Endocrine disrupting environmental chemicals EVELINE DIRINCK 2016

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Endocrine Disrupting Environmental Chemicals

From accumulation to their role in the global "neuro-endocrine" epidemic of obesity and its metabolic consequences

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Chapter 7

Endocrine disrupting polychlorinated biphenyls in metabolically healthy and unhealthy obese subjects before and after weight loss

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7.1. Introduction

The prevalence of obesity, defined as a body mass index (BMI) of > 30 kg/m², has reached alarming proportions with 30-80 percent of the European adult population currently being overweight (BMI > 25 kg/m²) and obesity affecting up to a third of the population (World Health Organisation) Obesity, in particular abdominal obesity, is often accompanied by unfavorable medical conditions such as arterial hypertension, dyslipidemia and glucose metabolism abnormalities (Van Gaal et al. 2006). This cluster of conditions is described as the metabolic syndrome, and causes a severely increased risk for cardiovascular morbidity and mortality (International Diabetes federation, American Diabetes Association). Interestingly, however, a subset of obese individuals does not display these metabolic abnormalities. For example, in the 1999–2004 National Health and Nutrition Examination Survey (NHANES), around 30% of obese US adult did not display any cardiometabolic abnormalities (Wildman et al. 2008).

It remains unclear what factors trigger the development of a metabolically unhealthy phenotype in an obese individual. It has been suggested that body fat distribution or birth weight are important determinants (Camhi et al. 2014, Muller et al. 2012, Primeau et al. 2011), but recently some researchers postulate that polychlorinated biphenyls (PCBs) are contributing to the unhealthy phenotype too (Gauthier et al. 2014). PCBs are known endocrine disrupting chemicals, that have been linked to the development of obesity and type 2 diabetes mellitus (Dirinck et al. 2011, Dirinck et al. 2014). In animal models, exposure to PCBs accelerates the development of insulin resistance, glucose intolerance, low-grade inflammation, and visceral obesity (Ibrahim et al. 2011, Ruzzin et al. 2010). PCBs were used worldwide since the 1930s for various industrial purposes. More than 100 individual PCB congeners have been identified in commercial mixtures, whose chemical and toxicological properties are related to the number and position of the chlorine atoms. Despite the ban on their production in the USA and Europe since the 1970s, their ongoing use, chemical stability, resistance to degradation and lipophilicity has led to significant bioaccumulation in most compartments of the ecosystem and human tissues (Den Hond et al. 2009). This bioaccumulation leads to an ongoing human exposure to PCBs through a variety of pathways, with the most important being dietary intake (Bilau et al. 2008). Several studies have shown an increase in PCB serum levels during weight loss, due to their release from the fat compartment (Hue et al. 2006, Kim et al. 2011, Pelletier et al. 2002). To date, weight loss remains the most important tool to reverse obesity-associated co-morbidities (Sjoholm et al. 2015). It is unclear to what extent the increase in serum PCB levels can hinder the resolution of co-morbidities during and after weight loss.

In order to determine the contribution of PCBs to the metabolically unfavorable features of obesity, this study raised five related questions:

1) Do serum levels of PCBs at baseline differ between metabolically healthy and metabolically unhealthy individuals in our obese cohort?
2) Is there a significant difference in PCB serum levels increment after 6 and 12 months of weight loss between metabolically healthy and unhealthy obese individuals?
3) Is there a significant difference in PCB serum levels increment between metabolically unhealthy obese individuals that remain metabolically unhealthy after weight loss and individuals who become metabolically healthy after weight loss?
4) Do serum levels of PCBs before weight loss and/or their rise during weight loss predict the metabolic health after weight loss?
5) Does the rise in PCB serum levels predict the change in the individual components of metabolic health (fasting glucose, waist, triglycerides, hdl cholesterol, systolic blood pressure, diastolic blood pressure, c-reactive protein)?

7.2. Subjects and methods

7.2.1. Population

A cohort of 184 overweight and obese men (n = 53) and women (n = 131) was prospectively selected from patients visiting the weight management clinic of the Antwerp University Hospital between 2010 and 2012. All participants were older than 18 years of age. The subject characteristics are described in Table 7.1. Participants were treated with a weight loss protocol including dietary counselling and physical activity, or with bariatric surgery. According to local Belgian criteria, patients are eligible for bariatric surgery if their BMI ≥ 40 kg/m² or if their BMI ≥ 35 kg/m² with the presence of diabetes mellitus, therapy resistant arterial hypertension or obstructive sleep apnea. In the dietary intervention group, almost all patients started on a hypocaloric protein enriched 700 kcal diet, 2 patients started on a hypocaloric 1500 kcal diet. For those patients on a 700 kcal diet, the caloric intake was gradually increased after 6 weeks, by 200 kcal per month. During the last 6 months of the study, caloric intake was stabilized at 1200 kcal daily. For this study, both groups were pooled in the same analyses. After bariatric surgery, the nutritional advice patients received consisted of a balanced low calorie diet. Patients were re-evaluated after 6 and 12 months. This study was approved by the ethical committee of the Antwerp University Hospital (Belgian Registry number B30020097009) and registered at clinicaltrials.gov (number NCT01778868). All participants provided their written informed consent.

7.2.2. Anthropometric data

Anthropometric measures were taken in the morning with patients in a fasting state and undressed. Height was measured to the nearest 0.5 cm and body weight was measured with a digital scale to the nearest 0.2 kg. Waist circumference was measured at the mid-level between the lower rib margin and the iliac crest. Anthropometric measures were performed at baseline and 6 and 12 months.

7.2.3. Blood sampling

Venous blood samples were obtained in fasting state from an anticubital vein between 08 AM and 10 AM into sterile BD Vacutainer tubes. Blood samples for chemical analysis of PCBs were immediately centrifuged at 2500-3000 rpm during 15 minutes. Serum was stored in glass vials at -20°C. In obese subjects without a known history of type 2 diabetes mellitus, an oral glucose tolerance test (OGTT) with 75g of glucose was performed with sampling at 0, 15, 30, 60, 90, 120, 150 and 180 minutes. HbA1c, glucose and insulin were measured at the hospital laboratory. Diabetes was classified according to the American Diabetes Association definition (American Diabetes Association 2015). OGTT was repeated in all subjects after 12 months. Total cholesterol, triglycerides and high-sensitivity c-reactive protein (crp) were measured at the hospital laboratory, using a validated method (Dimension Vista 1500 Systems, Siemens) at baseline and 6 and 12 months follow-up.

7.2.4. Fat sampling

Among 66 individuals undergoing bariatric surgery, 50 agreed to provide adipose tissue samples: these were collected during surgery from both the visceral and subcutaneous fat compartment. Samples were stored in glass vials at -20°C until analysis.

7.2.5. Determination of PCBs

Analyses of PCBs, in both serum and adipose tissue samples, were performed at the Toxicological Centre (University of Antwerp). The samples were analyzed for 27 PCB congeners (IUPAC nrs 28, 74, 95, 99, 101, 105, 118, 149, 146, 153, 138, 187, 183, 128, 167, 174, 177, 171, 172, 156, 180, 170, 199, 196/203, 194, 206, 209). PCB levels were expressed on a lipid adjusted basis (ng/g lw). All levels were added to create the sum of all PCBs (ΣPCBs). Total lipids were calculated using the formula: Total lipids (g/L) = total cholesterol (g/L) * 2.27 + triglycerides (g/L) + 0.62 (Philips et al. 1989). Levels below the

method level of quantification (LOQ) were assigned a value of DF×LOQ, with DF being the proportion (%) of measurements with levels above the LOQ or the detection frequency. The analytical methods and quality assurance and quality control have been published previously (Dirtu et al. 2013, Malarvannan et al. 2013).

7.2.6. Metabolic health status

Participants were classified as metabolically healthy or unhealthy based on 3 different sets of criteria: 1) the metabolic syndrome as defined by the Adult Treatment Panel III criteria (ATPIII) (National Cholesterol Eduction Program 2002), 2) the metabolic syndrome as defined by the International Diabetes Federation (IDF) (International Diabetes Federation) and 3) the metabolically healthy phenotype, a combination of criteria based on ATPIII criteria, extended by HOMA-IR as a marker for insulin resistance and c-reactive protein (crp) as a marker for inflammation, as proposed by Karelis and Wildman (Wildman et al. 2008, Karelis et al. 2004). A HOMA-IR > 1 was defined as metabolically unhealthy in this study. Subjects with the presence or absence of the metabolic syndrome are represented as MetS+ and MetS- respectively. Participants were considered metabolically unhealthy obese (MUO) if they fulfilled ≥ 2 out of 7 criteria, or metabolically healthy obese (MHO) if they fulfilled < 2 criteria.

7.2.7. Statistical analysis

Statistical calculations were performed using IBM SPSS, version 21.0 (IBM SPSS, Chicago, IL). Levels below the method level of quantification (LOQ) were assigned a value of DF×LOQ, with DF being the proportion (%) of measurements with levels above the LOQ or the detection frequency. Normality of distribution was verified using the Kolmogorov–Smirnov test. All PCB levels displayed a skewed distribution. After transformation (y = log (x+1)), all PCB levels were transformable to normality. To detect differences in serum PCB levels between MetS+ versus MetS- and MUO versus MHO, independent T-tests or Mann-Whitney-U tests were performed, depending on the normality of the distribution of the variables. A probability value $P < 0.05$ was considered statistically significant. Subjects were identified who were MUO or MetS+ at baseline, and remained MUO or MetS+ at 6 and 12 months follow-up (persistent MUO or MetS+). Likewise, subjects were identified who were MUO or MetS+ at baseline, but were no longer MUO or MetS+ at 6 or 12 month follow-up (resolved MUO or MetS). A binary logistic regression was performed to assess the impact of age, smoking behaviour, BMI, PCB serum levels at baseline on the likelihood of being a persistent or resolved MUO or MetS. Finally, regression analyses were performed, using ΔBMI and ΔPCB

(PCB153, PCB138, PCB180 and ∑PCB separately) as independent variables and Δglucose, Δwaist, Δtriglycerides, ΔHDL cholesterol, Δ systolic blood pressure, Δ diastolic blood pressure and Δcrp as dependent variables. These regression analyses were performed with the 6 month follow-up data and the 12 month follow-up data.

7.3. Results

7.3.1. Study population

184 subjects were included in the study, 53 men and 131 women with a mean age of 41 ± 13 years (Table 7.1). Using the traditional criteria, about 40% of subjects were diagnosed with the metabolic syndrome (MetS+). Given the almost perfect match between groups defined with the IDF or ATPIII criteria for metabolic syndrome, all further analyses presented are restricted to the APTIII criteria for readership convenience. Using the more elaborate criteria with HOMA-IR and crp, 74% were considered MUO. Follow-up data at 6 and 12 months were available for a subgroup of patients (see Table 7.1). BMI, waist, triglycerides and crp levels were significantly higher in this subgroup ($p < 0.05$), compared to the complete baseline group.

7.3.2. PCB levels

The most prevalent PCB congeners identified in our study population are PCB153, PCB138 and PCB180, both in serum as in adipose tissue samples. Detailed information on the serum and adipose tissue levels of all measured congeners, the limit of quantification and detection frequency has been published previously (Dirtu et al. 2013, Malarvannan et al. 2013). PCB153, PCB138 and PCB180 make up 60% of the total PCB profile. Therefore, we limited further statistical analysis of individual PCBs to these three PCBs. PCB levels, both in serum and in adipose tissue, increased significantly with age (Figure 7.1). Since the absolute PCB levels did not differ between visceral and subcutaneous adipose tissue, as described previously (Malarvannan et al. 2013), further statistical analyses is restricted to visceral adipose tissue levels.

7.3.3. Differences in serum PCB levels at baseline

Subjects with the metabolic syndrome (n = 75) had significantly higher serum levels of PCB138 (median 31.3 versus 21.4 ng/g lw) (Figure 7.2A). MUO (n = 136) had significantly higher serum levels of PCB153 (median 60.7 versus 43.1 ng/g lw), PCB180 (48.4 vs. 24.9 ng/g lw) and ΣPCB (228.4 vs 155.7 ng/g

Table 7.1. *Descriptive statistics of study population at baseline and follow-up.*

	Baseline (n= 184)	**Baseline with follow-up data (n= 71)**	**6 month follow-up (n= 71)**	**12 month follow-up (n= 50)**
Sex (Male/Female)	53/131	24/47	24/47	17/33
Age (years)	41 ± 13	44 ± 13	44 ± 13	47 ± 12
Active smoker (yes/no)	29/155	10/61	10/61	3/47
BMI (kg/m²)	35.4 ± 8.6	40.2 ± 5.2	34 ± 5	32 ± 6
Waist (cm)	108 ± 21	121 ± 13	107 ± 14	104 ± 15
Male	120 ± 19	127 ± 11	112 ± 14	107 ± 13
Female	103 ± 20	117 ± 13	104 ± 13	102 ± 16
Fasting glucose (mg/dl)	89 ± 24	94 ± 31	87 ± 11	88 ± 11
HOMA-IR	3.1 ± 2.3	4.0 ±2.5	2.7 ± 2	2.5 ± 1.4
Glucose tolerance status				
NGT	110	28		33
IFG	3	2		0
IGT	46	22		24
IFG + IGT	6	4		3
Type 2 Diabetes mellitus	19	15		2
BP systolic (mmHg)	124 ± 14	125 ± 13	116 ± 12	118 ± 13
BP diastolic (mmHg)	76 ± 10	75 ± 9	72 ± 9	71 ± 9
Triglycerides (mg/dl)	138 ± 81	171 ± 90	123 ± 17	118 ± 58
HDL (mg/dl)	53 ± 17	47 ± 15	52 ± 14	56 ± 14
Male	44 ± 13	40 ± 12	45 ± 10	49 ± 10
Female	57 ± 17	80 ± 16	56 ± 14	60 ± 14
CRP (mg/dl)	0.62 ± 0.65	0.75 ± 0.64	0.52 ± 0.82	0.27 ± 0.30
Met Σ ATPIII (present/absent)	75/108	47/23*	24/47	18/32
MUO / MHO	136/48	61/7*	51/15*	43/5*
PCB153 (ng/g lipid weight)	45.3 (2.5 – 624.0)	48.2 (2.5 – 624.0)	67.4 (7.8 – 276.5)	79.1 (17.3 – 364.6)
PCB138 (ng/g lipid weight)	23.6 (0.3 – 317.0)	26.4 (0.3 – 317.0)	36.0 (4.7 – 151.9)	39.8 (9.6 – 162.7)
PCB180 (ng/g lipid weight)	27.9 (1.6 – 432.0)	27.4 (1.6 – 404.0)	40.9 (3.6 – 204.6)	54.7 (9.9 – 252.5)
ΣPCB (ng/g lipid weight)	172.2 (14.3 – 2189.0)	174.9 (14.3 – 2189.0)	254.0 (32.4 – 1249.5)	284.3 (65.8 – 1572.4)

Clinical data are presented as mean ± standard deviation, PCB serum levels are presented as median (minimum – maximum) given their not-normal distribution Abbreviations: BMI= body mass index; HOMA-IR= homeostatis model assessment of insulin resistance; Diabetes was classified according to the American Diabetes Association Definition: NGT= normal glucose tolerance, IGT= impaired glucose tolerance, IFG= impaired fasting glucose; HDL= high density lipoprotein; crp= c-reactive protein; Met Σ ATPIII= metabolic syndrome according to Adult Treatment Panel III criteria;
MUO / MHO= metabolically unhealthy obese / metabolically healthy obese, please see Methods section for criteria
** data were missing for some subjects*

lw) (Figure 7.2B). There was a significant age difference between individuals with or without the metabolic syndrome (44 ± 13 years vs 39 ± 12 years, p = 0.014), whereas this was not the case for MUO versus MHO subjects (41 ± 10 years vs 41 ± 13 years).

These analyses were repeated in the subgroup of patients (n = 71) of whom

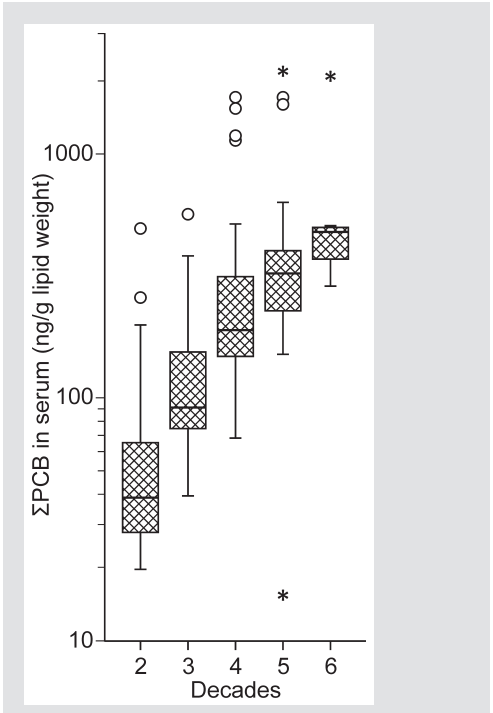

Figure 7.1. *Serum PCB levels in entire study population (n= 184), according to decade*

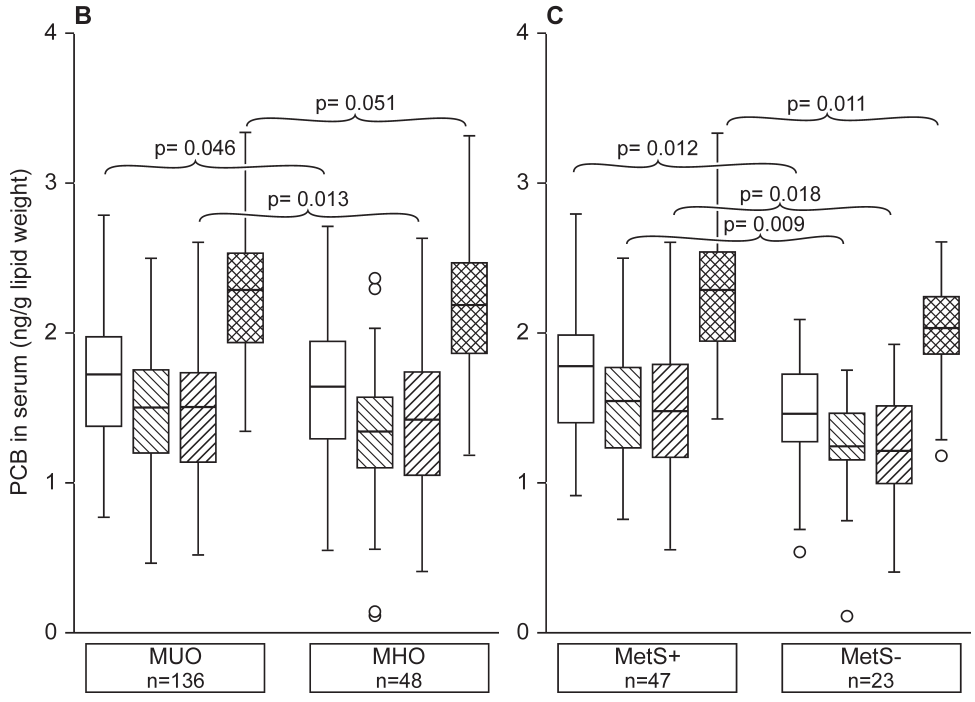

Figure 7.2. Serum levels of PCBs of individuals with versus without the metabolic syndrome and metabolically healthy versus unhealthy individuals at baseline.

MetS - / + = metabolic syndrome according to Adult Treatment Panel III criteria absent / present
MUO / MHO = metabolically unhealthy obese / metabolically healthy obese, please see Methods section for criteria
Serum PCB levels were logarithmically transformed for normality.
A and B represent the entire study population (n= 184), C represents the study population with follow-up data available (n= 71)
*○ indicate values within 3 standard deviations, * indicate values outside 3 standard deviations (also in figure 7.1)*

follow-up data were available. Serum levels of PCB153, PCB138, PCB180 and ΣPCB were significantly higher in MetS+ subjects (n = 47), compared to MetS- subjects (n = 23), 40 ± 32 vs 98 ±127, 22 ± 15 vs 56 ± 74, 24 ± 20 vs 59 ± 82, 138 ±105 vs 365 ± 481 ng/g lw respectively with $p < 0.02$ for all (Figure 7.2C). MUO subjects (n = 61) did not have significantly different serum PCB levels compared to the MHO subjects (n = 7). Age was not significantly different between MetS+ vs MetS- and MUO vs MHO groups.

Table 7.2. *Descriptive statistics of subgroup with adipose tissue sampling*

	Entire population (N= 50)	**MetS+ (N= 30)**	**MetS- (N= 20)**	**MUO (N= 45)**	**MHO (N= 5)**
Sex (Male/Female)	17/33	12/18	5/15	17/28	1/4
Age (years)	40 ± 12	43 ± 11	34 ± 12	40 ± 12	38.4 ± 14
Active smoker (yes/no)	7/43	6/24	1/19	6/39	1/4
BMI (kg/m²)	42.1 ± 3.8	42.5 ± 4.2	41.4 ± 2.9	42.4 ± 3.8	39.8 ± 1.7
Waist (cm)	122 ± 12	126 ± 12	117 ± 8	124 ± 12	112.8 ± 4.7
Male	131 ± 10	135 ± 11	124 ± 4	132 ± 10	NA
Female	118 ± 10	121 ± 10	114 ± 8	119 ± 10	113 ± 5
Fasting glucose (mg/dl)	98 ± 42	109 ± 52	83 ± 10	100 ± 44	82 ± 8
HOMA-IR	4.3 ± 2.7	4.9 ± 2.8	3.5 ± 2.3	4.5 ± 2.8	3.3 ± 1.7
Glucose tolerance status					
NGT	22	9	13	17	5
IFG	1	0	1	1	0
IGT	17	12	5	17	0
IFG + IGT	3	2	1	3	0
Type 2 Diabetes mellitus	7	7	0	7	0
BP systolic (mmHg)	126 ± 19	131 ± 21	118 ± 12	127 ± 19	118 ± 11
BP diastolic (mmHg)	76 ± 11	78 ± 10	72 ± 12	76 ± 11	71 ± 11
Triglycerides (mg/dl)	169 ± 103	214 ± 108	101 ± 36	177 ± 105	99 ± 32
HDL (mg/dl)	47 ± 17	41 ± 14	57 ± 17	45 ± 17	68 ± 8
Male	35 ± 11	32 ± 7	44 ± 16	36 ± 11	NA
Female	53 ± 17	47 ± 15	61 ± 16	51 ± 17	68 ± 8
CRP (mg/dl)	0.85 ± 0.67	0.84 ± 0.70	0.87 ± 0.63	0.87 ± 0.69	0.75 ± 0.45
PCB Adipose tissue levels (ng/g lipid weight)					
PCB153	66.8 (9.1 – 268.2)	78.8 (13.1 – 268.2)	36.9 (9.1 – 207.5)	70.1 (10.0 – 268.2)	57.4 (9.1 – 96)
PCB138	33.3 (5.9 – 141.9)	42.4 (8.1 – 141.9)	21.9 (5.9 – 92.9)	35.8 (7.2 – 141.9)	29.7 (5.9 – 33.6)
PCB180	44.2 (4.9 – 186.5)	58.3 (6.4 – 162.9)	23.1 (4.9 – 186.5)	45.8 (5.4 – 186.5)	35.7 (4.9 – 68.6)
ΣPCB	284.6 (44.7 – 1232.8)	337.1 (56.9 – 1232.8)	154.6 (44.7 – 807.2)	298.6 (50.1 – 1232.8)	224.3 (44.7 – 350.0)

Clinical data are presented as mean ± standard deviation, PCB adipose tissue levels are presented as median (minimum – maximum) given their not-normal distribution

Abbreviations: BMI= body mass index; HOMA-IR= homeostatis model assessment of insulin resistance; Diabetes was classified according to the American Diabetes Association Definition: NGT= normal glucose tolerance, IGT= impaired glucose tolerance, IFG= impaired fasting glucose; HDL= high density lipoprotein; crp= c-reactive protein; NA= not applicable; MetS+= metabolic syndrome according to Adult Treatment Panel III criteria present; MetS-= metabolic syndrome according to Adult Treatment Panel III criteria absent; MUO / MHO= metabolically unhealthy obese / metabolically healthy obese, please see Methods section for criteria

7.3.4. Differences in adipose tissue PCB levels at baseline

Subjects with the metabolic syndrome had significantly higher adipose tissue levels of PCB153, PCB138, PCB180 and ΣPCB, whereas this was not the case for MHO vs MUO (Table 7.2). MetS+ subjects were significantly older then MetS- subjects, whereas this was not the case for MUO vs MHO (Table 7.2).

7.3.5. Differences in serum PCB increments during weight loss

The increment in serum levels of PCB153, PCB138, PCB180 and ΣPCB, expressed as %, did not differ significantly between individuals with or without the metabolic syndrome or MUO versus MHO at baseline (Figure 7.3 + Table 7.3). The weight loss, expressed as a reduction in BMI, between the two

Table 7.3. *Body mass index and PCB serum levels at baseline and follow-up in metabolically healthy and unhealthy individuals*

		Number	BMI	Serum PCB 153	Serum PCB 138	Serum PCB 180	Serum ΣPCB
Baseline	**MetS pos**	47	40.3 (31.5 – 51.4)	59.2 (7.3 – 624.0)	34.4 (4.8 – 317)	29.4 (2.6 – 404.0)	215 (27 – 2189)
	MetS neg	23[1]	39.6 (27.4 – 50.5)	28.4 (2.5 – 122.3)	16.9 (0.3 – 56.5)	15.4 (1.6 – 84.6)	107 (14 – 409)
	MUO	61	40.3 (29.9 – 51.4)	48.5 (2.5 – 624.0)	29.3 (0.3 – 317)	27.4 (2.1 – 404.0)	178 (14 – 2189)
	MHO	7	38.0 (27.4 – 42.2)	33.3 (3.9 – 89.3)	21.0 (4.7 – 45.5)	16.8 (1.6 – 51.7)	117 (19 – 302)
6 month follow-up[2]	**MetS pos at baseline**	47	34.2 (22.5 – 48.9)	71.3 (10.4 – 276.5)	43.1 (6.4 – 151.9)	53.2 (4.6 – 204.6)	264 (41 – 1249)
	% change		-18 (-41 – 7)	58 (-87 – 385)	50 (-88 – 304)	76 (-85 – 532)	58 (-87 – 422)
	MetS neg at baseline	23	33.0 (23.6 – 46.3)	40.5 (7.8 – 213.5)	21.2 (4.7 – 103.3)	22.9 (3.5 – 181.8)	142 (32 – 740)
	% change		-17 (-27 - -4)	43 (-10 – 4972)	24 (-24 – 24800)	45.9 (-22 – 3244)	43 (-13 – 3144)
12 month follow-up[2]	**MetS pos at baseline**	34	31.7 (20.3 – 46.7)	81.4 (19.6 – 364.6)	43.2 (12.8 – 162.7)	63.3 (11.4 – 252.5)	314 (77 – 1572)
	% change		-21 (-54 – 3)	37 (-87 – 985)	27 (-87 – 620)	54 (-86 – 1203)	31 (-87 – 984)
	MetS neg at baseline	16	31.2 (23.8 – 48.1)	66.8 (17.3 – 196.5)	28.0 (9.6 – 90.5)	42.9 (9.9 – 166.6)	240 (66 – 703)
	% change		-19 (-39 – 0)	63 (1 – 5170)	31 (-23 – 22003)	76 (14 – 3261)	69 (-2 – 3171)

Data are represented as median (minimum, maximum)
BMI= body mass index; ∑PCB sum of 27 PCB congeners (please see methods for details)
MetS pos / neg= metabolic syndrome according to ATPIII criteria present / absent
MUO / MHO= metabolically unhealthy obese / metabolically healthy obese, please see Methods section for criteria
[1] Data were missing for one subject
[2] Differences between groups were assessed using a Mann Whitney U analysis, but no statistically significant differences were detected

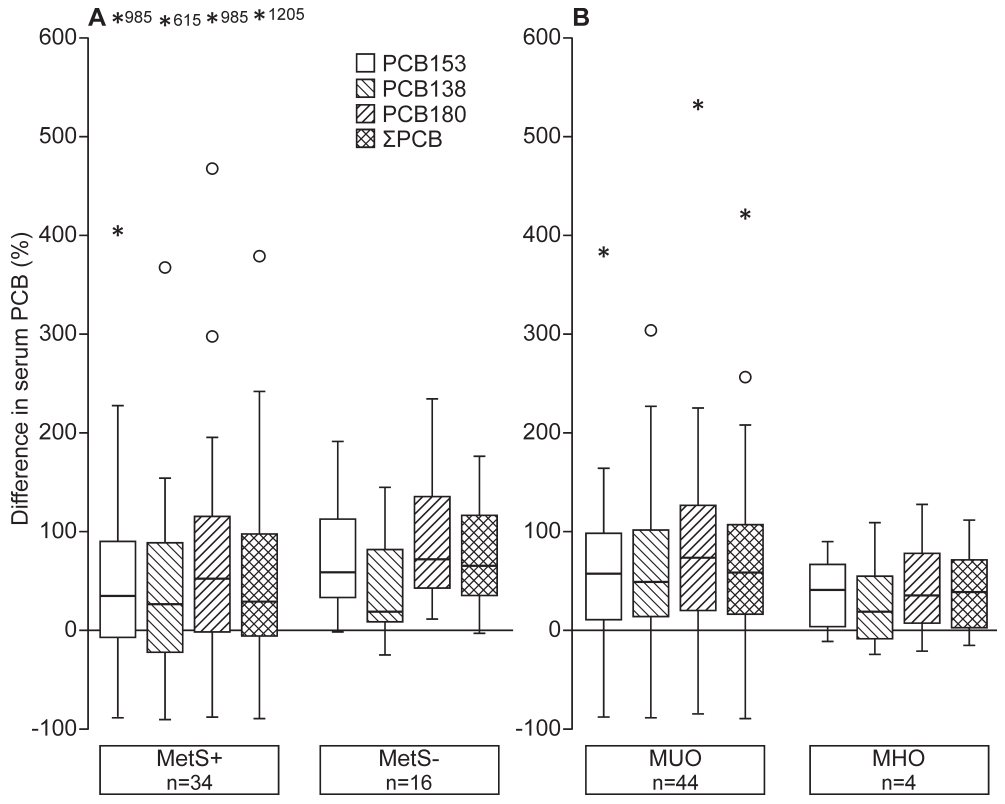

Figure 7.3. *The increment in serum PCB levels, after 12 months of weight loss*

MetS - / + = metabolic syndrome according to Adult Treatment Panel III criteria absent / present at baseline
*MUO / MHO = metabolically unhealthy obese / metabolically healthy obese (please see Methods section for criteria) at baseline. Difference between groups was assessed using the Mann-Whitney-U test, but no statistically significant differences were detected. ○ indicate values within 3 standard deviations, * indicate values outside 3 standard deviations.*

groups did not differ significantly either (-16 ± 7% vs -17 ± 10% at 6 months, -20 ± 13% vs -19 ± 14% at 12 months).

7.3.6. Differences in serum PCB increments between persistent MetS / MUO and resolved MetS / MUO subjects after weight loss

After 6 and 12 months, 18 and 15 subjects respectively were identified as persistent MetS patients. Twenty-seven and 20 subjects were identified as resolved MetS patients. We did not detect a significant difference in percentage increment in serum levels of PCB153, PCB138, PCB180 or ΣPCB at either time (Figure 7.4A and 7.4C, Table 7.4). At 6 months, the group in which the metabolic syndrome persisted had a significantly lower weight loss (-13 ± 8% vs -19 ± 11 %, p = 0.046), but this was not the case at 12 months. After 6 and 12 months, 11 and 3 individuals respectively were identified as resolved MUO,

Table 7.4. *Body mass index and PCB serum levels at baseline and follow-up in persistent and resolved metabolically healthy and unhealthy individuals.*

		Number	BMI	Serum PCB 153	Serum PCB 138	Serum PCB 180	Serum ΣPCB
Baseline	**MetS pos**	47	40.3 (31.5 – 51.4)	59.2 (7.3 – 624.0)	34.4 (4.8 – 317)	29.4 (2.6 – 404.0)	215 (27 – 2189)
	MetS neg	23[1]	39.6 (27.4 – 50.5)	28.4 (2.5 – 122.3)	16.9 (0.3 – 56.5)	15.4 (1.6 – 84.6)	107 (14 – 409)
	MUO	61	40.3 (29.9 – 51.4)	48.5 (2.5 – 624.0)	29.3 (0.3 – 317)	27.4 (2.1 – 404.0)	178 (14 – 2189)
	MHO	7	38.0 (27.4 – 42.2)	33.3 (3.9 – 89.3)	21.0 (4.7 – 45.5)	16.8 (1.6 – 51.7)	117 (19 – 302)
6 month follow-up	**Persistent MetS**	20	36.5 (28.2 – 43.6)	88.2 (15.3 – 276.5)	52.2 (10.5 – 151.9)	54.9 (9.6 – 204.6)	341 (71 – 1249)
	% change		-12[1] (-24 – -3)	40 (-85 – 141)	40 (-86 – 116)	55 (-86 – 180)	47 (-87 – 145)
	Resolved MetS	27	31.8 (22.5 – 48.9)	67.4 (10.4 – 201.4)	35.4 (6.3 – 83.4)	53.1 (4.6 – 150)	260 (41 – 681)
	% change		-20[1] (-41 – 7)	76 (-87 – 385)	68 (-88 – 304)	95 (-86 – 532)	83 (-87 – 422)
	Persistent MUO[2]	46	34.3 (24.3 – 48.9)	71.8 (10.4 – 276.5)	43.1 (6.4 – 151.9)	44.9 (4.6 – 204.6)	262 (41 – 12479)
	% change		-16 (-30 – 7)	43 (-85 – 4972)	43 (-87 – 24800)	56 (-86 – 3244)	49 (-87 – 3144)
	Resolved MUO	11	31.9 (25.8 – 39.8)	46.6 (14.4 – 213.5)	32.2 (10.1 – 103.3)	34.0 (7.3 – 181.8)	205 (55 – 740)
	% change		-20 (-41 – -1)	62 (-86 – 385)	53 (-88 – 304)	95 (-85 – 532)	58 (-87 – 422)
12 month follow-up	**Persistent MetS**	15	33.3 (26.7 – 44.6)	79.4 (25.0 – 364.6)	40.9 (15.5 – 162.7)	51.2 (17.9 – 252.5)	272 (99 – 1572)
	% change		-14 (-35 – 0)	26 (-87 – 154)	24 (-87 – 94)	39 (-86 – 196)	21 (-88 – 148)
	Resolved MetS	20	29.2 (20.3 – 46.7)	98.6 (19.6 – 223.4)	49.2 (-83 – 620)	65.4 (11.4 – 180.5)	351 (76 – 795)
	% change		-21 (-53 – 3)	57 (-82 – 985)	50 (-83 – 620)	70 (-79 – 1203)	56 (-82 – 984)
	Persistent MUO	40	32.2 (23.8 – 48.1)	80.9 (17.3 – 364.6)	42.1 (9.6 – 162.6)	52.6 (9.9 – 252.5)	288 (65 – 1572)
	% change		-18 (-39 – 3)	40 (-87 – 5170)	22 (-87 – 22003)	59 (-86 – 3261)	39 (-88 – 3172)
	Resolved MUO	3	27.4 (20.3 – 27.9)	41.8 (19.6 – 150.9)	23.3 (12.8 – 64.1)	21.9 (11.4 – 113.3)	156 (77 – 574)
	% change		-32 (-53 – -30)	84 (73 – 985)	94 (43 – 620)	93 (79 – 1203)	86 (75 – 984)

Data are represented as median (minium, maximum)
BMI = body mass index; ∑PCB sum of 27 PCB congeners (please see methods for details)
MetS pos / neg = metabolic syndrome according to ATPIII criteria present / absent
MUO / MHO = metabolically unhealthy obese / metabolically healthy obese, please see Methods section for criteria
Persistent MetS / MUO = metabolic syndrome / metabolically unhealthy present at baseline and still present after 6 or 12 months follow-up
Resolved MetS / MUO = metabolic syndrome / metabolically unhealthy no longer present after 6 or 12 months follow-up
Mann-Whitney U test was performed to assess differences between persistent and resolved MetS or MUO.
[1] = difference between persistent and resolved MetS significant with $p < 0.05$
[2] = difference between persistent and resolved MUO not statistically significant

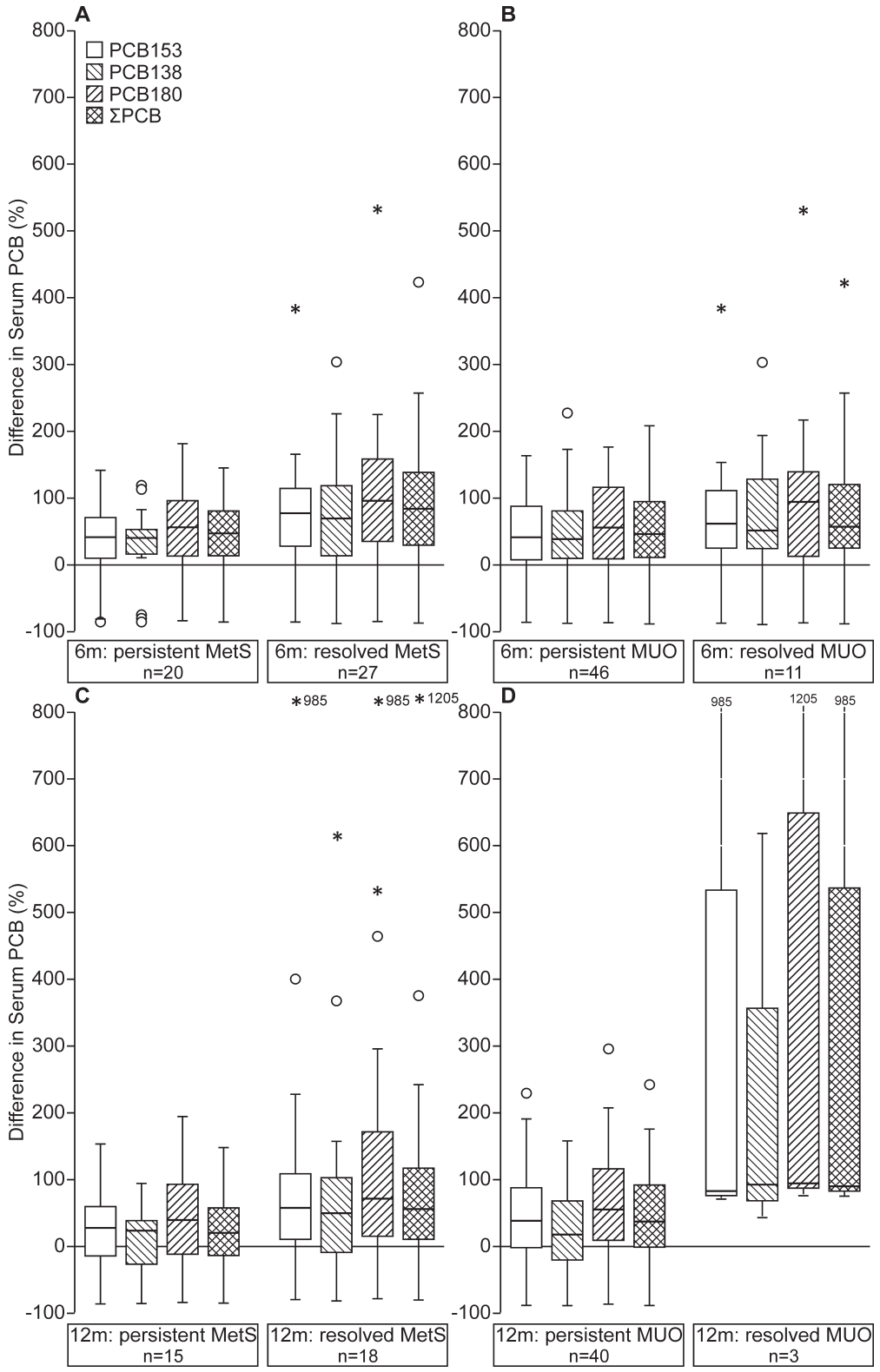

Figure 7.4. *Differences in serum PCB increments between individuals with persistent versus resolved metabolic syndrome or persistent versus resolved metabolically healthy individuals.*

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while 46 and 40 subjects respectively were considered persistent MUO. We detected no difference in percentage increment in serum levels of PCB153, PCB138, PCB180 or ΣPCB at either moment (Figure 7.4B and 7.4D).

7.3.7. Predictive power of PCB serum levels for the resolution of the metabolic syndrome or metabolically unhealthy status after weight loss

We performed a binary logistic regression to assess the impact of age, smoking behavior, BMI, PCB serum levels at baseline on the likelihood of being a persistent or resolved MetS or MUO. Neither the serum levels of PCB153, PCB138, PCB180 or ΣPCB were a significant factor in distinguishing between a persistent or resolved MUO or MetS. In this regression analyses, serum levels of PCB153, PCB138, PCB180 or ΣPCB were not identified as a significant factor in distinguishing between a persistent or resolved MetS or MUO. Additionally, in regression analyses, ΔPCB serum levels could not be identified as significant predictors in the change of the individual components of the MetS and MUO.

7.4. Discussion

Exposure to chemicals with endocrine disrupting properties such as PCBs, has recently been brought forward as a contributing factor in the increasing prevalence of obesity and related metabolic disorders such as insulin resistance and type 2 diabetes mellitus (Dirinck et al. 2011, Dirinck et al. 2013, Lee D-H et al. 2007, Lee D-H 2006). Our study is in line with these previous studies, indicating higher serum levels of PCBs in MUO subjects and individuals with the MetS. The biochemical persistence of PCBs causes significant accumulation within the human body throughout the lifetime. This trend is also present in our cohort (figure 7.4). Since MetS+ subjects were significantly older than their MetS- counterparts, it could be argued that the statistical significance in serum and adipose tissue levels is attributable to this age difference. However, in the entire baseline group (n = 184) MHO were not significantly younger than the MUO, and they displayed a significantly lower PCB serum and adi-

Legend figure 7.4:
6/12m: persistent MetS = individuals with metabolic syndrome according to Adult Treatment Panel III criteria present both at baseline and 6/12 month follow-up
6/12m: resolved MetS = individuals with metabolic syndrome according to Adult Treatment Panel III criteria present both at baseline, but no longer present at 6/12 month follow-up
6/12m: persistent MUO = metabolically unhealthy obese (please see Methods section for criteria) both at baseline and 6/12 month follow-up
6/12m: resolved MetS = metabolically unhealthy obese (please see Methods section for criteria) at baseline that became metabolically healthy obese at 6/12 month follow-up
Difference between groups was assessed using the Mann-Whitney-U test, but no statistically significant differences were detected.
*○ indicate values within 3 standard deviations, * indicate values outside 3 standard deviations*

pose tissue levels nonetheless. The absence of this difference in the subgroup with follow-up data (n = 71) and with adipose tissue sampling might be due to the very small number of MHO (n =7 and 5, respectively). Interpreting these results should be done with caution. Our findings are however in line with the few available studies that have also established higher serum PCB levels in patients with the metabolic syndrome or MUO (Gauthier et al. 2014, Lee D-H et al. 2007). In a recent Korean study, serum PCB levels were predictive for the development of the MetS during a 4-year follow-up study (Lee YM et al. 2014). It should be noted that PCBs have recently been linked to trunk and visceral adipose tissue, the fat compartment that exerts a crucial role in the negative metabolic consequences of obesity (Dirinck et al. 2015, Zong et al. 2015). Given the variety of possible endocrine disrupting mechanisms of different groups of PCB congeners (e.g. dioxin versus non-dioxin like), trying to identify the specific group of PCB congeners responsible for the metabolically detrimental effect is particularly interesting. However, due to a very high intercorrelation between the different PCB congeners in our study ($r > 0.7$ and $p < 0.01$ for most congeners), detailed analysis of separate PCB groups was statistically not feasible.

Weight loss is a powerful method to reduce or abolish obesity associated co-morbidities. During weight loss, however, lipophilic PCBs are released from the adipose tissue into the bloodstream, causing a rise in PCB serum levels (Hue et al. 2006, Kim et al. 2011, Pelletier et al. 2002, Dirtu et al. 2013) To our knowledge, no investigations have yet been carried out to determine if this rise in PCB serum levels is different depending on metabolic status, and whether this rise is capable of blunting the beneficial effects of weight loss. In our study, no difference was detected in percentage increase in PCB serum levels in MUO vs MHO or MetS pos vs neg subjects. There were also no differences in the serum PCB level increments between versus resolved MUO or MetS pos subjects. Neither were PCB serum levels at baseline predictive for the persistence of resolution of a metabolically unfavourable state.

From a clinical point of view, individuals with the metabolic syndrome or the MUO phenotype are considered at elevated risk for cardiovasculair morbidity and mortality. Several studies have investigated the link between cardiovascular disease and PCBs. In the NHANES study, PCB serum levels increased the risk for cardiovascular mortality only in elderly subjects with low fat mass (Kim S et al. 2015). Another sub-analysis of NHANES indicated an increased mortality risk in individuals over 40 years of age with higher dioxin exposure (Lin et al. 2012). A review by Humblet also suggested an increased risk of cardiovascular mortality with higher dioxin exposure, but was not powered to control for other confounding factors (Humblet et al. 2008). Moreover, it has also been suggested that PCB exposure can modulate traditional risk factors

such as cigarette smoking (Lee D-H et al. 2014)

Although we report very detailed data on PCBs and metabolic health, we cannot rule out the possibility of reverse causality given the cross-sectional design of the study. Other chemicals of a less persistent nature, such as phthalates and their metabolites, have also been linked to obesity and disturbances of glucose metabolism but were not taken into account (Dirinck et al. 2015). In addition, factors such as diet, parity and breastfeeding, may influence the serum levels of PCBs as well. This information was, however, not collected in our study.

For now, the concept of metabolically healthy obesity has not been clearly defined yet (Rey-Lopez et al. 2014) but nonetheless studies have indicated that a large part of these metabolically healthy obese individuals are still at increased risk to develop the metabolic syndrome or type 2 diabetes mellitus after several years (Bell et al. 2014, Durward et al. 2012, Eshtiaghi et al. 2015). Our study seems to suggest that despite increased serum levels of PCBs during and immediately after weight loss, the beneficial health effects of weight reduction still far outweigh the possible risks associated with elevated exposure to these endocrine disrupting chemicals. This study raises the questions whether subjects with high PCB levels might have a higher chance of relapse of the obesity after weight loss or a higher chance of evolving from a MHO state into a MUO state. Indeed, PCB release has been shown to negatively affect resting metabolic rate after weight loss, possibly making individuals more prone to weight regain (Tremblay et al. 2004). It would require a large scale follow-up study to answer these questions. The overwhelming health consequences and costs associated with obesity underscore the importance of identifying risk factors that can be modified through public health or policy interventions.