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Accumulation of distinct persistent organic pollutants is associated with adipose tissue inflammation

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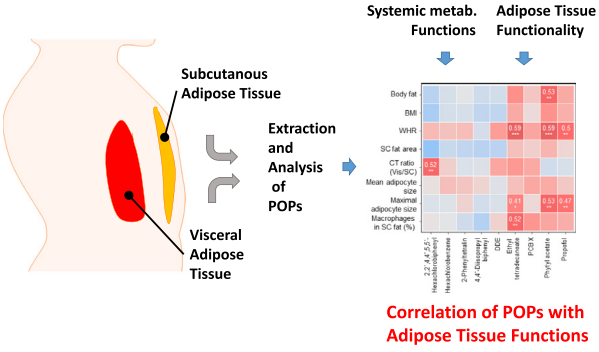
HIGHLIGHTS

- POPs accumulate in human adipose tissue
- Identification of sex and BMI specific correlations
- Distinct POPs correlate with adipose tissue macrophage infiltration
- POPs may contribute to adipose tissue dysfunction

GRAPHICAL ABSTRACT



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ABSTRACT

Hydrophobic environmental chemicals bio-accumulate in adipose tissue (AT) in animals and humans, but their impact on diseases related to adipose tissue dysfunction remains unclear. Moreover, visceral rather than subcutaneous (SC) fat deposition is more closely associated with cardio-metabolic diseases such as type 2 diabetes, fatty liver and cardiovascular diseases. We therefore tested the hypotheses that environmental chemicals bio-accumulate in a fat depot specific pattern and that these patterns are related AT inflammation and obesity comorbidities.

First, we developed an extraction method for detecting and quantifying a set of 9 persistent organic pollutants (POPs) in human AT. The quantified chemicals exhibit K_{OW} coefficients from 4 to 7. Paired abdominal omental and SC AT samples were obtained from 54 individuals (30 women, 24 men) with a wide range of body mass index (BMI, 16–70 kg/m^2) during laparoscopic abdominal surgeries. Among the POPs are classical halogenated substances like Dichlorodiphenyldichloroethylene (DDE) and polychlorinated biphenyls (PCBs), but also fragrance substances.

We find that AT concentrations of these chemicals are neither significantly different between visceral and SC fat depots nor between women and men. However, AT bio-accumulation of distinct POPs significantly correlates with AT macrophage infiltration, adipocyte size and parameters of glucose metabolism. In both fat depots, the strongest correlations of POPs (Ethyl- tetradecanoate, 4,4′-Diisopropylbiphenyl, 2-Phenyltetralin, 2,2′,4,4′,5,5′-Hexachlorobiphenyl, Hexachlorobenzene) and AT macrophage infiltration were detected in lean individuals. In

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men with obesity, abundance of POPs correlated with the duration of obesity. Additional significant associations between AT POPs and parameters of glycemia, insulin sensitivity, and inflammation suggest that specific environmental chemicals may contribute to AT dysfunction, adipocyte hypertrophy, impaired glucose metabolism, systemic inflammation and variation in fat distribution, but not to obesity.

1. Introduction

Over the past decades, obesity has become a pandemic (Berrington de Gonzalez et al., 2010; Collaboration, 2016). Obesity is a major health concern because it contributes to premature mortality and disability as a result of the obesity-associated increased risk to develop type 2 diabetes mellitus, fatty liver disease, cardiovascular diseases, osteoarthritis, Alzheimer's disease, depression and certain types of cancer (Berrington de Gonzalez et al., 2010). The obesity pandemic is closely associated with changes in behavior, lifestyle, but also the wide use of chemicals in agriculture (e.g. pesticides), for food preservation and packaging (e.g. plasticizer), cosmetics and other uses in daily life (Le Magueresse-Battistoni et al., 2018; Swinburn et al., 2011; Veiga-Lopez et al., 2018). Although obesity is a heritable trait, twin studies (Borjeson, 1976; Stunkard et al., 1990) and recent large genome-wide association studies (Speliotes et al., 2010; Turcot et al., 2018) revealed that genetic variation may only account for up to 50% of the risk to develop obesity. Therefore, environmental factors (in addition to behavior) may play an important role as obesogens.

The risk of obesity-related cardio-metabolic diseases is more closely associated with adverse fat distribution characterized by high visceral and trunk fat and low leg fat deposition (Bluher and Laufs, 2019; Chen et al., 2019; Pischon et al., 2008). In this context, dysfunction of adipose tissue (AT) is an important factor contributing to impaired glucose and lipid metabolism as well as to a pro-inflammatory and atherogenic state (Bluher, 2020; Kloting and Bluher, 2014; Scherer, 2019). Cohort studies revealed associations between POPs and the risk for obesity and type 2 diabetes (Lee et al., 2014; Tornevi et al., 2019). A large study with 1016 participants reported associations between blood concentrations of some POPs with visceral and subcutaneous AT distribution (Roos et al., 2013). However, the mechanisms causing adverse fat distribution and impaired AT function are not fully understood. We therefore hypothesize that accumulation of environmental chemicals may contribute to heterogeneity in fat distribution and AT function.

Since hydrophobic substances bio-accumulate in AT, the question arises whether environmental chemicals can affect adipocyte and AT function subsequently leading to adverse systemic effects. Postulated effects of environmental chemicals on AT function may be independent of whole body fat mass or BMI and could therefore help to explain why some individuals with obesity do not develop cardio-metabolic abnormalities (Bluher, 2020; Stefan et al., 2018), whereas lean people with impaired AT function and adverse fat distribution are at increased risk for obesity related diseases (Eckel et al., 2015; Stefan et al., 2017). Furthermore, it was found that lower levels of 21 POPs in plasma were associated with metabolically healthy obesity, whereas in individuals with metabolically unhealthy obesity significant higher POPs levels were detected (Gauthier et al., 2014).

In respect to toxicokinetic, the uptake of POPs into AT from the blood depends on flow rate, tissue volume, and anatomical localisation. The diffusion across the AT of POPs with high K_{OW} is assumed to be diffusion-limited (Levitt, 2010). The same holds true for the release of POPs from AT induced by weight loss. Indeed, significant weight loss after bariatric surgery seems to be associated with increased POP concentrations such as PCBs and perfluorochemicals (PFs) in the circulation (Brown et al., 2019). The correlation between the concentration of POPs in AT and in blood was analyzed in more detail, and it was described that after extensive weight loss the concentration in blood increased, leading to a reduction of the total POP amount in the body (Kim et al., 2011). The reduction of adipocytes' lipid droplet size also resulted in an increased POP concentration in AT. In this respect, AT can have a protective effect since POP accumulation may reduce circulating POP levels and thus decrease the exposure of potential other target organs. This study also revealed more insight into the POP concentration dependent expression of pollutant target genes. They found an increase of AhR target genes in obese patients as well as markers for low grade inflammation. These findings could not be shown for blood cells, indicating a tissue specific effect. Interestingly, although the strong weight loss resulted in an overall loss of POP abundance in AT but did not lead to significant decrease of AhR target genes. In contrast they found a clear correlation of POP abundance with negative liver and metabolic parameters that significantly improved with a delay maybe associated to the temporarily increase of POP abundance after weight loss. This supports a higher sensitivity of the liver in comparison to AT.

Beyond the effects of POPs on AT, mobilisation of chemicals from AT (or increased circulating POPs from other sources) may affect the function of other organs. As an example, Lee et al. reported a direct effect of POPs on pancreatic islets, which could be linked to the pathogenesis of type 2 diabetes (Lee et al., 2017).

Taken together, bio-accumulation of environmental chemicals may affect AT function, fat distribution and could thereby represent one mechanistic link between impaired AT function and cardio-metabolic diseases even independently of whole body fat mass. Here, we tested the hypotheses that AT concentrations of POPs are fat-depot and gender specific, related to AT function and associated with BMI, fat distribution and parameters of systemic inflammation, glucose and lipid metabolism. We took advantage of a newly developed method to detect POPs in AT and the large adipose tissue biobank at the University of Leipzig.

2. Material and methods

2.1. Cohort characteristics

Adipose tissue was investigated in 54 donors of paired omental visceral and abdominal SC adipose tissue samples, who underwent abdominal surgery for cholecystectomy, weight reduction surgery, or explorative laparotomy. Collection of AT and human phenotyping has been approved by ethics committee of the University of Leipzig (approval numbers: 159-12-21052012 and 017-12-23012012) and all study participants gave written informed consent before taking part in the study. Clinical parameters were assessed as described previously (Kloting et al., 2010). In brief, percentage body fat was measured by bioimpedance analysis (BIA). Abdominal visceral and subcutaneous fat areas were calculated using magnetic resonance imaging at the level of L3–L5. Because of this scan window, we were not able to calculate total abdominal visceral and subcutaneous fat volume. Insulin sensitivity was assessed using the HOMA-IR index or with the euglycemic-hyperinsulinemic clamp method. All blood samples were collected between 8 and 10 am after an overnight fast and up to 4 weeks prior to surgery. At the time of blood sampling, participants were free of clinical symptoms for acute infections. Plasma insulin was measured with an enzyme immunometric assay for the IMMULITE automated analyzer (Diagnostic Products Corporation, Los Angeles, CA, USA). Serum high-sensitive CRP, interleukin-6 (IL-6) as well as parameters of lipid and glucose metabolism were measured as previously described (Kloting et al., 2010). All subjects had a stable body weight, defined as the absence of fluctuations of >2% of body weight for at least 3 months before surgery.

Adipose tissue was immediately frozen in liquid nitrogen after explantation. Histologic analysis of AT sections was performed by two investigators who were blinded, and it included at least three slides with three sections each per patient. Total number of AT macrophages (ATM) was visualized by staining for CD68. To further distinguish between M1 and M2 macrophages subtypes, we performed immunomorphological analysis in analogy to a previously described method (Aron-Wisnewsky et al., 2009) and using antibodies against CD40 (R&D Systems, Minneapolis, MN) to detect M1 ATMs and CD163 (AbD Serotec, Raleigh, NC) for M2 ATM quantification. Number of AT macrophages was normalized to 100 adipocytes as previously described (Cancello et al., 2006). To determine adipocyte size, 200 μl aliquots of adipocytes from both SC and visceral AT were fixed with osmic acid, incubated for 48 h at 37 °C, and counted in a Coulter counter (Multisizer III; Beckman Coulter, Krefeld, Germany).

2.2. Sample preparation for measurement of POPs in adipose tissue

54 paired visceral and SC AT samples were prepared. 250 mg lipid tissue were homogenized with 1 ml n-hexane and 12 steel bullets (2.381 mm, Isometall Handelsgesellschaft Schmidt u. Co., Pleidelsheim, Germany) for 25 min at 30 Hz using the TissueLyser II (Qiagen, Hilden, Germany). After centrifugation at 1200*g* for 2 min at room temperature the supernatant was collected. The cell debris was solved in 600 μl ethyl acetate (EMD Millipore, Darmstadt, Germany) and homogenized again for 10 min at 30 Hz. The solvent of the homogenate was removed using SpeedVac concentrator plus (Eppendorf, Hamburg, Germany) for 15 min at 45 °C. The dried cell pellet was re-suspended in 800 μl n-hexane (EMD Millipore), homogenized for another 10 min at 30 Hz, and followed by a centrifugation at 1200*g* for 2 min at room temperature. The supernatant was collected and combined with the previously collected supernatant (primary extract).

The primary extract was spiked with 10 μl of 100 μg/ml PCB 116-d5 and α-HCH-D6 each (internal standards). After freezing the extract at 20 °C for 2 h, the supernatant was immediately separated in a precooled centrifuge at 2292*g* for 2 min and transferred into a 750 × 280 mm membrane bag, prepared from a lay-flat polyethylene (PE) tube of 50 μm wall thickness (Polymer-Synthese-Werk GmbH, Rheinberg, Germany). The PE-bag was sealed with Sealboy 235 (Audion Electro, Kleve, Germany) and stirred for 17 h at room temperature in a prepared 30 ml-centrifuge tube with 20 ml n-hexane using a magnetic stirrer at 200 rpm. After removing the PE- bag, 50 μl of nonane (Sigma-Aldrich, Munich, Germany) were added as keeper to the gained secondary extract. The extract was evaporated in the SpeedVac at 45 °C for 32 min to 1 ml leftover. 1 ml of n-hexane was used to wash the walls of the centrifuge tube. The resulting 2 ml of extract were transferred into a GC-vial and evaporated at 30 °C V-AQ until 50 μl nonane remained. 10 μl of 100 μg/ml DDT-D8 (qualifier) and the sample extract were combined in the insert of a GC-Vial. N-hexane was added for reconstitution to obtain a volume of 100 μl.

2.3. GC–MS measurement

The GC/MS analyses of the prepared fat samples were performed using the 7890A GC system coupled to the 5975C inert XL MSD with Triple-Axis Detector (both Agilent Technologies, Santa Clara, USA). The GC-system was equipped with the 7693A Automatic Liquid Sampler (Agilent Technologies) and a Pressure Controlled Tee (PCT) backflushing system. Two columns were used in the GC instrument set up: The first column Optima 5MS (30 m × 250 μm × 0.25 μm, Macherey-Nagel, Düren, Germany) was installed between the inlet and the Auxiliary Electronic Pressure Control (Aux EPC) Module. The second one was the Backflush Transfer column (1 m × 100 μm × 0 μm, Agilent Technologies), placed between the Aux EPC Module and the MS source (vacuum outlet). Helium was used as carrier gas with a flow rate of 1 ml/min for column 1 and 1.2 ml/min for column 2. The oven temperature program was set as follows: The initial temperature of 80 °C was held for 1 min and then increased for 11.5 min by 20 °C/min to a final temperature of 310 °C for 4.5 min. Mass spectral data were obtained in full scan mode after EI at −70 V with a solvent delay of 6.1 min. The full scan was performed for a mass range from 50 to 500 *m/z* with a detection gain factor of 1.0. The Ion source temperature was kept at 230 °C, the Quadrupole temperature at 150 °C. The identification was done by NIST library (Version 2011). When we established the method it turned out to be difficult for most of the substances to order isotopically labelled standards with the exception of DDE. Thus we used isotopically labelled DDE and obtained an absolute quantification for it. This is not possible for the other substances for which we only measured relative quantifications given by the peak area.

2.4. Statistical analyses

Peak intensities from the LC-MS measurements were normalized using the peak intensity of the spiked internal standard. This was done by dividing the contaminants peak intensity by the peak intensity of the spike-in standard of the sample and then multiplying by the median of the peak intensities from the spike-in standard from all samples. Statistical analysis of POPs normalized peak intensities was performed in R using the Kruskal-Wallis test with the Dunn test used for post hoc pairwise analysis. The post hoc test results were corrected for multitesting by the Benjamini-Hochberg method. Correlation analysis was done by calculating the Pearson's product moment coefficient between variables using the corrplot package in R. Figures were constructed using the ggplot package from R. Error bars in bar plots represent SEM.

3. Results

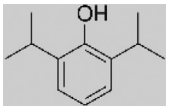
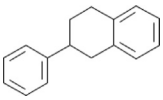
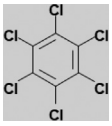
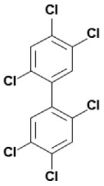
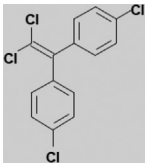
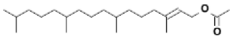
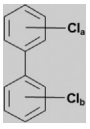
3.1. Study participants

The characteristics of the 54 individuals included into the cross-sectional study are summarized in Table 1. Selection of study participants was based on availability of sufficient amounts of paired abdominal omental (visceral) and subcutaneous adipose tissue biopsies and complete data sets from euglycemic-hyperinsulinemic clamps and abdominal MRI scans. In addition, we aimed for a wide representation of BMI categories including lean individuals and participants with overweight and obesity in both women and men. We did not include people with early-onset or childhood obesity. In our cohort, duration of obesity was age-dependent and ranges from 6 to 57 years. Lean individuals have always been lean. In each sex, subgroups of lean, overweight and obesity displayed the expected phenotypic differences in anthropometric (body weight, BMI, % body fat, waist and hip circumferences, visceral and SC fat areas), biochemical parameters (e.g. glucose infusion rate during the steady state of the clamp, fasting plasma insulin, HDL-cholesterol), and adipokines (leptin, adiponectin) (Table 2). Despite significantly higher BMI, men with overweight did not have higher body fat mass compared to lean men (Table 2). This finding is explained by the body composition of two men in the overweight group who have unexpectedly low fat and high lean mass. Participants in the obesity subgroups were significantly younger compared to the other groups (Table 2). Importantly, leucocyte counts and circulating liver enzymes were not significantly different among the groups (Table 2). Only in women with obesity, CRP serum concentrations were significantly higher in the obesity compared to other groups.

3.2. Detection of nine environmental chemicals in human adipose tissue

For assessing POPs from human adipose tissue, we developed a new method combining an extraction of AT by acetonitrile, a dialysis step and the subsequent untargeted measurement by GC–MS. In order to obtain reliable and reproducible results, we filtered for those chemicals that were detectable in all samples. In a second step of quality control,

Table 1
Chemical structures, molecular weight, theoretical K_{ow} values and potential sources of detected persistent organic pollutants.

Contaminant	Structure	CAS-Nr.	MW (g/mol)	Log Kow/* XLogP3-AA	Source
Propofol		2078-54-8	178.27	4,0	Drug, Anesthetic
2-Phenyltetralin		29422-13-7	208.3	4,8*	Cosmetics, Household cleaner, Detergents
Hexachlorobenzene		118-74-1	284.8	5,73	Fungicide
2,2′,4,4′,5,5′-Hexachlorobiphenyl (PCB153)		35065-27-1	360.9	6,34/7,62*	Plasticizer, Adhesive, Sealant, Catalyst, Intermediate for pharmaceutical and veterinary drugs
Dichlorodiphenyldichloroethane (DDE)		72-55-9	318.02	6,51	Insecticide
Ethyl tetradecanoate (Myristic acid ethyl ester)		124-06-1	256.42	6,7*	Palm kernel oil, Coconut oil
4,4-Diisopropylbiphenyl		18970-30-4	238.4	6,6*	Plasticizer
Phytol, acetate		7541-49-3	338.57	6,93	Food additive
Hexachlorobiphenyl (PCB X)		Not applicable (class of compound)	360.9	7*	Plasticizers in plastics, paints and resins, lubricants, flame retardant

we eliminated all substances, which we detected also in the method and instrument blanks. Out of 50 detectable chemicals in AT, the method yielded reliable results for eight POPs and the anesthetic Propofol (Table 1). But only for DDE we were able to use isotopically labelled standards so that we ended with relative quantifications based on the peak area for all compounds with the exception of DDE. Detection of Propofol in all samples could be considered a positive control, because this intravenous anesthetic has been used for induction and maintenance of general anesthesia in our AT donors. The nine compounds were found in all AT samples, but in a wide range of relative concentrations as illustrated by normalized MS peak areas (Fig. 1). For AT bioaccumulation of DDE, we could absolutely quantify the concentration and the distribution (Fig. 2). The nine environmental compounds reflect a range of K_{OW} from 4 to 7. Thereby they represent a broad range of K_{OW} values indicating that the results might also be representative for other environmental chemicals.

3.3. Equal bioaccumulation of all POPs in subcutaneous and visceral adipose tissue

The nine environmental POPs were analyzed in paired AT biopsies from 54 women and men with a wide range of BMI, fat distribution, glucose and lipid metabolism parameters (Table 2). AT relative concentrations of all POPs and Propofol were not significantly different between women and men or between subcutaneous and visceral AT (Fig. 3). In addition, AT relative POPs concentrations were not significantly different between subgroups of men and women with BMI < 25 kg/m^2 (lean), BMI 25-30 kg/m^2 (overweight) and BMI > 30 kg/m^2 (obesity) (Fig. 3). All subsequent analyses were performed separately for men and women and for subcutaneous AT (SCAT) and visceral AT (VAT).

3.4. Correlation of POPs concentration with adipose tissue macrophage infiltration in women with normal body weight

We analyzed associations between POPs relative concentrations in subcutaneous and visceral AT and phenotypic traits including BMI, body fat mass, waist-to-hip ratio (WHR), subcutaneous and visceral fat depot volume, the ratio of visceral to subcutaneous fat as well as AT histological parameters (mean and maximal adipocyte size, AT macrophage (ATM) infiltration) in women (Fig. 4). In SCAT of all women, we found correlations between WHR, body fat, adipocyte size and ATM with the relative concentration of Ethyltetradecanoate, Phytyl acetate and/or Propofol (Fig. 4a). Significant associations between phenotypic traits and AT POPs were of the same frequency in VAT but rather clustered to the traits of WHR and CT than to distinct chemical compounds as in SCAT (Fig. 4a). BMI subgroup analyses revealed only in lean women significant correlations between several POPs and ATM in SCAT (Fig. 4b) and VAT (Fig. 4c). In addition, WHR was almost exclusively associated with distinct AT POPs in the lean subgroup (Fig. 4b, c). We find some significant correlations between the relative concentration of distinct POPs and maximal or mean adipocyte size suggesting

Table 2
Characteristics of the study participants.

BMI subgroup	Women				Men			
	Total	<25 kg/m^2	25–29.9 kg/m^2	>30 kg/m^2	Total	<25 kg/m^2	25–29.9 kg/m^2	>30 kg/m^2
n	30	13	5	12	24	6	6	12
Age (years)	62 ± 16	68 ± 11	68 ± 11	53 ± 19*#	64 ± 14	70 ± 6	70 ± 12	59 ± 17#
Body weight (kg)	83.9 ± 31.5	61.4 ± 6.8	72.8 ± 7.0	113 ± 31*#	89.3 ± 20	74.5 ± 6.1	83.6 ± 7.3	103 ± 22#
BMI (kg/m^2)	30.8 ± 11.5	22.6 ± 2.0	26.2 ± 0.6	41.7 ± 11.2*#	29.5 ± 5.9	24 ± 0.51	27.3 ± 1.1	34.4 ± 5.2#
Duration of obesity (years)	30.6 ± 14.4	NA	37.8 ± 8.7	27.7 ± 15.5	36.4 ± 13.5	NA	44.6 ± 10.6	32.6 ± 13.4
Body fat (%)	29.5 ± 11.4	20.9 ± 4.5	25.1 ± 2.5	40.5 ± 9.4*#	25.9 ± 7.1	21.6 ± 5.7	21.6 ± 2.4	31 ± 6.0#
Visceral fat area (cm^2)	133 ± 139	65 ± 63	74 ± 21	250 ± 163*#	154 ± 106	77 ± 67	125 ± 41	223 ± 110#
Subcutaneous fat area (cm^2)	431 ± 560	58 ± 41	182 ± 42*	1023 ± 541*#	397 ± 372	67 ± 28	241 ± 72	710 ± 331#
Waist circumference (cm)	94 ± 30*	73 ± 13	81 ± 9	124 ± 26*#	107 ± 12	99 ± 5.2	102 ± 3	116 ± 12#
Hip circumference (cm)	101 ± 23	84 ± 9	99 ± 7*	122 ± 21*#	108 ± 14.5	100 ± 3.1	104 ± 8.1	114 ± 18.6#
FPG (mmol/l)	5.7 ± 0.8	5.5 ± 0.8	5.5 ± 0.4	6.1 ± 0.9	6.08 ± 1.22	6.3 ± 1.37	5.65 ± 0.31	6.12 ± 1.39
FPI (pmol/l)	68.2 ± 94.9	10.8 ± 12.5	23.2 ± 3.3	127 ± 111*#	54.4 ± 60.7	17.7 ± 23.3	47 ± 17.3	85 ± 78.4#
HbA1c (%)	5.7 ± 0.5	5.5 ± 0.6	5.6 ± 0.2	5.92 ± 0.5*	5.75 ± 0.5	5.6 ± 0.57	5.7 ± 0.28	5.89 ± 0.53
Clamp GIR (μmol/kg/min)	81 ± 28	88 ± 25	89 ± 13	69 ± 33*#	77 ± 32	101 ± 14	75 ± 21	57 ± 36#
Cholesterol (mmol/l)	5.48 ± 1.06	5.61 ± 1.04	5.46 ± 0.6	5.27 ± 1.5	5.1 ± 0.94	5.35 ± 0.78	5.57 ± 1.37	5.89 ± 0.53
HDL-Cholesterol (mmol/l)	1.39 ± 0.53	1.53 ± 0.58	1.5 ± 0.26	1.18 ± 0.53#	1.21 ± 0.23	1.29 ± 0.15	1.29 ± 0.05	1.1 ± 0.31#
LDL-Cholesterol (mmol/l)	3.72 ± 1.28	3.47 ± 1.43	3.34 ± 0.4	4.16 ± 1.32	3.22 ± 0.89	3.69 ± 0.69	3.36 ± 0.97	2.59 ± 0.77
Triglycerides (mmol/l)	1.06 ± 0.51	1.24 ± 0.55	0.95 ± 0.14	1.04 ± 0.45	1.2 ± 0.41	1.37 ± 0.38	0.84 ± 0.12	1.16 ± 0.46
Free fatty acids (mmol/l)	0.34 ± 0.36	0.24 ± 0.23	0.2 ± 0.07	0.57 ± 0.48#	0.39 ± 0.35	0.45 ± 0.41	0.19 ± 0.13	0.42 ± 0.37
hsCRP (mg/l)	2.43 ± 1.89	1.13 ± 1.03	1.87 ± 0.74	4.28 ± 1.67#	3.15 ± 2.12*	3.1 ± 2.75	3.02 ± 2.74	3.25 ± 2.06
IL-6 (pg/ml)	3.21 ± 4.78	1.97 ± 1.87	1.6 ± 0.42	6.46 ± 6.12#	3.72 ± 4.81	1.4 ± 1.47	0.98 ± 1.15	6.1 ± 5.88#
Leucocytes (Gpt/l)	8.89 ± 4.8	7.3 ± 2.4	8.3 ± 4.5	8.4 ± 1.7	7.47 ± 2.46	7.1 ± 2.69	7.54 ± 1.39	7.71 ± 2.81
ALAT (μkat/l)	0.37 ± 0.28*	0.32 ± 0.15	0.5 ± 0.47	0.44 ± 0.31	0.59 ± 0.73	0.44 ± 0.17	0.37 ± 0.14	0.80 ± 1.05#
ASAT (μkat/l)	0.44 ± 0.17*	0.42 ± 0.15	0.39 ± 0.19	0.5 ± 0.17	0.57 ± 0.52	0.42 ± 0.18	0.58 ± 0.43	0.69 ± 0.7#
GGT (μkat/l)	0.7 ± 0.83	0.71 ± 1.02	0.93 ± 0.72	0.62 ± 0.65	0.91 ± 0.7	1.0 ± 0.75	0.74 ± 0.41	0.93 ± 0.83
TSH (mU/l)	1.81 ± 1.42	1.17 ± 1.3	2.36 ± 1.8	2.2 ± 1.2	1.44 ± 1.27	0.83 ± 0.31	0.96 ± 0.77	2.11 ± 1.58#
Adiponectin (μg/ml)	8.3 ± 4	11.4 ± 3.5	7.5 ± 4.1	6.61 ± 3.5#	7.6 ± 4.3	10.3 ± 4.5	5.4 ± 3#	6.34 ± 3.9#
Leptin (pg/ml)	27.4 ± 21.3*	8.8 ± 4.9	15.6 ± 5.7	41.9 ± 18.5#	12.2 ± 10*	5.6 ± 6.8	5.3 ± 2.8	18.4 ± 9.2#
Macrophages in visceral fat (n/100 adipocytes)	17.4 ± 8.7	12.9 ± 5.8	13.4 ± 3.8	23.8 ± 9.1#	19.0 ± 9.8	13.5 ± 6.9	16.4 ± 11.4	17.8 ± 5.8#
CD40 Macrophages visceral (n/100 adipocytes)	10.8 ± 6.9	7.2 ± 4.0	8.6 ± 3.4	15.7 ± 7.6#	11.4 ± 6.5	4.8 ± 3.2	4.4 ± 1.8	9.2 ± 5.3#
CD163 Macrophages visceral (n/100 adipocytes)	5.6 ± 2.7	5.8 ± 2.1	4.8 ± 0.8	8.2 ± 2.9#	5.1 ± 2.4	5.2 ± 3.0	5.0 ± 2.8	5.9 ± 1.7
Macrophages in SC fat (n/100 adipocytes)	12.8 ± 7.3	9.6 ± 5.6	8.6 ± 1.8	14.7 ± 5.1#	11.2 ± 2.7	8.5 ± 3.5	8.6 ± 1.8	14.3 ± 6.7
CD40 Macrophages SC (n/100 adipocytes)	7.4 ± 5.3	4.8 ± 3.2	4.0 ± 1.2	11.6 ± 5.5#	6.6 ± 5.5	4.8 ± 3.2	4.4 ± 1.8	9.2 ± 5.3#
CD163 Macrophages SC (n/100 adipocytes)	5.4 ± 2.7	4.8 ± 2.4	4.6 ± 1.1	6.4 ± 3.2#	5.0 ± 2.7	4.0 ± 1.5	4.2 ± 1.3	6.1 ± 3.5#

Data are means ± SD. *$p < 0.05$ for comparisons between women and men in each subgroup. #$p < 0.05$ compared to lean within one gender. Abbreviations: ALAT -Alanine-Aminotransferase; ASAT -Aspartate-Aminotransferase; BMI – body mass index; FPG - fasting plasma glucose; FPI - fasting plasma insulin; GGT - gamma-glutamyl transpeptidase; HbA1c - glycated haemoglobin; HDL - high density lipoprotein; hsCRP - high sensitivity C-reactive protein; IL-6 – Interleukin 6; LDL – low density lipoprotein.

that bio-accumulation of 2,2′,4,4′,5,5′-Hexachlorobiphenyl, Hexachlorobiphenyl, (PCB X) and Phytyl acetate may be associated with regulation of adipocyte size regulation (Fig. 4b, c). Independently of the subgroup or fat depot, AT POPs abundance did not correlate with BMI (Fig. 4).

3.5. Associations of adipose tissue POPs with obesity parameters in men

Analogous to women, we performed correlation analyses between AT the relative concentration of POPs and parameters of obesity, fat distribution and AT morphology in men (Fig. 5). In the combined analysis of all male AT donors, we did not find any significant correlations between AT POPs accumulation and those parameters (Fig. 5a). However, SCAT and VAT specific analyses of BMI subgroups revealed significant associations between adipocyte size parameters and POPs in AT (Fig. 5b, c). Only in lean men, we found positive correlations between body fat mass and the relative concentration of Hexachlorobenzene in both fat depots (Fig. 5b, c). In SCAT of lean men, 2-Phenyltetralin and 4,4-Diisopropylbiphenyl significantly correlate with mean adipocyte size (Fig. 5b). AT relative concentration of the same POPs were significantly negatively associated with BMI only in overweight men (Fig. 5b, c). Moreover, in overweight men, the relative concentration of Ethyltetradecanoate and PCB X AT levels are positively correlated with adipocyte size. Phytyl acetate In contrast to women, ATM are either not significantly (VAT) or in overweight men inversely correlated (SCAT) with the relative concentration of distinct chemicals (Propofol, 2-Phenyltetralin) (Fig. 5b).

3.6. Association of adipose tissue POPs with parameters of glucose metabolism, CrP and the duration of obesity

We further tested the hypothesis that AT relative concentration of POPs in subcutaneous and visceral AT are associated with parameters of glucose metabolism (fasting plasma glucose and insulin, 2hour OGTT glucose, HbA1c, glucose infusion rate (GIR) in an euglycemic-hyperinsulinemic clamp), creatinine and CrP separately for women (Fig. 6) and men (Fig. 7). For the entire group of women, we find significant associations between the relative concentration of SCAT 2,2′,4,4′,5,5′-Hexachlorobiphenyl and HbA1c, associations between Ethyltetradecanoate, phytyl acetate and Propofol with 2 h OGTT glucose (Fig. 6a). VAT 2-Phenyltetralin abundance positively correlates with FPG, FPI, HbA1c, 2 h OGTT glucose and negatively with glucose infusion rate in the clamp (Fig. 6a). We find additional associations between the relative concentration of AT POPs with glucose metabolism parameters in BMI subgroups, in a distinct fat depot pattern. Strongest associations between distinct the relative concentration of AT POPs and HbA1c as well as Clamp GIR were found in lean women (Fig. 6b, c). In SCAT and VAT biopsies of lean men, the relative concentration of 2,2′,4,4′,5,5′-Hexachlorobiphenyl, Hexachlorobenzene and DDE were significantly associated with fasting plasma insulin. In general, associations between the relative concentration of AT POPs and phenotypic traits were more pronounced in women compared to men. Most pronounced in lean men, serum CrP significantly correlates with distinct POPs' abundance in SCAT and/or VAT (Fig. 7).

The sex-dependent effect is much stronger when we analyzed the correlation between the duration of obesity and the relative concentration of POPs (Fig. 8). We found strong positive correlations for almost all

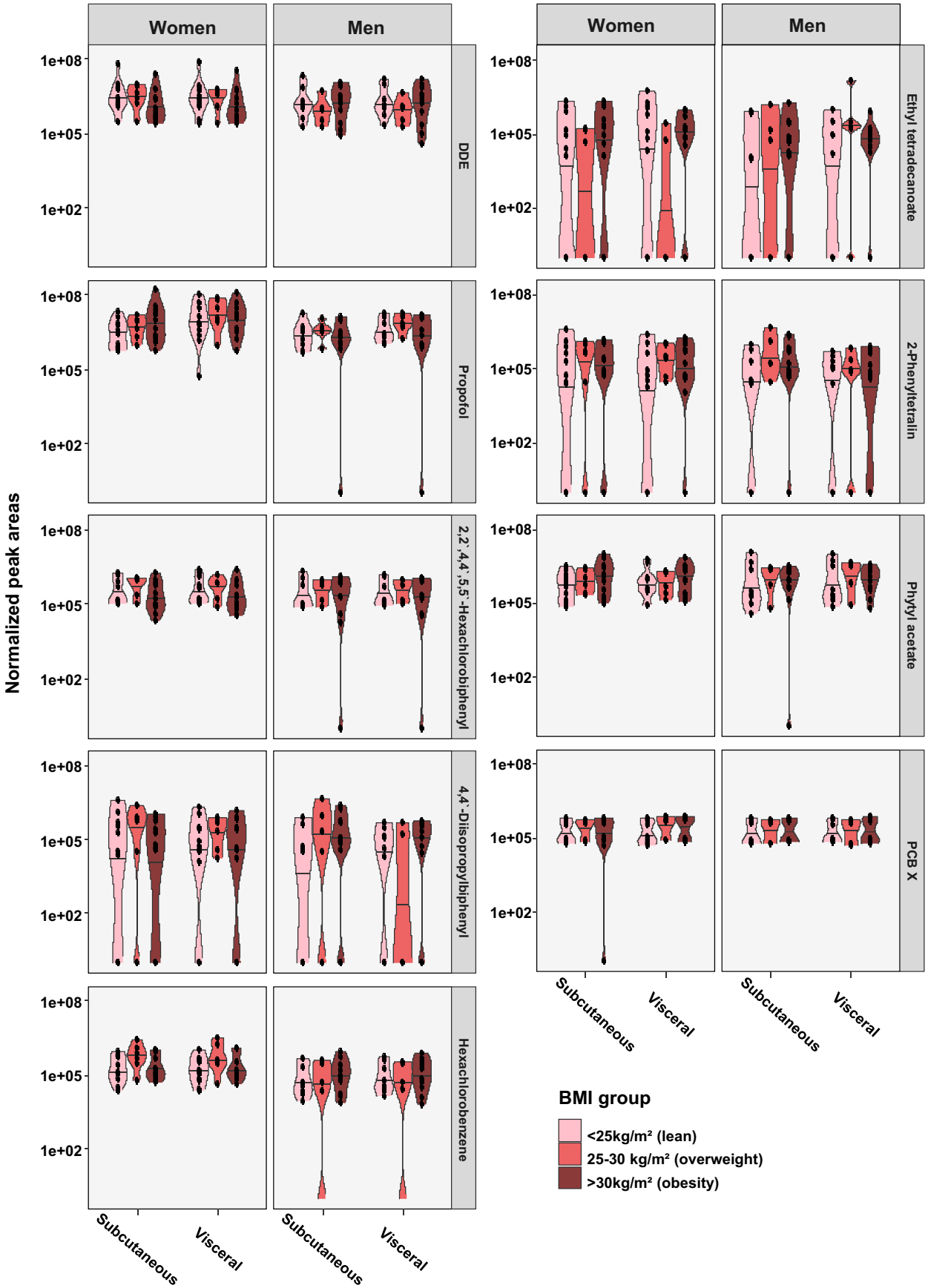


Fig. 1. Detection of persistent organic pollutants (POPs) and Propofol in human adipose tissue. Violin plots of normalized MS peak intensities of contaminant detected in subcutaneous and visceral adipose tissue from women (n = 30) and men (n = 24) with normal weight (BMI < 25 kg/m^2, lean), overweight (BMI 25-30 kg/m^2, OW) and obesity (BMI > 30 kg/m^2, O).

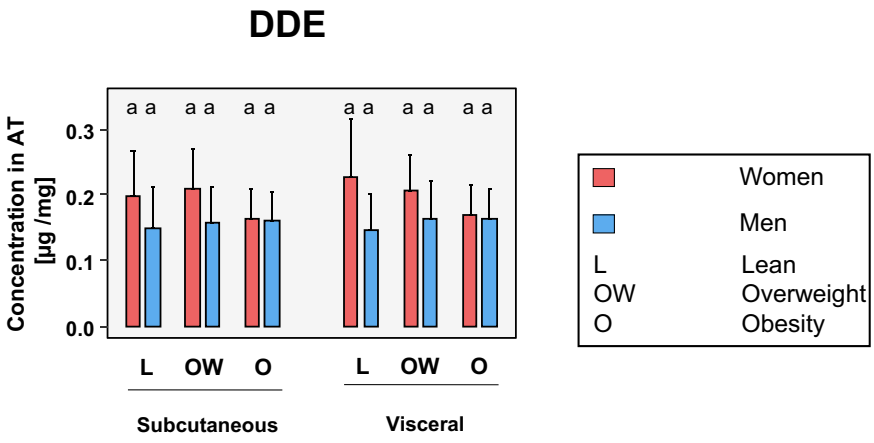


Fig. 2. Bio-accumulation of Dichlorodiphenyldichloroethane (DDE) in adipose tissue. DDE concentrations were measured by Mass Spectrometry in subcutaneous and visceral adipose tissue from women (n = 30) and men (n = 24) with normal weight (BMI < 25 kg/m^2, lean = L), overweight (BMI 25-30 kg/m^2, OW) and obesity (BMI > 30 kg/m^2, O). Significance calculated by Kruskal-Wallis test post hoc test by Dunn test.

POPs in men with obesity, but only a negative correlation for women. We do not have information on breast feeding of the women but given the average age of men and women (men: 64.9 ± 3.7 years; women: 62.3 ± 4.0 years) we assume that at least some women have given birth to children and might have been breastfeeding as well. During lactation, some fat depots might have been mobilized and previously bioaccumulated chemicals might therefore have been reduced.

4. Discussion

4.1. Sources and toxicokinetic of POPs

The chemicals analyzed in our study belong to different substance classes. The detection of Propofol in both fat depots illustrates the reproducibility of our extraction and detection method, because all AT donors received Propofol for general anesthesia during the laparoscopic surgery (Alvarez et al., 2000). Detected POPs include 2,2′,4,4′,5,5′-hexachlorobiphenyl, 4,4-Diisoprpylbiphenyl, Hexachlorobiphenyl and Dichlorodiphenyl-dichloroethane (DDE), which is a transformation product of the insecticide DDT. We also found a group of PCB accumulating in AT (Table 1). The exposure to PCBs occurs mostly by ingestion of contaminated food (Shields, 2006). Until the 1980s, PCBs were mainly used in transformers, electrical capacitors, as hydraulic fluids and as plasticizers in paints, sealants, insulating materials and plastics. Related to polyaromatic substances is the aromatic chemical Hexachlorobenzene B, which features a single aromatic ring system. Unlike the aromatic structures, Phytol acetate and Ethyl tetradecanoate do not contain aromatic structures. Their hydrophobicity stems from long alkyl chains or terpene related structures. Both Phytol acetate and Ethyl tetradecanoate are fragrance substances and as such are present in consumer care products and exposure happens most likely mostly over the skin.

Even though the exposure paths might have been different for the two classes of chemicals, the accumulation obviously occurs equally in different fat depots. The distribution and transformation of xenobiotics like pharmaceuticals have been studied extensively (Mao et al., 2018). It has been shown that especially at organ and tissue barriers, many suitable transporters for POPs are present and thus act as gatekeeper and allow for a whole body distribution. Although that has been shown for many pharmaceuticals, the evidence is much weaker for highly hydrophobic substance with K_{OW} values >5 like those analyzed here. For highly hydrophobic substances, it has been described that the transport through aqueous boundary layers in the gastrointestinal tract and in the blood is the limiting process for accumulation in tissues (Larisch and Goss, 2018). And on top of that they found that high hydrophobicity does result in extremely slow clearance kinetics.

4.2. No differences between subcutaneous and visceral AT in abundance of POPs

Against our a priori hypothesis, we did not find significant differences in the relative concentration of AT POPs between subcutaneous and visceral AT. Our data may therefore suggest that AT bioaccumulation of these POPs already represents a saturated state of equal distribution. Noteworthy, the cross-sectional design of our study does not allow investigating potential effects of dynamics in AT POPs accumulation. We can therefore not exclude the possibility that POPs may play a role in the regulation of fat distribution before a steady state concentration is reached. Visceral fat accumulation has been shown to be more closely related to the risk of cardiometabolic diseases than subcutaneous fat mass (Stefan, 2020). However, impaired ability to expand subcutaneous fat in the lower part of the body is also important for predicting the incidence of these cardiometabolic diseases (Stefan, 2020). Our observation that POPs bio-accumulate equally in visceral and subcutaneous fat depots does not exclude that POP-related effects on adipose tissue function are different between these depots.

4.3. Sex-specific effects on AT distribution and in respect to the duration of obesity

Although we do not find significant differences in AT POPs accumulation between women and men, several significant associations between distinct POPs and phenotypic traits are sex-specific. For example, waist-to-hip ratio (WHR), a surrogate parameter for fat distribution significantly correlates with the accumulation of Ethyltetradecanoate and Phytyl acetate in SCAT and with 2-Phenyltetralin, 4,4-Diisopropylbiphenyl, Hexachlorobiphenyl (PCB X) and Phytyl acetate accumulation in VAT of women. In addition, visceral fat area and the ratio of visceral to subcutaneous abdominal fat determined in MRI or CT scans is significantly associated with the accumulation of five out of the nine POPs in women. Interestingly, in men there were no correlations between POPs bio-accumulation in SCAT or VAT with WHR, visceral or subcutaneous fat area. This suggests that some POPs may contribute to the sex-specific regulation of fat distribution. Indeed, many POPs are classified as endocrine disruptors and exert effects on estrogen, androgen, and thyroid receptors (Bonefeld-Jorgensen et al., 2001; Langer, 2005). In particular, the positive associations between distinct POPs' AT abundance (PCB153, 2-Phenyltetralin,

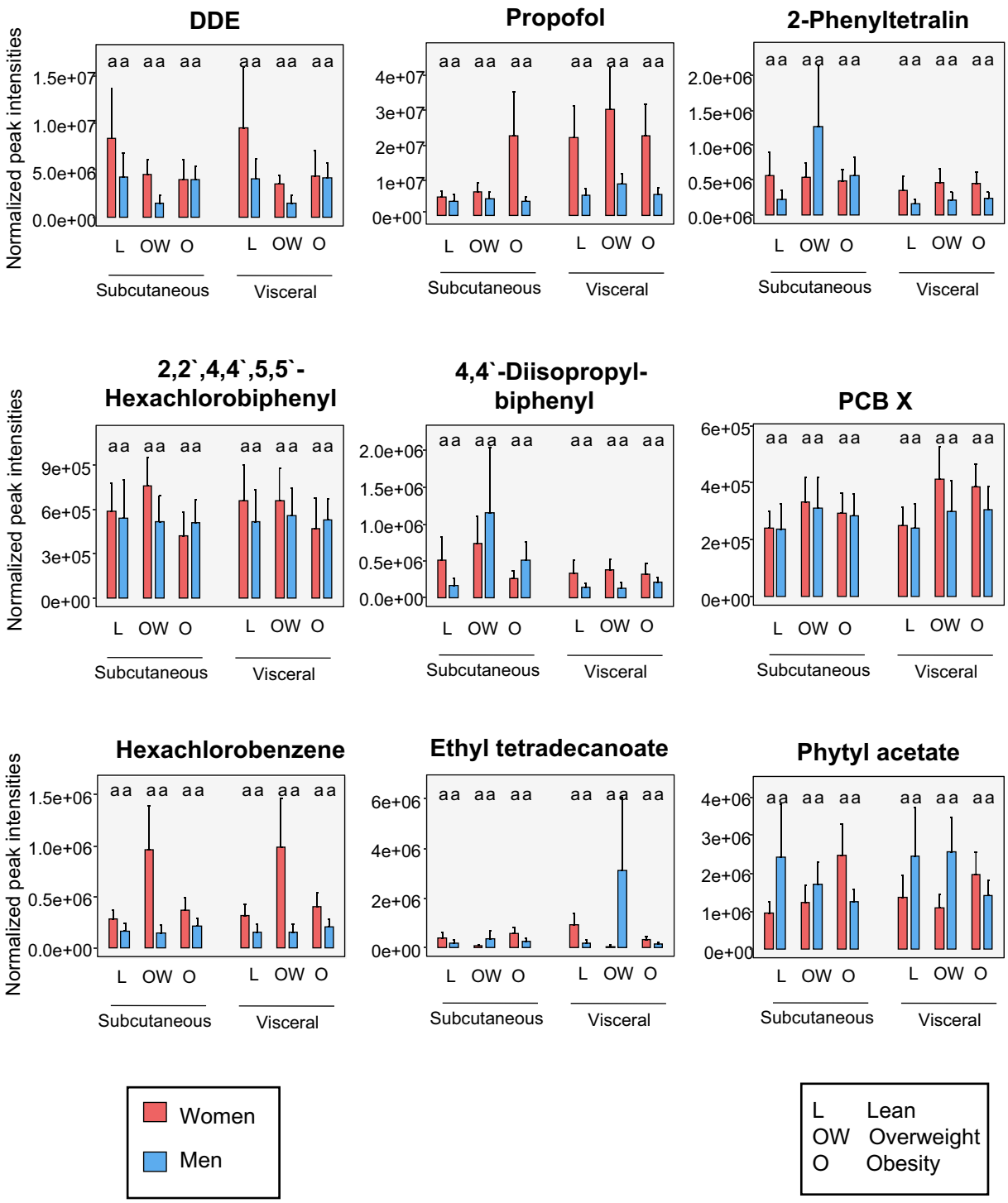


Fig. 3. Relative quantification of persistent organic pollutants (POPs) and Propofol in human adipose tissue. Normalized MS peak intensities of POPs in subcutaneous and visceral adipose tissue from women (n = 30) and men (n = 24) with normal weight (BMI < 25 kg/m^2, lean), overweight (BMI 25-30 kg/m^2, OW) and obesity (BMI > 30 kg/m^2, O). Significance calculated by Kruskal-Wallis test post hoc test by Dunn test.

4,4-Diisopropylbiphenyl, DDE, Ethyl tetradecanoate) and visceral fat area in lean women may suggest a contribution of these POPs to potentially harmful visceral fat distribution. For PCB153 and 2-Phenyltetralin, these significantly positive associations were also detected in women with obesity.

Another sex-specific effect was that the relative concentration of POPs correlated strongly with duration of obesity only for men. We do not have information on breast feeding of the women but given the average age of men and women (64,9 y ± 3.7y SEM; 62.3 y ± 4.0 SEM) we assume that at least several women have given birth to children and might have been breastfeeding as well. In this phase fat depots might have been mobilized and so also bio-accumulated chemicals be secreted as reviewed elsewhere (van den Berg et al., 2017).

4.4. Sex-specific effects on the immune-modulation in AT

In order to analyze the effects of POPs' abundance on the tissue level of AT, we analyzed adipose tissue histology including AT macrophage

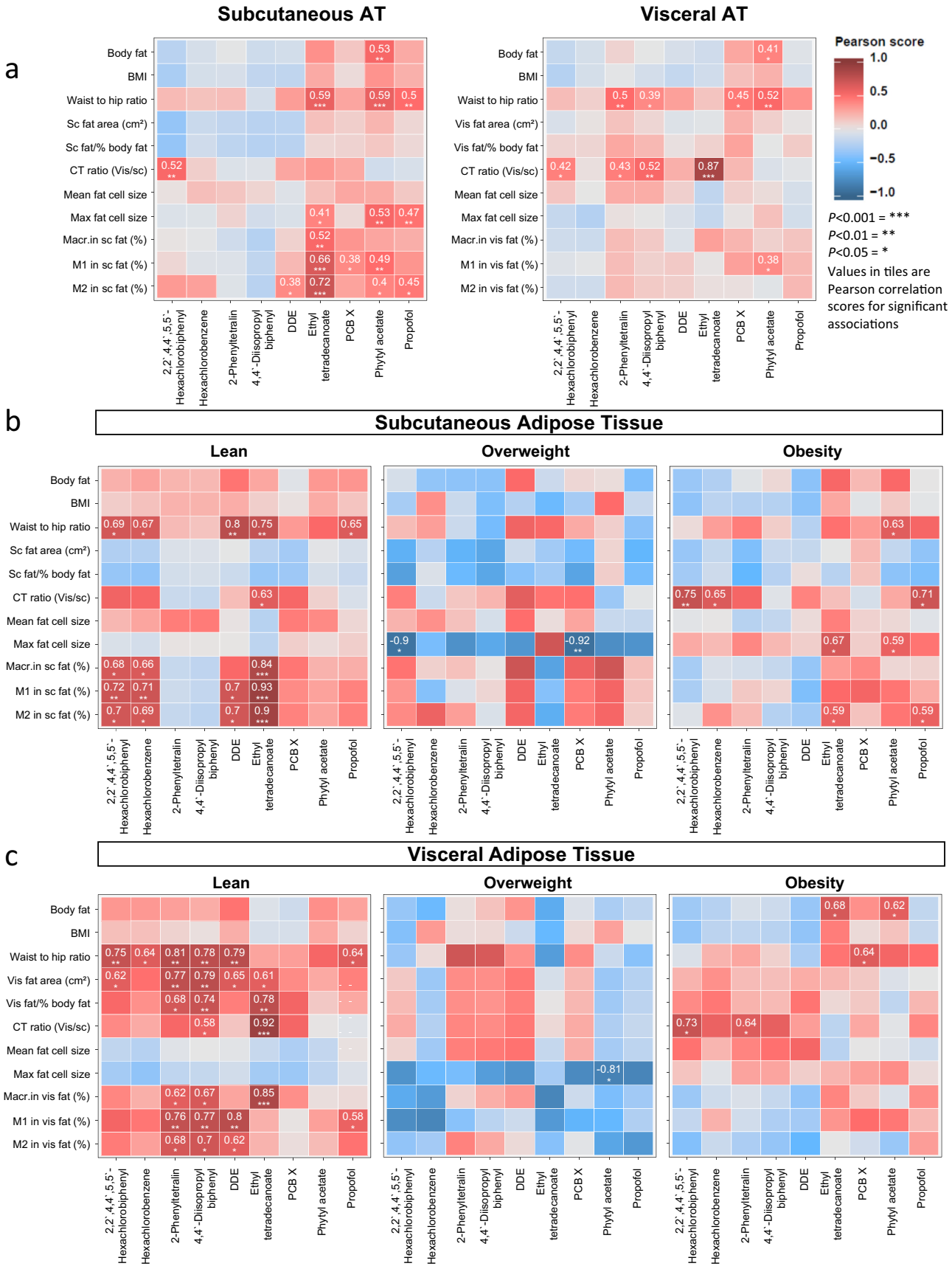


Fig. 4. Correlation analyses of the relative concentration of adipose tissue persistent organic pollutants (POPs) with obesity and fat distribution parameters in women. (a) Correlation matrix between distinct POPs, Propofol and phenotype parameters for all women (n = 30). (b) Subcutaneous adipose tissue correlations in subgroups with normal weight (BMI < 25 kg/m^2, n = 13), overweight (BMI 25-30 kg/m^2, n = 5) or obesity (BMI > 30 kg/m^2, n = 12). (c) Visceral adipose tissue correlations in the same BMI-subgroups. Values in tiles are Pearson's correlation coefficients. (***$P < 0.001$, **$P < 0.01$,*$P < 0.05$). BMI, body mass index; sc, subcutaneous; CT, computed tomography; vis, visceral; Macr, macrophage; M1, M1 Macrophages; M2, M2 Macrophages.

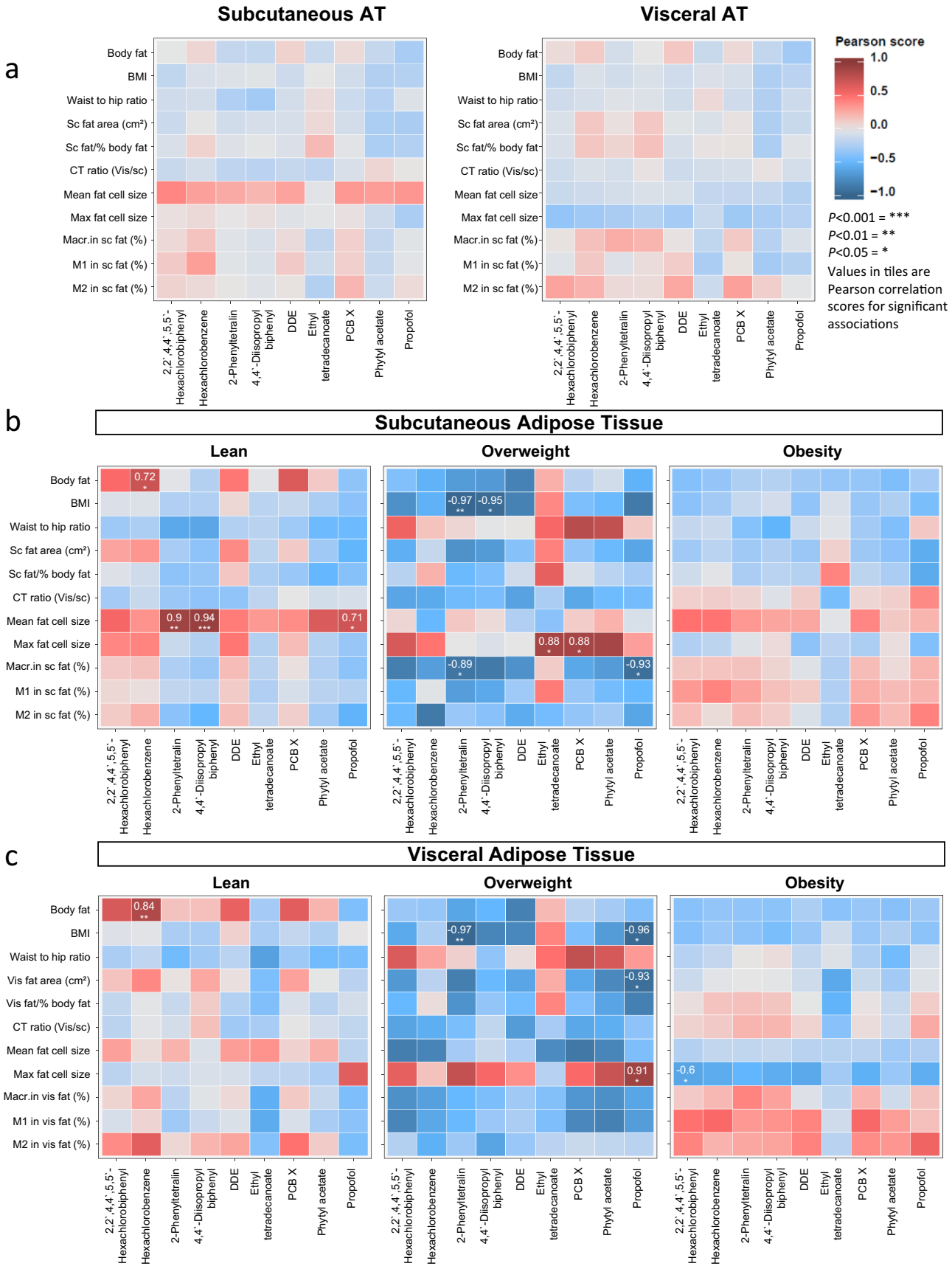


Fig. 5. Correlation analyses of the relative concentration of adipose tissue persistent organic pollutants (POPs) with obesity and fat distribution parameters in men. (a) Correlation matrix between distinct POPs, Propofol and phenotype parameters for all men (n = 24). (b) Subcutaneous adipose tissue correlations in subgroups with normal weight (BMI < 25 kg/m^2, n = 6), overweight (BMI 25-30 kg/m^2, n = 6) or obesity (BMI > 30 kg/m^2, n = 12). (c) Visceral adipose tissue correlations in the same BMI-subgroups. Values in tiles are Pearson's correlation coefficients. (***$P < 0.001$, **$P < 0.01$, *$P < 0.05$). BMI, body mass index; sc, subcutaneous; CT, computed tomography; vis, visceral; Macr, macrophage; M1, M1 Macrophages; M2, M2 Macrophages.

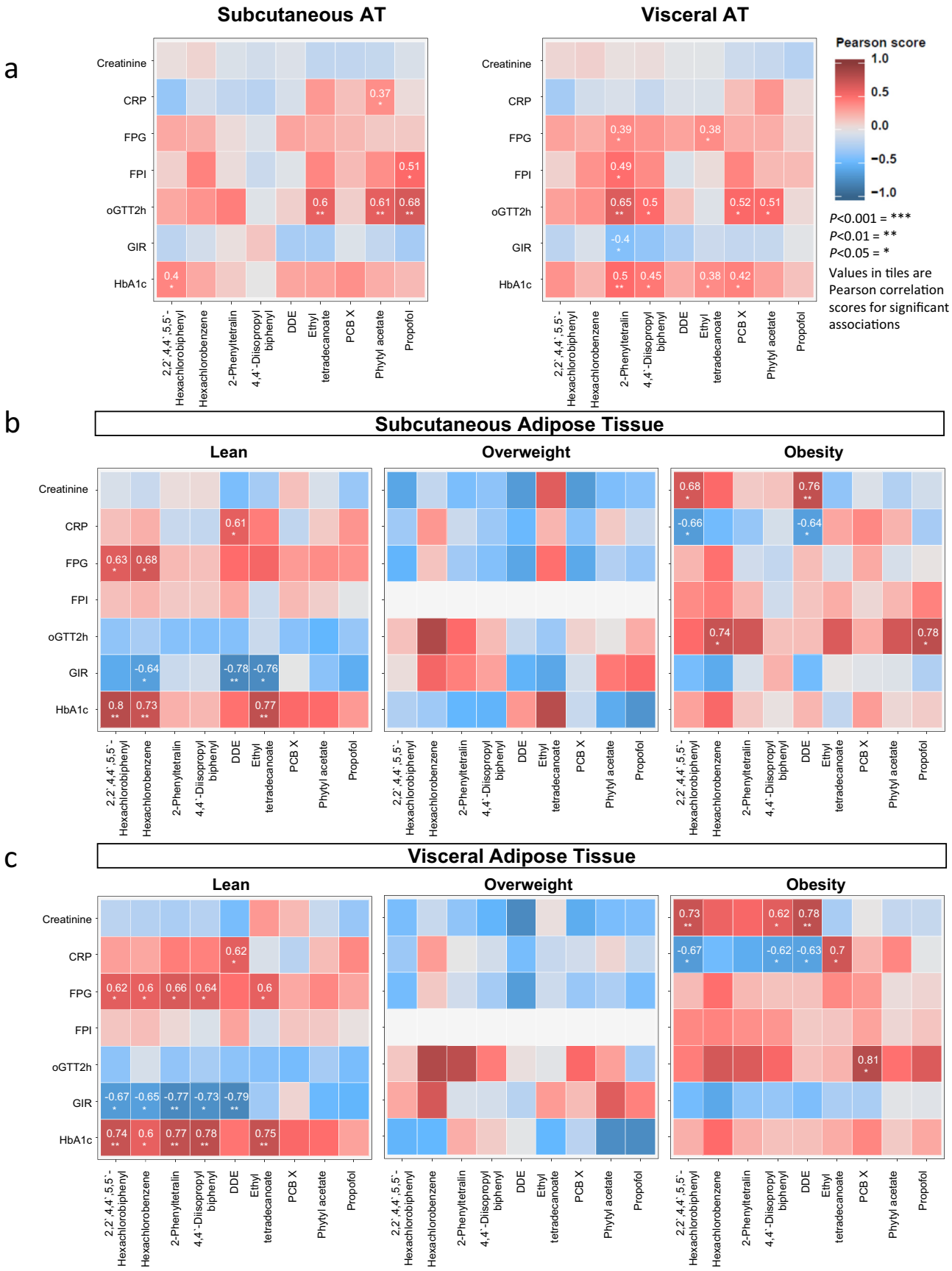


Fig. 6. Correlation analyses of the relative concentration of adipose tissue persistent organic pollutants (POPs) with parameters of glucose metabolism, insulin sensitivity, creatinine and CrP in women. (a) Correlation matrix between distinct POPs, Propofol and phenotype parameters for all women (n = 30). (b) Subcutaneous adipose tissue correlations in subgroups with normal weight (BMI < 25 kg/m^2, n = 13), overweight (BMI 25-30 kg/m^2, n = 5) or obesity (BMI > 30 kg/m^2, n = 12). (c) Visceral adipose tissue correlations in the same BMI-subgroups. Values in tiles are Pearson's correlation coefficients. (***$P < 0.001$, **$P < 0.01$, *$P < 0.05$) CRP, C reactive protein; FPG, fasting plasma glucose, FPI, fasting plasma insulin; oGTT2h; 2 hour oral glucose tolerance test glucose plasma concentration, GIR, glucose infusion rate during the steady state of an euglycemic-hyperinsulinemic clamp.

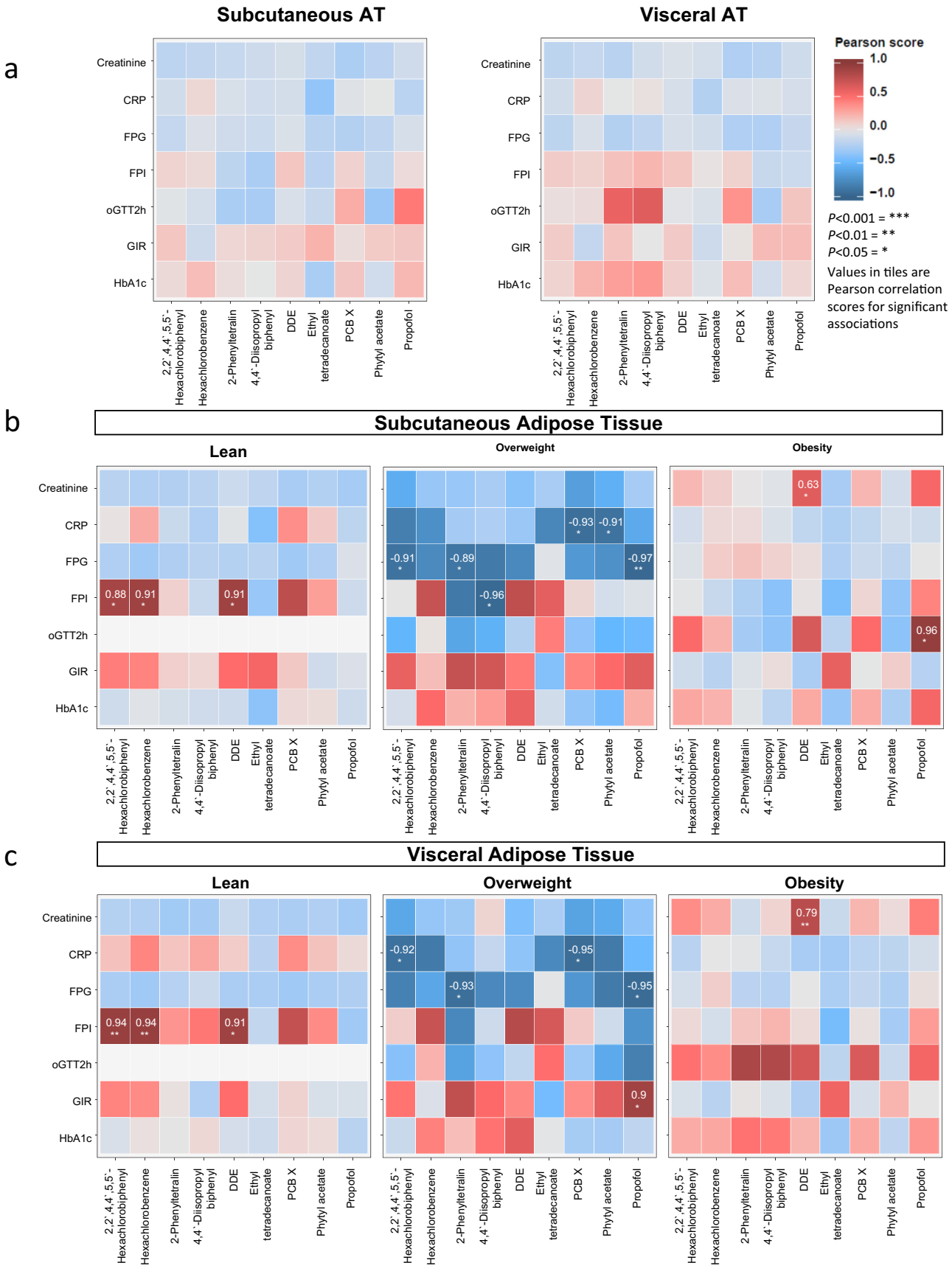


Fig. 7. Correlation analyses of the relative concentration of adipose tissue persistent organic pollutants (POPs) with parameters of glucose metabolism, insulin sensitivity, creatinine and CrP in men. (a) Correlation matrix between distinct POPs, Propofol and phenotype parameters for all men (n = 24). (b) Subcutaneous adipose tissue correlations in subgroups with normal weight (BMI < 25 kg/m^2, n = 6), overweight (BMI 25-30 kg/m^2, n = 6) or obesity (BMI > 30 kg/m^2, n = 12). (c) Visceral adipose tissue correlations in the same BMI-subgroups. Values in tiles are Pearson's correlation coefficients. (***$P < 0.001$, **$P < 0.01$, *$P < 0.05$). CRP, C reactive protein; FPG, fasting plasma glucose, FPI, fasting plasma insulin; oGTT2h; 2 hour oral glucose tolerance test glucose plasma concentration, GIR, glucose infusion rate during the steady state of an euglycemic-hyperinsulinemic clamp.

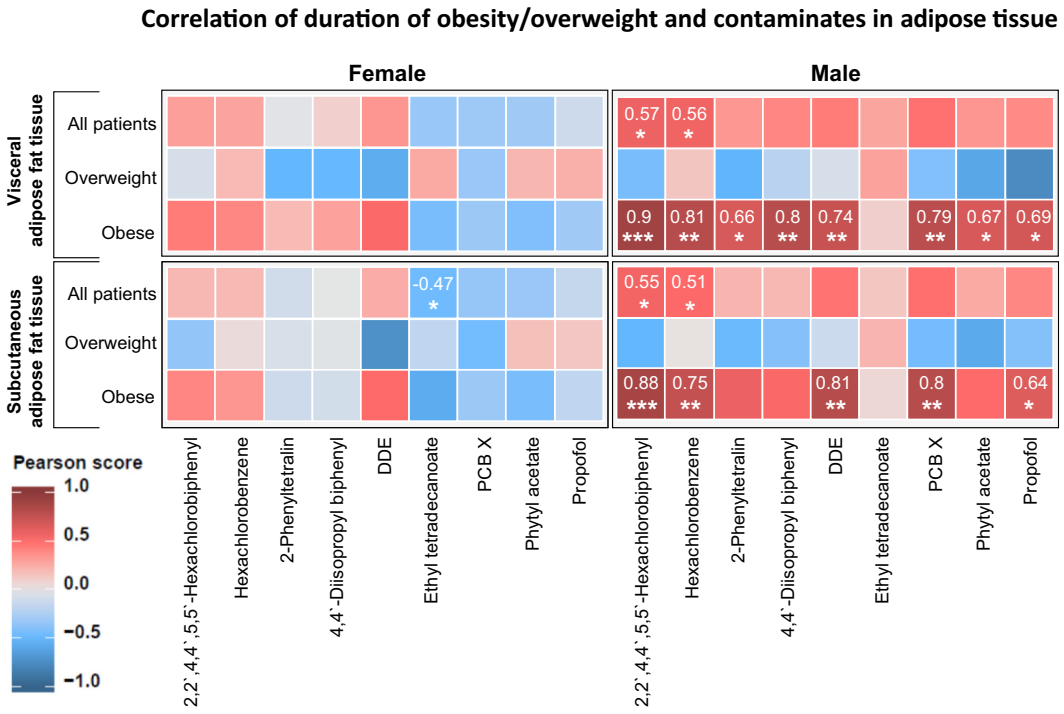


Fig. 8. Correlation analyses of the relative concentration of adipose tissue persistent organic pollutants (POPs) with the duration of obesity/overweight (in years). Separate analysis for subcutaneous and visceral fat tissue for all patients (overweight and obesity), and separately for the overweight and obese groups. Female patients overweight (BMI 25-30 kg/m^2, n = 5) or obese (BMI > 30 kg/m^2, n = 12) and male patients overweight (BMI 25-30 kg/m^2, n = 5) or obese (BMI > 30 kg/m^2, n = 12). Values in tiles are Pearson correlation scores for significant associations. (***$P < 0.001$, **$P < 0.01$, *$P < 0.05$).

infiltration, mean and maximal adipocyte size. Particularly in lean women, there are significant correlations between the abundance of POPs and the number of macrophages in SCAT (2,2′,4,4′,5,5'-Hexachlorobiphenyl, Hexachlorobenzene, Ethyl tetradecanoate) as well as VAT (2-Phenyltetralin, 4,4-Diisopropylbiphenyl, Ethyl tetradecanoate). In independent studies, associations between macrophages in (visceral) AT with obesity, insulin resistance, type 2 diabetes and fatty liver disease have been a consistent finding (Cancello et al., 2005; Kloting et al., 2010; Weisberg et al., 2006; Zatterale et al., 2019). Higher macrophage infiltration in AT reflects AT dysfunction and can be induced by adipocyte apoptosis or secretion of chemoattractant molecules from AT. Importantly, AT inflammation does not necessarily correlate with body fat mass or BMI and can occur even in individuals with low fat mass or lipodystrophy (Morris et al., 2011). The correlation of AT POP accumulation and ATM in lean women indicates that POPs could independently of BMI and fat mass contribute to AT inflammation. Whether POPs may thereby contribute to an increased risk for diseases related to AT dysfunction needs to be investigated in model systems or prospective human trials. The associations between distinct AT POP accumulation and number of ATMs in both fat depots almost exclusively in lean women and even inversely related in overweight men (2-Phenyltetralin) supports the notion that increased fat accumulation does not result in more POP-associated AT dysfunction. Our data leave room for the speculation that higher fat mass (at least in women) may protect against the potentially harmful effects of some POPs on AT function. Whether expansion of AT depots may in part represent an adaptation of AT to buffer increasing POPs needs to be investigated in suitable model systems like rats or mice.

Activation of G-protein-coupled receptors for lipid mediators and metabolites has been shown to affect the inflammatory response (Recio et al., 2018). Noteworthy, ligands of long-chain fatty acid or middle-chain fatty acid specific GPCRs share structural similarities with POPs for which we found associations to ATMs (Ethyl tetradecanoate, Phytol acetate).

In contrast to women, we find significant associations between distinct AT POP accumulation and circulating CrP in men with normal body weight and obesity. Sex specific differences in the systemic inflammatory response have been reported (Rathod et al., 2017). The inflammatory response to typhoid vaccine resulted in different abundance of immune cells. More specifically, in females an elevated number of non-activated neutrophils were found at baseline and 8 h. Interestingly the flow-dilation rate was increased in females but decreased in males. These results indicate the cell type specific response in both sexes.

In the context of AT inflammation, it has been proposed that higher estrogen levels in women counteract the link between AT inflammation and a systemic proinflammatory state (Ghosh et al., 2019). Our data are in accordance with a previous study demonstrating that VAT and SCAT relative concentration of POPs show heterogeneous and sex-specific associations to parameters of obesity, metabolism and adipose tissue function (Pereira-Fernandes et al., 2014).

4.5. Correlation of AT accumulation and adipocyte parameters

We find further significant correlations between AT accumulation of certain POPs and parameters of adipocyte size in both depots, in men and women and across different BMI subgroups. For some POPs, we detected negative correlations with adipocyte size (PCB X, PCB153, Phytyl acetate) depending on sex and BMI group. Whether POP accumulation in AT positively or inversely correlates with adipocyte size seems to be substance, but not sex or fat depot specific. Positive associations between the relative concentration of AT POPs and adipocyte size are of particular interest, because adipocyte hypertrophy is AT-related factor, which confers a higher risk to develop type 2 diabetes (Cotillard et al., 2014). Our data that distinct POPs' accumulation in AT correlates with adipocyte size and AT inflammation supports previous findings that certain POPs contribute to an adipocyte phenotype characterized by hypertrophy, an increased production of pro-inflammatory cytokines, oxidative stress and lipolytic rates (Artacho-Cordon et al., 2016; Cotillard et al., 2014; Howell 3rd and Mangum, 2011).

Fibrosis of AT plays an important role in AT dysfunction and subsequent systemic metabolic abnormalities (Sun et al., 2013) In obesity, rapidly expanding AT is frequently associated with abnormal collagen deposition, a central process in the development of AT fibrosis that is associated with AT inflammation (Spencer et al., 2011; Sun et al., 2011). Upon quick fat expansion, interstitial AT fibrosis has been shown to develop from the response of the tissue to a hypoxic state with an upregulation of pro-fibrotic genes including collagens and their biosynthetic enzymes (Halberg et al., 2009). Because of limited availability of AT biopsy material, we were not able to assess AT fibrosis in the context of this study. However, based on our data that abundance of distinct POPs correlates with AT macrophage infiltration and adipocyte hypertrophy, we hypothesize that similar associations may exist with parameters of AT fibrosis. In this context, it has been shown in both rodent (Liu et al., 2009) and human obesity (Henegar et al., 2008; Keophiphath et al., 2009) that low-grade inflammation in AT can further stimulate accumulation of interstitial fibrosis. Because immune cell Toll-like receptor 4 represents a mediator of obesity associated AT fibrosis (Vila et al., 2014) future studies should investigate whether and which POPs may activate pathways of the local immune response. The observed sex-differences in correlations between POPs accumulation and AT dysfunction parameters could be caused by recently characterized sex-differences in oxidative metabolism and tissue remodeling or adaptability state (Varghese et al., 2020). Taken together, the role of POPs on AT fibrosis requires further systematic studies.

In contrast to these parameters of adipose tissue morphology and function, we do not find significant correlations between the relative concentration of AT POPs and BMI in analyses of the entire cohort. However, in some BMI subgroups, there are sex-related significant correlations with distinct POPs. We acknowledge that our experimental setting prohibits the drawing of conclusions with regard to causality chains between the accumulation of a specific POP and phenotypic traits. Our data may therefore stimulate further mechanistic studies to test the hypothesis that certain POPs may contribute to the regulation of fat distribution, adipose tissue function and cardio-metabolic diseases.

5. Conclusions

The relationship between the relative concentration of POPs in AT (and or serum) with AT function and whole body glucose metabolism has been studied previously (Kim et al., 2014; Pereira-Fernandes et al., 2014). Other studies found fat depot specific differences in the abundance of certain POPs (Pestana et al., 2014) correlating with dysglycaemia. In our study, the variance and heterogeneity of AT POP accumulation was very high for certain POPs so that we were not able to detect statistically significant differences between women and men, BMI subgroups or different fat depots with our given sample size. However, in accordance with previous reports on the abundance of other POPs in serum and adipose tissue we find significant correlations between the relative concentration of five POPs and parameters of insulin sensitivity, fasting and chronic hyperglycemia mainly in lean women. Higher glucose parameters (fasting plasma glucose, 2 hour OGTT glucose, HbA1c) are associated with AT abundance of PCB153, Hexachlorobenzene, 2-Phenyltetralin, 4,4-Diisopropylbiphenyl, Ethyl tetradecanoate, whereas glucose infusion rate during the steady state of an euglycemic-hyperinsulinemic clamp was inversely correlated to AT POPs abundance. Together with the associations between distinct POPs and parameters of AT dysfunction, our data suggest that POPs' AT bioaccumulation negatively affects AT function and the link between AT and whole body glucose metabolism.

We consider as strengths of our analyses that we were able to reliably relatively quantify AT POPs that we include AT donors with a wide range of BMI (including lean healthy individuals), fat distribution and metabolic parameters and that AT specific parameters were deeply characterized. The major limitation of our study is that we do not unravel the precise mechanisms how bioaccumulation of POPs in human AT may affect AT function and systemic glucose metabolism. However, we narrowed down the number of POP candidate molecules which need to be systematically studied in rodent exposure studies to elucidate the dynamics of POP effects on adipose tissue function and to define the molecular mechanisms altered by increasing accumulation of POPs in adipose tissue. Collectively, our data suggest that specific environmental chemicals may contribute to AT dysfunction, adipocyte hypertrophy, systemic inflammation and variation in fat distribution, but not to obesity.

CRediT authorship contribution statement

Ulrike Rolle-Kampczyk: Conceptualization, Formal analysis, Methodology, Project administration, Supervision, Writing - original draft, Writing - review & editing. **Scarlett Gebauer:** Data curation, Formal analysis, Investigation, Methodology. **Sven-Bastiaan Haange:** Data curation, Writing - original draft, Writing - review & editing. **Kristin Schubert:** Writing - original draft, Writing - review & editing. **Matthias Kern:** Investigation, Writing - review & editing. **Yusef Moulla:** Data curation, Writing - review & editing. **Arne Dietrich:** Data curation, Supervision, Methodology, Writing - review & editing. **Michael R. Schön:** Formal analysis, Writing - review & editing. **Nora Klöting:** Data curation, Supervision, Writing - review & editing. **Martin von Bergen:** Conceptualization, Funding acquisition, Project administration, Resources, Writing - original draft, Writing - review & editing. **Matthias Blüher:** Conceptualization, Funding acquisition, Project administration, Resources, 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.

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