. Model average estimate of the dose–response relationship between adipose tissue Cd levels and PON1 activities in serum of an averaged subject according to the multivariable linear regression models with interaction by using the $PON1_{Q192R}$ functional phenotype as a covariate.
The representative covariate profile was a human (man and woman) with non-alcohol consumption.
Suffix *i* in the three variables PALSi, PAHSi and CMPAi means PON1 activities as calculated from the multivariable linear regression models with interaction coming from the assayed PON1 activities PALS, PAHS and CMPA towards phenyl acetate, phenyl acetate in 2 M NaCl and 4-(chloromethyl)phenyl acetate, respectively.
$PON1_{Q192R}$ functional phenotypes are represented by dashed lines, using the following symbols and colours △ (QQ, black), X (QR, red) and ⋄ (RR, green).

1997; Tavori et al., 2011).

In any case, the mechanism behind these observations might involve the free sulfhydryl group of Cysteine 284 (Cys284) in PON1. The free sulfhydryl groups of cysteines are known to react with ROS (Sies et al., 2017), oxidized LDL (Fig. 4E) (Aviram et al., 1999; Tavori et al., 2011), and metals, including Cd (Fig. 4C,F) (Bjørklund et al., 2019; Jalilehvand et al., 2009). Of note, cigarette smoke extracts inhibited PON1 activities while glutathione and sulfhydryl compounds such as acetylcysteine, 2-mercaptoethanol, DL-dithiothreitol and L-cysteine protected PON1 activity against those extracts (Fig. 4C). Both superoxide dismutase and catalase, as well as compounds with redox capability such as ascorbate, vitamin E and butylated hydroxytoluene did not protect PON1 activity in the presence of cigarette smoke extracts (Nishio and Watanabe, 1997). Although Cys284 is not located at the active centre, it is situated alongside His285 within a highly conserved region, where it is surrounded by four other highly conserved amino acids from adjacent strands (Leu267, Val268, Glu303 and Leu305) (Harel et al., 2004; Josse et al., 1999; Sorenson et al., 1995). Consequently, reactions with Cys284 are considered to cause steric tensions (Fig. 4E,F) that alter the PON1 conformation and result in a deterioration of its activity (Fig. 4G). Moreover, Cys284 is considered to be involved in the stabilisation of the substrate and hence it is critical for the antioxidant properties of PON1 (Aviram et al., 1999, 1998a; Rozenberg and Aviram, 2006; Tavori et al., 2011).

$PON1_{Q192R}$ functional phenotype as a susceptibility biomarker

Herein, the assayed activities showed different susceptibility to Cd inhibition according to the $PON1_{Q192R}$ phenotype. Allele frequencies in this cohort were found to be within previously published ranges for Caucasian populations, which showed higher frequencies of the Q allele, whereas Asian, African or African-American populations showed higher frequencies of the R allele (Coombes et al., 2011; Dardiotis et al., 2019; Mackness and Mackness, 2015; McDaniel et al., 2014; Wang et al., 2012). Thus, the use of non-organophosphorus compounds enabled the classification of participants on the basis of the graphical representation of the PON1 activities PAHS versus CMPA (Richter et al., 2008).

With regard to exposure to Cd, Pb and Hg, PON1 activities of RR individuals were more susceptible than those of QQ individuals (Almeida Lopes et al., 2017; Li et al., 2006; Pollack et al., 2014), as in the GraMo cohort. Particularly, workers ($n = 597$) in a Pb-acid battery manufactory and in a Pb recycling plant showed reduced PON1 activities (arylesterase, paraoxonase and diazoxonase) as their Pb levels in blood increased (Li et al., 2006). Moreover, the association between paraoxonase activity and Pb levels in blood was stronger for RR workers ($r = –0.251$, $p < 0.001$) compared with QR ($r = –0.101$, $p = 0.122$) and QQ cases ($r = –0.007$, $p = 0.959$) (Li et al., 2006). In healthy premenopausal women, non-monotonic reductions in PON1 activities were observed for Cd and Pb terciles, but not for Hg (Pollack et al., 2014). The combined exposure, according to tertile of composite blood metal level, was found to result in a greater reduction of PALS and paraoxonase activity (towards paraoxon) activities among women with the RR and

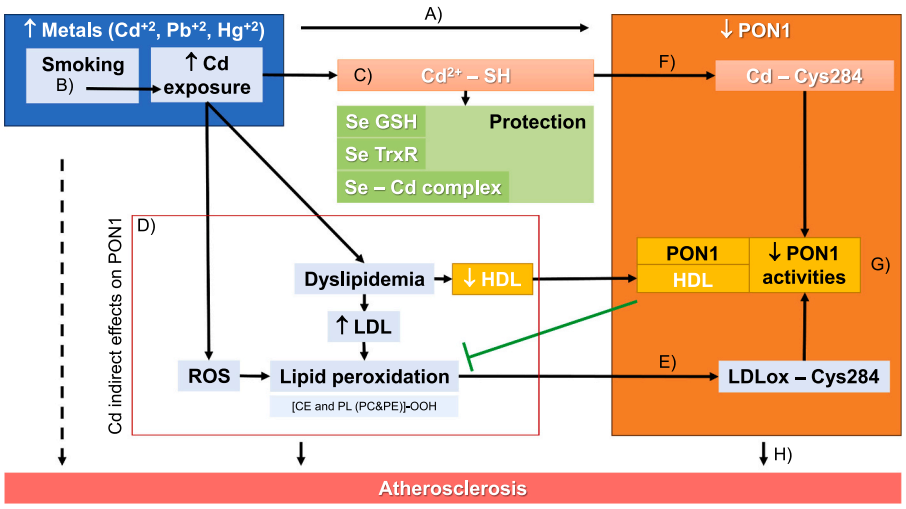

Fig. 4. Conceptual framework for the associations of adipose tissue cadmium levels with the PON1 activities found within the GraMo cohort.
Cd, Cadmium; CE, Cholesterol esters; Cys, Cysteine; GSH, Glutathione; HDL, High-density lipoprotein; Hg, Mercury; HOO-, peroxide group; LDL, Low-density lipoprotein; LDLox, Oxidized low-density lipoprotein; Pb, Lead; PC, Phosphatidylcholine; PE, Phosphatidylethanolamine; PL, Phospholipids; PON1, Paraoxonase 1; ROS, Reactive Oxygen Species; Se, Selenium; SH-, Sulfhydryl group; TrxR, Thioredoxin reductase. For both references and figure Section (4A-4H), the reader is referred to the discussion section.

QR phenotypes than among women with the QQ phenotype (Pollack et al., 2014). Moreover, increased Cd concentrations were associated with lower PON1 activity in serum of women carrying the RR phenotype, according to cluster analysis [cluster 2 in (Almeida Lopes et al., 2017)]. Those results suggested that low levels of Cd exposure might reduce PON1 activities (Almeida Lopes et al., 2017). Conversely, although the levels of Hg in blood of Inuits from Nunavik (Canada) were associated with a reduction of paraoxonase activity (towards paraoxon), the $PON1_{Q192R}$ polymorphism was not a significant susceptibility factor (Ayotte et al., 2011). On the other hand, *in vitro* studies showed a higher susceptibility to metal-mediated PON1 inhibition for the $PON1_{R192}$ when compared with the $PON1_{Q192}$ isoform (Costa et al., 2017; Gençer and Arslan, 2009).

Clinical relevance of the $PON1_{Q192R}$ functional phenotype differential susceptibility to Cd

Findings in the current study are of clinical relevance as reduced PON1 activities are also associated with higher risks of cardiovascular diseases (Ferretti and Bacchetti, 2012; Jarvik et al., 2003; Jiang et al., 2011; Kunutsor et al., 2016; Mackness et al., 2003; Mackness and Mackness, 2015; Moya and Máñez, 2018). In fact, knock-out PON1 mice showed increased atherosclerosis and oxidized LDL levels (Shih et al., 2000, 1998). Additionally, lower paraoxonase activity (towards paraoxon) in human serum was associated with higher levels of fatty acid oxidation products, and hence, higher risk of all-cause mortality and major adverse cardiac events (Bhattacharyya, 2008). Conversely, transgenic mice over-expressing human PON1 (2.2–3.8-fold increase in PON1 activity) showed reduced atherosclerotic lesions and increased protection against LDL oxidation (Tward et al., 2002). Thus, the mechanism by which PON1 protects against atherosclerosis is related to the PON1 activity for the detoxification of oxidised species of cholesterol and phospholipids (phosphatidylcholine and phosphatidylethanolamine) carried by LDL (Aviram et al., 1998b, 1998a; Rosenblat et al., 2006; Shih et al., 2000) (Fig. 4H). Complementary to this, carriers of the $PON1_{Q192}$ allele experienced a reduced risk of cardiovascular diseases when compared to homozygous for the $PON1_{R192}$ allele (Durrington et al., 2023; Mackness et al., 2001).

Of note, the observed associations between the $PON1_{Q192R}$ functional phenotype and various medical conditions might be, at least in part, mediated by the selective inhibition of PON1 by metals such as Cd, Pb and Hg, discussed above. In this study, individuals with the RR phenotype showed an increased susceptibility when compared to those with the QR phenotype and even more with respect to those with the QQ phenotype on the basis of PON1 activity reductions associated with Cd concentrations in adipose tissue.

The relevance of smokers to the current study relies on the previously-reported association between smoking and Cd levels in adipose tissue within GraMo cohort (Echeverría et al., 2019). In other studies, RR smokers showed the highest odds ratios for increased atherogenic plasma index (4.9, 95 % CI 1.4–17) and Framingham risk score (7.90, 95 % CI 1.81 – 34.60) (Souza-Nogueira et al., 2016).

Limitations and strengths of the study

Despite the relatively limited sample size and exploratory design of the present study, our results were seemingly plausible and showed novel associations that warrant further confirmation in future studies. In particular, this limited sample size increases the uncertainty of the results, especially for the interaction terms, which show very wide confidence intervals. Therefore, these findings should be interpreted as hypothesis-generating and require verification in future studies. The use of a hospital-based cohort may certainly bias the representativity of our findings, although a wide variety of surgeries were included in order to maximize representativity, and our exposure and effects of interest are *a priori* independent of the surgery received. The cross-sectional design precludes us to assume causality. However, our previous findings in GraMo support the use of adipose tissue as a highly novel long-term exposure assessment matrix for Cd (ATSDR-EPA, 2012; Echeverría et al., 2019; EFSA, 2009). As blood Cd also reflects daily exposure fluctuations (ATSDR-EPA, 2012), urine Cd was considered more reliable (Tellez-Plaza et al., 2012) but still reflecting recent exposure (ATSDR-EPA, 2012). Although no direct evidence is available regarding its half-life in adipose tissue, the fact that Cd is a highly persistent chemical in humans (average half-life = 20–30 years) (Genchi et al., 2020), together with its partial lipophilicity, supports our hypothesis that Cd adipose tissue concentrations are a better biomarker of long-term exposure than their concentrations in blood which represent a short-term biomarker (Echeverría et al., 2019). Previously, potential adverse effects were found in the GraMo cohort as tobacco users with higher adipose tissue Cd concentrations showed higher risks of type 2 diabetes mellitus (Salcedo-Bellido et al., 2021).

However, the associations observed in the present study may be affected by residual confounding due to unmeasured (e.g., physical activity) or inadequately captured covariates through our questionnaires (e.g., smoking habit as categorical variable). In addition, we cannot rule out the confounding effect of co-exposure to pollutants potentially bioaccumulable in adipose tissue, such as lipophilic persistent organic pollutants (Pérez-Carrascosa et al., 2021; Requena et al., 2023; Zhu et al., 2024). In this regard, accurately assessing variability in alcohol consumption solely through self-reported questions is particularly challenging.

Different methodologies for PON1 phenotyping have been developed (Eckerson et al., 1983; La Du and Eckerson, 1984) and have been applied in a number of observational studies (Adilay et al., 2023; Sarioglu et al., 2020, 2015). However, the validated and widely accepted method employed in this study (Richter et al., 2008) allows for a direct comparison with other publications. This is further supported by the availability of established conversion factors (Richter et al., 2009) which enable PON1 activities to be calculated across various methods.

Conclusions

Our study is the first to report associations between higher adipose tissue Cd concentrations and reduced PON1 activity in human serum. Significantly, our models revealed an interaction with the functional $PON1_{Q192R}$ phenotype, indicating that the susceptibility to Cd-induced changes in PON1 activity increases from QQ to QR, and is most pronounced in RR individuals. These findings strongly suggest that an increased susceptibility of the RR phenotype to cardiovascular diseases could be mediated, at least in part, by its higher sensitivity to Cd, with adipose tissue Cd levels potentially serving as a crucial biomarker for chronic environmental exposure. If confirmed by further studies, our findings in the GraMo cohort could contribute to improving risk prediction algorithms in clinical practice and support the identification of individuals at increased risk of cardiovascular disease.

Ethics committee

The study was approved by the Biomedical Research Ethics Committee of Granada (Comité de Ética de la Investigación Provincial de Granada, 8/2016).

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CRediT authorship contribution statement

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix B. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.etap.2025.104827.

Data availability

Data will be made available on request.

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