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Accumulation of organophosphorus pollutants in adipose tissue of obese women - metabolic alterations

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ABSTRACT

Organophosphorus pesticides (OPPs) and organophosphate esters (OPEs) are organophosphorus compounds created as substitutes for persistent environmental pollutants, namely organochlorines pesticides and brominated flame retardants, respectively. However, there is evidence that organophosphorus compounds are also widespread across the environment and have adverse effects on biota. In humans, OPPs and OPEs were reported to be carcinogenic, neurotoxic, hepatotoxic, nephrotoxic, amongst others. As lipophilic compounds, these accumulate in fat tissues as adipose tissue. Yet biomonitoring studies and analytical methodologies to assess these compounds in the human body are scarce, particularly in adipose tissue. In this study, the presence of six OPPs and seven OPEs was determined in samples of subcutaneous adipose tissue (scAT) and visceral adipose tissue (vAT) from 188 adult obese women. OPPs and OPEs were quantified by gas chromatography (GC) flame photometric detection and confirmed in GC tandem mass spectrometry. The detection frequencies ranged between 0.5-1.6% and 48–53%, respectively for OPPs and OPEs. Organophosphorus pollutants were present in both adipose tissues and median concentrations were 0.008 ± 0.020 μg/g scAT and 0.009 ± 0.020 μg/g vAT. A total of 32 Spearman's correlations were found between organophosphorus pollutants concentrations in adipose tissue and several biochemical parameters (18 positive and 14 negative). Our results show that anthropometric and hormonal parameters, cholesterol, glycaemia, macrominerals, urea and sedimentation velocity might be influenced by the presence of these compounds. The presence of organophosphorus pollutants in the environmental and their possible effect on female metabolic processes is concerning. Particularly because presently OPEs usage is not controlled or limited by any regulation. More studies are needed to fully understand these pollutants behaviour and hazard effects on human health, biota, and the environment so control regulations can be drawn to prevent and lessen their effects.

Abbreviations: 25th percentile (P25), 75th percentile (P75); acetonitrile (ACN), alanine aminotransferase (ALT); alkaline phosphatase (ALP), aspartate aminotransferase (AST); body index mass (BMI), C18 Endcapped Bulk (C18EC); celestolide (ADBI), dehydroepiandrosterone sulphate (DHEA-S); deoxyribonucleic acid (DNA), expanded combined uncertainty (*Ur,tot*); flame photometric detection (FPD), follicle-stimulating hormone (FSH); gama glutamil transpeptidase (GGT), gas chromatography (GC); glycated haemoglobin (HbA1c), high density lipoprotein (HDL); homeostasis model assessment of beta cell function (HOMA2-B), homeostasis model assessment of insulin resistance (HOMA-IR); homeostasis model assessment of insulin sensitivity (HOMA2-S), human biomonitoring for europe (HBM4EU); internal standard (IS), low density lipoprotein (LDL); luteinizing hormone (LH), mass spectrometry (MS); method detection limit (MDL), method quantification limit (MQL); organophosphorus ester flame retardants (OPEs), organophosphorus pesticides (OPPs); sex hormone binding globulin (SHBG), subcutaneous adipose tissue (scAT); supel QuE Z-Sep + Bulk (Z-Sep^{+}), tandem mass spectrometry (MS/MS); thyroid stimulating hormone (TSH), thyroxine (T4); triiodothyronine (T3), tri-isobutyl phosphate (TiBP); tri-n-butyl phosphate (TnBP), tri-o-tolyl phosphate or tri-o-cresyl phosphate (TCP); triphenylphosphate (TPP), tripropyl phosphate (TPrP); tris(2-butoxyethyl) phosphate (TBEP), tris(2-chloroethyl) phosphate (TCEP); tris(2-ethylhexyl) phosphate (TEHP), visceral adipose tissue (vAT).

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1. Introduction

Human are exposed every day to countless chemical compounds ubiquitous present in the environment. These entered the human body either by inhalation, ingestion, or dermal contact and most accumulate in the organism with low degradation or excretion (Sousa et al., 2022). Most of them are lipophilic compounds, meaning these accumulate in fat tissues (*e.g.* adipose tissue). In fact, adipose tissue has been pointed out as the more adequate matrix to understand the chronic accumulation of lipophilic compounds (Lee et al., 2017; Li et al., 2019; Mustieles and Arrebola, 2020; Rimkus et al., 1994; Venisse et al., 2019; Zani et al., 2013). Moreover, many environmental pollutants are also known as endocrine disruptors chemicals as these alter the normal function of endogenous hormones triggering an assort of severe health hazards (*e.g.* obesity and metabolic disorder). Additionally, studies have shown some endocrine disruptors capable of crossing the placental barrier and excreted through breast milk (Hernandez-Castro et al., 2023; Lao et al., 2023; Y. Liu et al., 2022; López Sanguos et al., 2023; Sousa et al., 2022).

Organophosphorus pollutants, as organophosphorus pesticides (OPPs) and organophosphorus flame retardants (or organophosphate esters, OPEs), are examples of environmental pollutants humans are exposed (Gbadamosi et al., 2021; Sousa et al., 2020). OPPs were created in 1930s and used as insecticide in substitution of organochlorine pesticides, representing 34% of the worldwide market share for pesticides production and sale (Ajiboye et al., 2022; Sousa et al., 2020). Although not as persistent, their toxicity is higher than organochlorine pesticides. OPPs present carcinogenic, neurotoxic, mutagenic, genotoxic, teratogenic, cytotoxic and immunotoxic effects. European Union regulation EC No.1107/2009 banned the OPPs chlorfenvinphos, parathion and parathion-methyl from use (European Union, 2009). Although, despite legislation to control or ban them, deaths by OPPs poisoning have been reported. OPPs are found in environmental and human samples as hair, liver, kidneys, and adipose tissue. Specifically in adipose tissue, studies are scarce, and concentrations have been found between 0.02 ng/g to 1.04 μg/g of fat (Akgür et al., 2003; Russo et al., 2002).

OPEs are man-made flame retardant chemicals employed as a replacement for brominated flame retardants in several products (*e.g.* textiles, paints, furniture, plastics, construction materials, food additives, and electronics), since the later were linked to severe health hazards (Bo and Zhu, 2022; M. Liu et al., 2022). However, the usage of OPEs is also cause for concern. These account for about 20% of the total flame retardant usage in Western Europe and are under the European Union Regulation EC No.1272/2008 (European Union, 2008). However, regulations branded OPEs as health and/or environmental hazards, their usage grows without control or restrictions. Furthermore, OPEs are easily released from materials to the environment (*e.g.* by leaching, volatilisation, or abrasion) since these are only physically added to materials and not chemically bonded to them. In addition, OPEs are more prevalent in indoor air than in the outdoors (Lao et al., 2023). Hence, these have been detected in several environmental matrixes and human samples, as plasma, blood, hair, nails, breast milk, adipose tissue, placenta, seminal fluid, and urine (Bo and Zhu, 2022; Gbadamosi et al., 2021; Guo et al., 2022; Ji et al., 2022; Lao et al., 2023; LeBel and Williams, 1983; Y. Liu et al., 2022; Yao et al., 2021; Zhao et al., 2017). Classified as emergent endocrine disruptor chemicals, these are inserted in the European Union (EU) priority compounds list and part of the Human Biomonitoring for Europe (HBM4EU) initiative (Bajard et al., 2021). OPEs exposure was linked to carcinogenicity, neurotoxicity, hepatotoxicity, nephrotoxicity, reproductive toxicity, endocrine disruption, oxidative stress, obesity, low-birth-weight infants, DNA damage, amongst others (Bo and Zhu, 2022; Gbadamosi et al., 2021; Guo et al., 2022; Ji et al., 2022; Lao et al., 2023; Y. Liu et al., 2022; Yao et al., 2021; Zhao et al., 2017).

Although called as non-persistent, these compounds still can remain in the environment for years (Campos and Freire, 2016). The ubiquitous presence of these organophosphorus pollutants in the environment and their impact on human health is a major concern. Even though researchers pointed to adipose tissue as the main target organ for their accumulation, studies that quantify OPPs and OPEs in human adipose tissue are scarce.

The present study measures the levels of OPPs and OPEs in samples of subcutaneous adipose tissue (scAT) and visceral adipose tissue (vAT) from 188 adult obese women. The aim is to evaluate and understand their possible effect on several biochemical parameters.

2. Materials and methods

2.1. Study design and sampling

Samples of scAT and vAT were collected between 2009 and 2011 from a cohort of 188 female patients undergoing bariatric surgery at Hospital de São João (Porto, Portugal). All participants provided written informed consent. This research followed the Declaration of Helsinki and was previously approved by the hospital's ethics committee (CE 146–09). Until analysis, samples were stored at −80 °C. All patients clinical and biochemical data was acquired and provided by the hospital as described in previous studies (Pestana et al., 2014; Teixeira et al., 2015).

The female population under study consists of obese patients (BMI>35 kg/m^2) with an age range from 19 to 65 years old (median 41 ± 16 years). The clinical, social, and biological characteristics of the patients are shown in Tables 1 and 2. Years of obesity (obesity evolution) were between 0 and 50 years and 6 months after patients underwent bariatric surgery the median BMI decreased 10 kg/m^2 (Table 1). All the patients were from Northern Portugal, where 78% lived in Oporto and more than half patients lived in a densely populated area (56%, Table 2). Moreover, 75% of the patients had children (Table 2).

2.2. Chemical and reagents

n-Hexane and acetonitrile (ACN) were chromatographic grade and obtained from Merck (Darmstadt, Germany). OPEs [tripropyl phosphate (TPrP), tri-iso-butyl phosphate (TiBP), tri-n-butyl phosphate (TnBP), tris (2-chloroethyl) phosphate (TCEP), tris(2-butoxyethyl) phosphate (TBEP), tris(2-ethylhexyl) phosphate (TEHP), tri-o-tolyl phosphate or tri-o-cresyl phosphate (TCP)], OPPs [dimethoate, chlorpyrifos-methyl, parathion-methyl, malathion, chlorpyrifos and chlorfenvinphos] were obtained from Sigma-Aldrich (St. Louis, MO, USA). Stock solutions were prepared in *n*-hexane at 2000 μg/L. Mixed fortification standards, containing seven OPEs or six OPPs were prepared by diluting stock standard solutions. Stock solutions were used for plotting calibration curves and prepared fortified adipose tissue samples, prior to utilization stock solutions were stored at 4 °C.

Dispersive SPE 2 mL Fatty Samples AOAC and C18 Endcapped Bulk (C18EC) sorbent were obtained from Agilent technologies (California, USA) and Supel QuE Z-Sep + Bulk (Z-Sep$^+$) from Sigma-Aldrich (St. Louis, MO, USA).

2.3. Gas chromatography flame photometric detection

A Shimadzu GC-2010 and flame photometric detection with phosphorus filter equipped with a ZB-XLB column (30 m × 0.25 mm, 0.25 m film thickness, Zebron), with ultrapure grade helium (Linde Sógas purity ≥99.999%) as carrier gas and operated by GCSolution Shimadzu software was used to quantified OPPs and OPEs. The injector was maintained at 250 °C in splitless mode and the detector at 290 °C. The gas chromatography (GC) oven temperature was programmed as follows: initial temperature of 50 °C (1 min hold), rising at 10 °C/min to 180 °C (1 min hold), increasing again at 10 °C/min to 220 °C (1 min hold), rising at 10 °C/min to 275 °C (1 min hold) and finally at 10 °C/min to 290 °C (7 min hold). The retention time for each OPPs and OPEs was determined by the injection of individual standard solutions of each

compound. OPPs and OPEs characteristics and retention times are shown in Supplementary Material Table SM1.

2.4. Gas chromatography tandem mass spectrometry

Positive samples were confirmed in a Trace GC Ultra gas chromatograph Polaris Q ion trap mass spectrometer (Thermo Fisher Scientific) with electron impact ionization mode (70 eV). Data acquirement and processing were performed with Xcalibur 1.3 software. Ultrapure grade helium (Linde Sógas purity ≥99.999%) was used as carrier gas (constant flow, 0.9 mL/min), in a Zebron ZB-5MSi column (30m × 0.25 mm, 0.25 mm film thickness, Phenomenex). The injector temperature was set at 260 °C. The GC oven temperature was programmed as followed: initial temperature of 100 °C (1 min hold), rising at 20 °C/min to 170 °C (1 min hold), rising at 4 °C/min to 210 °C (1 min hold) and finally at 15 °C/min to 290 °C (3.5 min hold). Transfer line and ion source

Table 1
Clinical and biological characteristics of the patients.

		n	Median (P25–P75)
Age information	Age (years)	188	41 (34–50)
	Obesity evolution (years)	181	19 (14–27)
Anthropometric parameters	BMI (kg/m^2)	186	44 (41–48)
	BMI 6 months after surgery (kg/m^2)	182	34 (32–37)
	Body fat (%)	51	49 (42–51)
	Torso body fat (%)	51	47 (43–49)
	Waist circumference (cm)	159	119 (113–128)
	Hip circumference (cm)	149	133 (126–140)
	vAT adipocyte area (mm^2)	186	4223 (3250–5001)
	scAT adipocyte area (mm^2)	185	6477 (5208–7546)
	vAT number of adipocyte (x10^9)	186	79 (67–99)
	scAT number of adipocyte (x10^9)	186	53 (44–63)
Plasma lipid profile	Total cholesterol (mg/dL)	157	197 (172–222)
	Total triglycerides (mg/dL)	157	120 (91–151)
	HDL cholesterol (mg/dL)	157	49 (42–56)
	LDL cholesterol (mg/dL)	157	126 (102–142)
	Apolipoprotein A1 (mg/dL)	39	158 (138–174)
	Apolipoprotein B (mg/dL)	39	93 (81–106)
	Lipoprotein A (mg/dL)	36	7 (3–18)
Blood pressure	Systolic blood pressure (mmHg)	149	135 (120–148)
	Diastolic blood pressure (mmHg)	149	80 (73–90)
Plasma glucose homeostasis and insulin sensitivity	Glycaemia (mg/dL)	178	87 (78–98)
	HbA1c (%)	140	5.6 (5.3–6.0)
	HOMA-IR	163	150 (110–191)
	HOMA2-B (%)	163	2 (1–3)
	HOMA2-S (%)	163	49 (35–71)
	C-peptide (ng/mL)	103	3 (3–4)
	Insulin (μUI/L)	122	16 (10–22)
Liver function	AST (U/L)	147	22 (18–28)
	ALT (U/L)	157	23 (18–31)
	GGT (U/L)	155	25 (18–34)
	ALP (U/L)	155	66 (57–84)
Minerals	Calcium (mEq/L)	152	4.7 (4.6–4.9)
	Phosphorus (mEq/L)	152	34 (31–37)
	Magnesium (mEq/L)	152	1.6 (1.5–1.7)
	Sodium (mEq/L)	174	139 (137–140)
	Potassium (mEq/L)	171	4.2 (4.0–4.5)
	Chlorides (mEq/L)	174	104 (103–105)

		n	Median (P25–P75)
Other parameters	FSH (mUI/mL)	121	6 (4–24)
	LH (mUI/mL)	123	7 (4–19)
	Estradiol (pg/mL)	114	49 (25–88)
	Progesterone (ng/mL)	42	0.4 (0.2–0.9)
	Testosterone (ng/mL)	127	0.4 (0.2–0.6)
	Androstenedione (ng/mL)	126	2 (1–3)
	SHBG (nmol/L)	118	44 (30–78)
	DHEA-S (μg/dL)	125	172 (116–239)
	Free T3 (ng/mL)	109	3.2 (2.8–3.5)
	Free T4 (ng/mL)	133	1.1 (1.0–1.2)
	TSH (μUI/mL)	136	2 (1–3)
	Albumin (g/L)	169	41 (40–43)
	Uric acid (mg/L)	153	53 (43–60)
	Creatinine (mg/L)	176	8 (8–9)
	Urea (g/L)	177	0.3 (0.3–0.4)
	C-reactive protein (mg/L)	53	6 (2–14)
	Cortisol (μg/dL)	59	18 (12–25)
	Sedimentation velocity (mm/1.ah)	55	22 (11–34)

ALP – alkaline phosphatase; ALT - Alanine aminotransferase; AST – aspartate aminotransferase; BMI - body index mass; DHEA-S - dehydroepiandrosterone sulphate; FSH - follicle-stimulating hormone; GGT – gama glutamil transpeptidase; HbA1c - glycated haemoglobin; HDL - high density lipoprotein; HOMA2-B - homeostasis model assessment of beta cell function; HOMA2-S – homeostasis model assessment of insulin sensitivity; HOMA-IR - homeostasis model assessment of insulin resistance; LDL - low density lipoprotein; LH - luteinizing hormone; P25 – 25th percentile 25; P75 – 75th percentile; scAT - subcutaneous adipose tissue; SHBG - sex hormone binding globulin; T3 – Triiodothyronine; T4 – Thyroxine; TSH - thyroid stimulating hormone; vAT - visceral adipose tissue.

Table 2
Social, clinical and biological characteristics of the patients and relation with the sum of OPP and OPE concentrations (μg/g AT).

				ΣOPP + OPE scAT		ΣOPP + OPE vAT		ΣOPP + OPE scAT + vAT	
	n	(%)	Characteristics	Median (P25–P75)	*p*	Median (P25–P75)	*p*	Median (P25–P75)	*p*
Area of residence	105	56%	Densely Populated Area	0.008 (0.005–0.02)	0.980	0.009 (0.005–0.03)	0.923	0.011 (0.006–0.04)	0.776
	61	32%	Moderately Populated Area	0.009 (0.005–0.03)		0.009 (0.005–0.03)		0.014 (0.007–0.06)	
	22	12%	Sparsely Populated Area	0.011 (0.005–0.01)		0.009 (0.006–0.02)		0.016 (0.007–0.03)	
Occupation	4	2%	Representatives of the legislative and executive bodies, directors and executive managers	0.011 (0.005–0.02)	0.268	0.019 (0.01–0.09)	0.096	0.029 (0.008–0.07)	0.092
	15	8%	Specialists in intellectual and scientific activities	0.010 (0.005–0.02)		0.006 (0.005–0.009)		0.009 (0.005–0.02)	
	13	7%	Intermediate level technicians and professions	0.008 (0.006–0.02)		0.007 (0.005–0.01)		0.011 (0.007–0.02)	
	9	5%	Administrative staff	0.005 (0.003-)		0.013 (0.007–0.1)		0.007 (0.005–0.02)	
	2	1%	Farmers and skilled workers in agriculture, fishing and forestry	0.005		nd		0.005	
	26	14%	Skilled workers in industry, construction and craftsmen	0.007 (0.005–0.05)		0.007 (0.005–0.07)		0.013 (0.009–0.09)	
	3	2%	Plant and machine operators and assembly workers	0.007 (0.005–0.01)		0.005 (0.005–0.01)		0.014 (0.009–0.03)	
	3	2%	Unskilled workers	0.009 (0.005–0.01)		0.009 (0.009–0.02)		0.019 (0.02–0.02)	
	45	24%	Without - Students, Retired and Unemployed	0.007 (0.005–0.01)		0.011 (0.005–0.03)		0.009 (0.007–0.03)	
Smoking	36	19%	Yes	0.012 (0.005–0.09)	0.380	0.013 (0.005–0.1)	0.202	0.024 (0.009–0.1)	0.091
	133	71%	No	0.008 (0.005–0.02)		0.009 (0.005–0.02)		0.013 (0.007–0.04)	
Parity (number of infants)	39	21%	0	0.006 (0.005–0.01)	**0.038**	0.005 (0.005–0.02)	**0.047**	0.009 (0.005–0.02)	0.089
	46	24%	1	0.007 (0.005–0.02)		0.009 (0.005–0.02)		0.013 (0.007–0.03)	
	64	34%	2	0.009 (0.005–0.03)		0.011 (0.005–0.03)		0.014 (0.007–0.05)	
	25	13%	3	0.014 (0.005–0.09)		0.011 (0.006–0.07)		0.016 (0.007–0.1)	
	7	4%	4	0.020 (0.005–0.4)		0.023 (0.007–0.3)		0.021 (0.005–0.3)	
Menopause	52	28%	Yes	0.013 (0.005–0.04)	**0.007**	0.014 (0.007–0.09)	**0.004**	0.014 (0.007–0.1)	0.09
	121	64%	No	0.007 (0.005–0.01)		0.009 (0.005–0.02)		0.014 (0.007–0.03)	
				ΣOPP + OPE scAT		ΣOPP + OPE vAT		ΣOPP + OPE scAT + vAT	
	n	(%)	Characteristics	Median (P25–P75)	*p*	Median (P25–P75)	*p*	Median P25–P75)	*p*
Age	101	54%	≤41	0.007 (0.005–0.02)	0.342	0.009 (0.005–0.02)	**0.04**	0.01 (0.007–0.03)	0.240
	87	46%	>41	0.009 (0.005–0.03)		0.01 (0.005–0.04)		0.01 (0.007–0.06)	
Elevated triglycerides	41	22%	Yes	0.009 (0.005–0.01)	0.278	0.009 (0.005–0.05)	0.801	0.014 (0.007–0.03)	0.498
	116	62%	No	0.006 (0.005–0.02)		0.009 (0.005–0.02)		0.011 (0.007–0.03)	
Reduced HDL	80	43%	Yes	0.007 (0.005–0.01)	0.117	0.007 (0.005–0.02)	0.066	0.009 (0.006–0.02)	0.105
	77	41%	No	0.010 (0.005–0.02)		0.011 (0.005–0.03)		0.014 (0.007–0.05)	
Elevated fasting glucose	39	21%	Yes	0.009 (0.005–0.03)	0.224	0.011 (0.005–0.08)	0.09	0.016 (0.007–0.1)	**0.03**
	140	74%	No	0.007 (0.005–0.02)		0.009 (0.005–0.02)		0.012 (0.007–0.03)	
Elevated blood pressure	98	52%	Yes	0.008 (0.005–0.01)	0.731	0.009 (0.005–0.02)	0.940	0.013 (0.007–0.03)	0.762
	51	27%	No	0.007 (0.005–0.02)		0.009 (0.005–0.03)		0.011 (0.005–0.06)	
Metabolic syndrome	83	44%	Yes	0.008 (0.005–0.02)	0.814	0.007 (0.005–0.02)	**0.03**	0.012 (0.007–0.03)	0.145
	101	54%	No	0.008 (0.005–0.02)		0.012 (0.005–0.03)		0.014 (0.007–0.06)	
Number of components of metabolic syndrome	40	21%	1	0.009 (0.005–0.03)	0.077	0.009 (0.005–0.02)	0.089	0.011 (0.006–0.05)	0.166

(*continued on next page*)

temperatures were set at 270 °C and 292 °C, respectively. Tandem mass spectrometry (MS/MS) isolation conditions were wideband application = 1 and isolation time = 12 ms. Fragmentation conditions and the selected ions for each analyte were selected according to (Castro et al., 2020; Maia et al., 2021; Sousa et al., 2020; Zeng et al., 2022) and are described in Supplementary Material Table SM1.

2.5. Extraction and clean-up

The extraction of OPEs and OPPs from adipose tissue samples was performed following the methodology described by (Sousa et al., 2020). Briefly, to 0.4 g of adipose tissue was added *n*-hexane (6 mL) and homogenised in an ultrasonic processor (Sonics & Materials VCX750). The samples were kept for 24 h at −18 °C, after which an aliquot was retrieved from the upper layer (1.5 mL), dried under nitrogen, and re-dissolved in ACN. After vortexing and centrifugation (10 min at 4500 rpm), the resulting upper layer was transferred to the d-SPE clean-up vial prepared according to (Sousa et al., 2020) with an extra 50 mg of C18EC and 50 mg of Z-Sep^{+}. From the upper layer, 450 μL were transferred into a vial, dry under nitrogen, and re-dissolved in 75 μL of *n*-hexane. Finally, the extract was analysed by GC flame photometric detection (FPD). Lipids were determined gravimetrically with a fraction of the initial extract. Positive samples were confirmed by GC-MS/MS.

2.6. Method validation

Method validation was performed according to (Sousa et al., 2020). The response in fortified human adipose tissue samples and in solvent solution were used to determine the signal suppression/enhancement (Paíga et al., 2021). Matrix-matched calibration curves were plotted from 10 to 200 μg/L and prepared in human adipose tissue extract (matrix without fortification or blank). Linearity was set to a coefficient of determination ≥0.99. Method detection (MDL) and quantification (MQL) limits were obtained, respectively, from 3 to 10 times the ratio of the standard deviation for the lowest standard and the calibration curve slope. Fortified human adipose tissue samples were used to determine accuracy (at 20, 30, and 50 μg/L, in triplicate). Repeatability and intermediate precision were assessed at four standard concentrations in triplicate (10, 20, 100, and 200 μg/L). The expanded combined uncertainty (*Ur,tot*) was calculated at 20 and 100 μg/L considering a confidence level of 95% and coverage factor k of 2, through the "top-down" approach (Nagyová and Tölgyessy, 2019).

2.7. Statistical analysis

Statistical Package for Social Sciences (SPSS, 21.0 version statistical software, IBMCorp.s, NewYork, USA) was used to conduct the statistical analysis. Data were presented as median, 25th percentile (P25) and 75th percentile (P75), due to the absence of normal distribution (verified by Kolmogorov-Smirnov or Shapiro-Wilk test, when n was <50). When OPPs or OPEs were found in concentrations below the MDL or the MQL, a value was assigned for statistical purposes. Therefore, to analytes found below the MDL was attributed the value of the ratio between MDL and the square root of 2 and to analytes found below the MQL was attributed the value of the ratio between MQL and the square root of 2 (Hornung and Reed, 1990). Wilcoxon test was used to compare medians of OPPs and OPEs between scAT and vAT. The Mann–Whitney and Kruskal–Wallis tests were used to compare medians of OPEs and OPPs between different patients' characteristics. Spearman correlation test were applied to find possible associations between the OPEs and OPPs and the biochemical parameters. Multivariate linear regression analysis adjusting for possible covariates was performed to the biochemical parameters that presented statistically significant Spearman correlations (dependent variable). Values of $p < 0.05$ were regarded as significant and all tests were two-tailed.

3. Results and discussion

3.1. Method validation

Method validation was performed to each individual analyte and the parameters assessed are shown in Supplementary Material Table SM2. The matrix displayed a minor effect on the determination of all OPPs and OPEs (between −17 and 18%), which follows in the guidelines set by the European Commission (signal suppression/enhancement <20%) (European Commission, 2021a). All matrix-matched calibration curves showed linearity (coefficient of determination >0.99). MDL were between 0.001 and 0.005 μg/g for OPPs and OPEs and MQL between 0.003 and 0.01 μg/g for OPPs and between 0.004 and 0.02 μg/g for OPEs. OPPs average recoveries ranged from 61 to 108% and for OPEs between 58 and 89%. According to the European Commission (European Commission, 2021a), average recoveries should be between 70 and 120% with a relative standard deviation of less than 20%. The OPPs, Chlorfenvinphos, and the OPEs, TPrP, TiBP, TnBP, TBEP, and TEHP achieved average recoveries between 58 and 68%, and so out of this criterion. However, the European Commission guidelines also state that between 30 and 140% are still acceptable recoveries if the relative standard deviation is less than 20% (European Commission, 2021b), which was verified in all the analytes (relative standard deviation between 2.2 and 10.3%). Good repeatability and intermediate precision were obtained for OPPs (1–9% and 5–17%, respectively) and OPEs (2–11% and 3–13%, respectively). Finally, *Ur, tot* were in the acceptable range (5–22% and 9–31% for OPPs and OPEs, respectively) according to the European Commission (*Ur, tot* <50%) (European Commission, 2021b).

3.2. Distribution of organophosphorus pollutants in scAT and vAT

Two OPPs and five OPEs were found in samples of the scAT and vAT (Table 3), namely dimethoate, malathion, TiBP, TnBP, TBEP, TEHP, and TCP. Dimethoate was only detected in vAT, whereas TEHP was only found in scAT. The detection frequencies for dimethoate, malathion, and TEHP were below 2.5%, for TCP, TnBP, and TBEP below 20%, and for TiBP below 30%. Overall, OPPs and OPEs were found in 91 samples of scAT (detection frequency = 48.4%) and in 100 samples of vAT

Table 2 (*continued*)

			ΣOPP + OPE scAT		ΣOPP + OPE vAT		ΣOPP + OPE scAT + vAT	
n	(%)	Characteristics	Median (P25–P75)	*p*	Median (P25–P75)	*p*	Median P25–P75)	*p*
49	26%	2	0.005 (0.005–0.01)		0.013 (0.006–0.03)		0.014 (0.008–0.03)	
48	26%	3	0.009 (0.005–0.02)		0.007 (0.005–0.02)		0.012 (0.007–0.03)	
24	13%	4	0.005 (0.005–0.01)		0.008 (0.005–0.02)		0.011 (0.005–0.02)	
11	6%	5	0.007 (0.005–0.02)		0.007 (0.005–0.09)		0.009 (0.007–0.1)	

HDL - high density lipoprotein; nd – not detected; P25 – 25th percentile 25; P75 – 75th percentile; Statistical analysis performed with Mann-Whitney and Kruskal Wallis tests, $p < 0.05$; Significant *p* values are shown in bold.

Table 3
OPP and OPE concentrations (μg/g AT) in scAT and vAT.

Compound	scAT			vAT			p^1	
	n	Frequency	Median (P25–P75)	n	Frequency	Median (P25–P75)		
Dimethoate		0.0%	nd	1	0.5%	0.2	na	
Chlorpyrifos-methyl		0.0%	nd		0.0%	nd		
Parathion-methyl		0.0%	nd		0.0%	nd		
Malathion	1	0.5%	<MDL	3	1.6%	<MDL	na	
Chlorpyrifos		0.0%	nd		0.0%	nd		
Chlorfenvinphos		0.0%	nd		0.0%	nd		
ΣOPP	1	0.5%	<MDL	3	1.6%	0.005 (<MDL-na)	na	
TPrP		0.0%	nd		0.0%	nd		
TiBP	53	28.2%	<MQL	56	29.8%	<MQL	na	
TnBP	25	13.3%	<MDL (<MDL-0.01)	35	18.6%	<MDL (<MDL-0.01)	na	
TCEP		0.0%	nd		0.0%	nd		
TBEP	29	15.4%	<MQL (<MDL-0.1)	37	19.7%	0.02 (<MQL-0.1)	na	
TEHP	4	2.1%	0.01 (0.01–0.04)		0.0%	nd	na	
TCP	7	3.7%	0.08 (0.03–0.1)	13	6.9%	0.02 (<MQL-0.05)	0.6	
ΣOPE	91	48.4%	0.008 (0.005–0.02)	100	53.2%	0.009 (0.005–0.02)	0.3	$\mathbf{r^2_{spearman}}$
ΣOPP + OPE	91	48.4%	0.008 (0.005–0.02)	100	53.2%	0.009 (0.005–0.02)	0.3	0.574

MDL – method detection limit; MQL – method quantification limit; OPE - Organophosphorus ester flame retardant; OPP - organophosphorus pesticide; P25 – 25th percentile 25; P75 – 75th percentile; scAT - subcutaneous adipose tissue; vAT - visceral adipose tissue; TBEP - tris(2-butoxyethyl) phosphate; TCEP - tris(2-chloroethyl) phosphate; TCP - tri-o-tolyl phosphate or tri-o-cresyl phosphate; TEHP - tris(2-ethylhexyl) phosphate; TiBP - tri-iso-butyl phosphate; TnBP - tri-n-butyl phosphate; TPrP - tripropyl phosphate; na – not analysed; nd – not detected; [1]Statistical analysis performed with Wilcoxon test, $p < 0.05$; [2]Statistical analysis with Spearman's correlation, $p < 0.05$; Significant p values are shown in bold.

(detection frequency = 53.2%). The mean lipid content of the samples was 90% and 95% for scAT and vAT, respectively. The median concentrations of individual organophosphorus pollutants were between <0.004 and 0.08 μg/g of scAT and between <0.004 and 0.2 μg/g of vAT and the sum of OPPs and OPEs was 0.008 ± 0.020 μg/g of scAT and 0.009 ± 0.020 μg/g of vAT. The levels of OPPs and OPEs in scAT and vAT did not differ nor these correlate to one another ($p > 0.05$, Table 3). Three other studies were found in the literature for the analysis of OPPs in adipose tissue, in which the most recent was a method validation study performed by the present authors (Sousa et al., 2020), where OPPs were not detected. As for the other two studies, Russo et al. (2002) found levels of dimethoate ranged from 0.0002 to 0.0004 μg/g of adipose tissue and chlorfenvinphos ranged from 0.00005 to 0.0002 μg/g in human adults. Whereas Akgür et al. (2003) found OPPs levels between 15 and 333 μg/g in cases of OPPs poisoning. To the authors knowledge, there is only one other study reporting levels of OPEs in human adipose tissue in literature. In which, LeBel et al. (LeBel and Williams, 1983) reported OPEs levels between 0.0005 and 0.1 μg/g. However, studies conducted in human blood, serum, and breast milk report levels of OPEs from 0.001 to 0.04 μg/mL of blood (Guo et al., 2022), 0.003–0.005 μg/g of serum (M. Liu et al., 2022), and 0.0002–0.02 μg/g of breast milk (values converted considering 3.4% of lipid content in breast milk) (Guo et al., 2022; Lao et al., 2023). Showing OPEs are transferable from breast milk to infants. Zhao et al. (2017) also found evidence of prenatal exposure, which is a cause of greater concern as the most vulnerable stages to endocrine disruption are embryonic and neonatal (Sousa et al., 2022). Hair and nails are matrixes also used to assess long-term exposure to pollutants. OPEs were found in hair with levels ranging from 0.002 to 10.8 μg/g, whereas in nails levels were reported between 0.09 and 233.7 μg/g (Guo et al., 2022). Furthermore, OPEs metabolites were found in urine samples from <0.0001 to 0.06 μg/mL (Chai et al., 2022; Gao et al., 2022; Guo et al., 2022). These studies, as our study, show the importance of biomonitoring OPEs in the human body. OPEs levels are within the same range as other hazard chemicals found in adipose tissue, *e.g.* their predecessor brominated flame retardants (4×10^{-6}-0.1 μg/g), or polychlorinated biphenyls (8.4×10^{-5}-3.9 μg/g), synthetic musk (0.002–0.6 μg/g) and organochlorine pesticides (0.1–24.8 μg/g) (Sousa et al., 2022). However, the concentrations reported are concerning. Let's not forget that at the moment there is not any legislation limiting the usage of OPEs.

3.3. Associations between organophosphorus pollutants and the clinical, social, and biochemical characteristics

Associations between organophosphorus pollutants and the clinical, social, and biological characteristics of the patients are shown in Tables 2 and 4 and Fig. 1.

The median concentration of organophosphorus pollutants in vAT was higher in older women (>41 years, Table 2) which was also corroborated by the positive Spearman's correlation found with patient age (Fig. 1). Moreover, the median of organophosphorus pollutants were statistically different according to the number of children (parity). Women with more children had the highest median of organophosphorus pollutants in scAT ($p = 0.038$) and in vAT ($p = 0.047$). Furthermore, female patients with the highest parity were also older (no children = 33 median age; 1 child = 36 median age; 2 children = 44 median age; 3 children = 47 median age; 4 children = 58 median age), which could explain this association. Even though, in scAT, median concentrations of organophosphorus pollutants were not statistically significant, the median was also higher in older women (Table 2). Likewise, the same can be said regarding menopausal status. Here women in menopause had the highest median of organophosphorus pollutants, and these women were also older (median age, 54 vs 36 years). Other studies either did not find an age-dependent association with OPEs or younger individuals showed the highest levels of OPEs metabolites (contrary to this study), which was associated with more usage of electronic devices and more time indoors due to occupational/lifestyle activities (Sun et al., 2018). However, OPEs biomonitoring studies are mainly performed in blood, serum or urine, hence representative of a recent exposure rather than long time accumulation of organophosphorus pollutants as the assessment conducted in our study. Boyle et al. (2019) suggested that OPEs accumulation in adipose tissue might decrease urinary OPEs concentrations. Moreover, usually these studies measure OPEs exposure in a single spot urine sample, which may not be indicate of an accurate long-time exposure to all OPEs (Boyle et al., 2019).

The outset of metabolic syndrome is caused by various factors such as overnutrition, physical inactivity, aging, sleep deficiency, and by environmental endocrine-disrupting chemicals (Luo et al., 2020b). Controversially in our study, the median of organophosphorus pollutants in vAT appeared higher in patients without metabolic syndrome

Table 4
Multivariate linear regression models between clinical and biological characteristics of the patients and the sum of OPP and OPE concentrations.

	ΣOPP + OPE scAT		ΣOPP + OPE vAT		ΣOPP + OPE scAT + vAT	
	β	*p*	β	*p*	β	*p*
Waist circumference						
age adjusted	0.207	**0.018**	0.142	0.093	0.154	**0.038**
multivariable adjusted[1]	0.202	**0.023**	0.142	0.094	0.151	**0.043**
vAT adipocyte area						
age adjusted	na	na	0.150	0.051	0.188	**0.005**
multivariable adjusted[1]	na	na	0.150	0.055	0.186	**0.006**
scAT adipocyte area						
age adjusted	−0.076	0.359	−0.101	0.210	−0.081	0.245
multivariable adjusted[1]	−0.070	0.404	−0.101	0.208	−0.078	0.269
vAT number of adipocyte						
age adjusted	na	na	na	na	−0.086	0.204
multivariable adjusted[1]	na	na	na	na	−0.080	0.244
scAT number of adipocyte						
age adjusted	0.177	**0.029**	0.084	0.288	0.124	0.072
multivariable adjusted[1]	0.163	**0.048**	0.084	0.289	0.117	0.092
Total cholesterol						
age adjusted	na	na	na	na	0.168	**0.025**
multivariable adjusted[1]	na	na	na	na	0.172	**0.023**
Glycaemia						
age adjusted	na	na	na	na	−1.116	0.266
multivariable adjusted[1]	na	na	na	na	−0.071	0.271
Magnesium						
age adjusted	−0.040	0.664	na	na	na	na
multivariable adjusted[1]	−0.036	0.702	na	na	na	na
Chlorides						
age adjusted	na	na	−0.098	0.223	na	na
multivariable adjusted[1]	na	na	−0.098	0.224	na	na
FSH						
age adjusted	0.075	0.343	−0.029	0.703	na	na
multivariable adjusted[1]	0.069	0.390	−0.029	0.705	na	na
Estradiol (ln transformed)						
age adjusted	−0.177	0.089	−0.111	0.262	−0.129	0.140
multivariable adjusted[1]	−0.184	0.082	−0.111	0.265	−0.132	0.136
Testosterone						
age adjusted	na	na	0.196	**0.031**	0.097	0.227
multivariable adjusted[1]	na	na	0.196	**0.031**	0.099	0.217
Androstenedione						
age adjusted	0.194	**0.032**	na	na	0.185	**0.015**
multivariable adjusted[1]	0.208	**0.023**	na	na	0.190	**0.012**
SHBG (ln transformed)						
age adjusted	na	na	−0.186	0.052	na	na
multivariable adjusted[1]	na	na	−0.186	0.052	na	na
Free T3						
age adjusted	na	na	−0.049	0.592	na	na
multivariable adjusted[1]	na	na	−0.049	0.595	na	na
Free T4						
age adjusted	na	na	−0.344	**<0.001**	−0.277	**0.001**
multivariable adjusted[1]	na	na	−0.344	**<0.001**	−0.274	**0.001**
Urea						
age adjusted	na	na	0.065	0.354	na	na
multivariable adjusted[1]	na	na	0.065	0.355	na	na
Sedimentation velocity						
age adjusted	na	na	na	na	0.067	0.577
multivariable adjusted[1]	na	na	na	na	0.073	0.544

na – not applicable, Spearman correlations (dependent variable) were not significant; OPE - Organophosphorus ester flame retardant; OPP - organophosphorus pesticide; scAT - subcutaneous adipose tissue; vAT - visceral adipose tissue; [1]Multivariable-adjusted model included age and parity; Statistical analysis performed by multivariate linear regression models, $p < 0.05$; Significant *p* values are shown in bold; β-values from a linear regression model are reported.

(Table 2). The classification of metabolic syndrome was assigned according to (Pestana et al., 2014), considering the following components: waist circumference ≥88 cm; HDL cholesterol <50 mg/dL; triglycerides ≥150 mg/dL; systolic blood pressure ≥130 mmHg and/or diastolic blood pressure ≥85 mmHg and glucose ≥100 mg/dL.

Waist circumference, scAT adipocyte area, and number were all correlated with organophosphorus pollutants concentration in scAT, vAT, and the overall concentration (scAT + vAT, Fig. 1). vAT adipocyte area and number were correlated with total organophosphorus pollutants concentration (scAT + vAT). vAT adipocyte area also shows a correlation with the concentration of organophosphorus pollutants in vAT. All the correlations found with anthropometric parameters were positive, except for adipocyte areas.

Endocrine disruptors alter lipid metabolism, size, and number of fat cells, which subsequently alter weight gain (Gupta et al., 2020). Urinary levels of OPEs metabolites were associated with increased BMI, waist circumference in women, and higher maternal weight gain (Boyle et al., 2019; Crawford et al., 2020; Luo et al., 2020b). Crawford et al. (2020) suggest, as a possible explanation for the weight gain associations, that OPEs impair nuclear receptor signalling and consequently alter lipid and glucose homeostasis and sex and thyroid hormones. Additionally, OPEs also can promote lipid accumulation and adipogenesis (Crawford et al., 2020; Siddique et al., 2020; Zhao et al., 2019). On the other hand, OPPs were also linked to weight gain and altered fat metabolism (Karami-Mohajeri and Abdollahi, 2011).

In fact, total cholesterol and glycaemia presented positive correlations with total organophosphorus pollutants concentration (scAT + vAT, Fig. 1). This was corroborated by the data in Table 2, patients with glycaemia higher than 100 mg/dL had the highest median of total organophosphorus pollutants (scAT + vAT). As mentioned before, OPEs were linked to impaired lipid and glucose homeostasis and hyperglycaemia (Crawford et al., 2020; Hoyeck et al., 2022) and OPPs have been associated with hyperglycaemia (Hoyeck et al., 2022; Karami-Mohajeri and Abdollahi, 2011), which was further associated with β-cell dysfunction (Hoyeck et al., 2022). A NHANES data study showed positive associations with insulin and HOMA-IR, while glucose, HOMA2-B, and HbA1c did not show any correlation with OPEs urinary levels (Bo and Zhu, 2022). Whereas for lipids, positive correlations between OPPs levels in blood and total and low-density lipoprotein (LDL) cholesterol were reported (Palaniswamy et al., 2021) as were correlations between urinary levels of OPEs and total cholesterol (Siddique et al., 2020; Zhao et al., 2019) and triglycerides (Zhao et al., 2019).

Magnesium showed a negative correlation with organophosphorus pollutants concentration in scAT, whereas chlorides showed a negative correlation with organophosphorus pollutants concentration in vAT (Fig. 1). Magnesium levels are affected by ovarian hormones (estrogen and progesterone), these diminish the levels of magnesium and alter the ratio of calcium/magnesium, which is linked to premenstrual syndrome whenever deficiency of these macrominerals is verified (Pokorska-Niewiada et al., 2022). Organophosphorus pollutants are endocrine disruptors that alter sex hormones as mentioned before (Crawford et al., 2020). Considering the above mentioned, the effect of organophosphorus compounds on hormones might have an indirect effect on macrominerals levels, which possibly explains the correlations found.

FSH and estradiol had positive and negative correlations, respectively with the concentration of organophosphorus pollutants in both tissues. Additionally, estradiol also presented a negative correlation with total organophosphorus pollutants concentration (scAT + vAT). Testosterone and SHBG showed a positive and negative correlation with

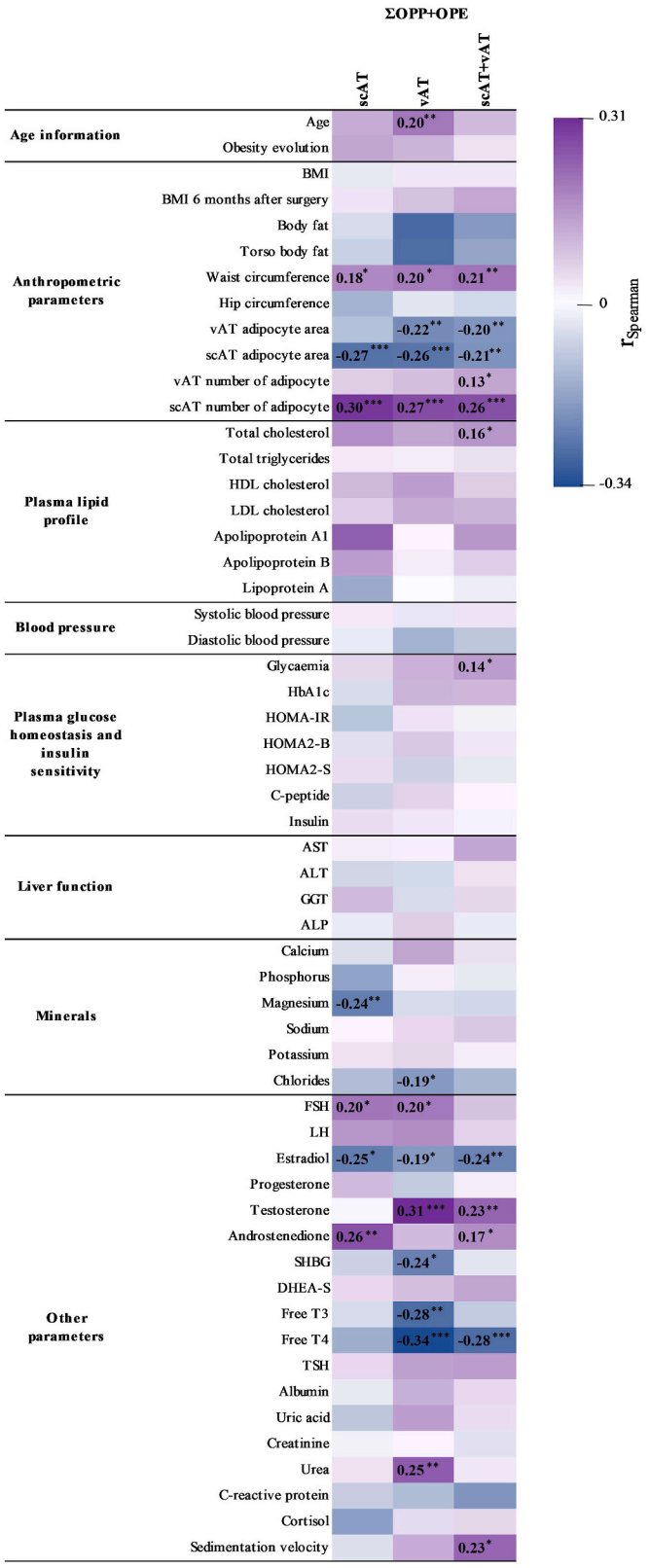

Fig. 1. Heatmap of the associations found between OPP and OPE in adipose tissue and the biochemical parameters. The colour code corresponds to Spearman's correlation coefficient (rSpearman), from lower values in blue to higher values in purple. Values in tiles are rSpearman for significant associations, $^{*}p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$.

organophosphorus pollutants concentration in vAT, respectively. Testosterone and androstenedione were positively correlated with total organophosphorus pollutants concentration (scAT + vAT). Androstenedione also had a positive correlation with organophosphorus pollutants concentration in scAT. Regarding other studies, Gao et al. (2022) reported a negative association between FSH and an OPE metabolite in the urine of fertile women. Likewise, most OPEs predecessors (brominated flame retardants) were associated with decreasing FSH levels in serum (Guo et al., 2018). However, levels of 1,2-bis-(2,4,6-tribromophenoxy)ethane (or BTBPE, a brominated flame retardant) showed a positive correlation with FSH (Guo et al., 2018) and Satar et al. (2004) found highest FSH levels in OPPs poising patients at the time of hospital admission than after these patients received treatment. Luo et al. (2020a) study achieved a negative correlation between urinary levels of OPE metabolites and estradiol in adolescent girls (similar to our study). Some OPEs were reported to have antagonist effects on estrogen, androgen, and progesterone receptors (Bajard et al., 2021). The sex hormone-binding globulin (SHBG) transports androgens and estrogens and manages serum sex hormones bioactivity. Low levels of SHBG are linked to high androgen levels. Testosterone is mostly bound to SHBG and free testosterone binds to androgen receptors. Furthermore, the synthesis of SHBG is triggered by estrogens and suppressed by testosterone (Luo et al., 2020a; Palaniswamy et al., 2021; Zhang et al., 2022). This intrinsic hormone relationship is concordant with the associations observed in our study. Other studies report different observations, OPPs metabolites and OPEs levels in urine showed negative correlations with testosterone in women (Guo et al., 2018; Zhang et al., 2022) and in female adolescents (Luo et al., 2020a). Moreover, OPEs showed a positive correlation with SHBG in adolescent girls (Luo et al., 2020a).

OPEs alter thyroid hormones, which are vital for keeping normal physiological functions (Crawford et al., 2020; Yao et al., 2021). In pregnant women, these hormones enable foetal development, and foetus is dependent on maternal thyroxine (T4). Target tissues enzymes transform free T4 into triiodothyronine (T3), its active form. The production of T4 is triggered by thyroid-stimulating hormone (TSH), hence levels of T4 and T3 are directly and indirectly managed by this hormone (Percy et al., 2021). In this study, free T3 and T4 showed negative correlations with organophosphorus pollutants concentration in vAT and free T4 also had a negative correlation with total organophosphorus pollutants concentration (scAT + vAT). Similar associations were found before in serum levels of OPEs (M. Liu et al., 2022) and OPPs (Pawitra et al., 2022). Moreover, Percy et al. (2021) studied the effects of maternal urinary OPEs metabolite levels on infant serum levels of T3, T4, and TSH hormones. Negative associations were found with free and total T3 and T4 and a positive association with TSH at birth was also reported (Percy et al., 2021). Whereas Yao et al. (2021) did not find any significant association between urinary levels of OPEs metabolites and free T3 and T4. In our study, TSH did not present any correlation with organophosphorus pollutants concentration. However other studies linked high levels of TSH to OPPs (Pawitra et al., 2022; Percy et al., 2021), and OPEs metabolites (Yao et al., 2021).

Urea had a positive correlation with organophosphorus pollutants concentration in vAT (Fig. 1). Some studies report high levels of urea after human OPPs poising (Aardema et al., 2008; Bereda, 2022; Farooqui et al., 2022). Moreover, *in vivo* studies, specifically in rats, showed an increase in urea levels after OPPs poising (Sobolev et al., 2022). The hepatoxicity of OPPs was suggested as a possible cause for the observed increase in urea levels (Karami-Mohajeri and Abdollahi, 2011).

Sedimentation velocity is an inflammatory parameter (Utami et al., 2019), and our study had a positive correlation with total organophosphorus pollutants concentration (scAT + vAT, Fig. 1). Utami et al. (2019) also reported a positive correlation between sedimentation velocity and levels of OPPs. Furthermore, animal studies reported the same observations in exposed rabbits (Salih, 2010) and fish (Malla et al., 2009).

The influence of age and parity on the above discussed correlations

was assessed by multivariate linear regression models adjusted for these covariates (Table 4). Organophosphorus pollutants concentration in scAT or/and total organophosphorus pollutants concentration (scAT + vAT) were significantly associated with waist circumference, vAT adipocyte area or/and scAT number of adipocyte. All these associations were positive. Total cholesterol displayed a positive significant association with total organophosphorus pollutants (scAT + vAT). Testosterone showed a positive association with organophosphorus pollutants concentration in vAT and androstenedione positive associations with organophosphorus pollutants concentration in scAT and total organophosphorus pollutants concentration (scAT + vAT). The correlations found previously for free T4 were corroborated by the multivariate linear regression analysis. The associations found in the multivariate linear regression analysis show that for these clinical parameters the achieved Spearman correlations are not a consequence nor linked to patient age or parity.

4. Conclusions

Organophosphorus pollutants are widespread in the environment and humans are in constant exposure. Seven compounds were detected (two OPPs and five OPEs) in the adipose tissue of obese women and median concentrations were 0.008 ± 0.020 μg/g scAT and 0.009 ± 0.020 μg/g vAT. Following within range levels of other lipophilic environmental pollutants found in adipose tissue (*e.g.* brominated flame retardants, polychlorinated biphenyls, synthetic musk, and organochlorine pesticides). However, contrary to these persistent environmental pollutants, OPEs are not presently restricted by any European legislation regarding quantities of usage. Hence, its use has been increasing freely and intensely.

For this cohort of obese females, several associations were found with biochemical parameters involved in metabolic processes (six Mann-Whitney and Kruskal Wallis associations, 32 Spearman's correlations and 20 multivariate linear regression models). Revealing the effect of these pollutants, specially OPEs, might have on an assort of parameters.

The present study has some limitations, the non-existent of a control cohort (*e.g.* under and normal weighted females or obese men) which keeps the study of being generalizable. Obesity is a disease that bears by itself metabolic consequences and the medications prescribed to treat those could altered the biochemical parameters under study. Additionally, this cross-sectional design does not permit temporal and causal variables relation assessment. Yet, few studies exist on OPPs assessment in adipose tissue and fewer on OPEs. Detection frequencies of OPEs were around 50%. Some authors had already pointed out that the assessment of OPEs metabolites in urine is not appropriate to assess long term exposure and that OPEs in adipose tissue may behave differently than their metabolites. Moreover, little is yet known about their action mechanisms. OPPs and OPEs had been shown to transfer to breast milk and cross the placental barrier, endangering the most vulnerable stage of life (embryonic and neonatal) and triggering unknown consequences. This is particularly concerning since the usage of OPEs is not regulated.

Our findings showed the impact of these pollutants on several biochemical parameters involving a wide range of metabolic processes in an obese female population. More studies are needed to further unveil the effects of organophosphorus pollutants on metabolism and understand how their effects can be blocked or minimized. The biomonitoring in adipose tissue is critical to determining long time accumulation of lipophilic pollutants. Which in turn may help prevent, and improve treatment and the follow-up of many metabolic-related diseases such as obesity. Additionally, control regulations for the usage of OPEs should be drawn to protect us and the environment from the effects of these pollutants.

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Institutional review board statement

The study was conducted in accordance with the Declaration of Helsinki and approved by Ethics Committee of Hospital de São João (CE 146–09, approved in 2009).

Informed consent statement

Informed consent was obtained from all subjects involved in the study.

CRediT authorship contribution statement

Sara Sousa: Conceptualization, Methodology, Validation, Investigation, Visualization, Formal analysis, Writing – original draft. **Diana Rede:** Investigation. **Virgínia Cruz Fernandes:** Writing – review & editing. **Diogo Pestana:** Writing – review & editing. **Gil Faria:** Resources, Writing – review & editing. **Cristina Delerue-Matos:** Resources, Writing – review & editing. **Conceição Calhau:** Writing – review & editing. **Valentina Fernandes Domingues:** Conceptualization, Validation, Supervision, Writing – review & editing.

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.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2023.117337.

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