CONCENTRATIONS OF CHLORINATED AND BROMINATED DIOXINS AND FURANS AND PBDES IN LIVER AND ADIPOSE TISSUE OF OBESE AND NON-OBESE SUBJECTS IN THE UNITED KINGDOM

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Introduction

The prevalence of obesity is increasing in many countries, and the frequency of bariatric surgery is also increasing. This study was initiated with several goals: 1) to provide baseline data at time of surgery on the concentrations of chlorinated and brominated dioxins and related compounds (PCDD/Fs, PBDD/Fs, selected polychlorinated and polybrominated biphenyls [PCBs and PBBs]) as well as polybrominated diphenyl ethers (PBDEs) to assess whether concentrations of these compounds are higher in obese than control subjects; and 2) to follow patients after bariatric surgery to assess the changes in measured concentrations and body burdens of these lipophilic compounds over the course of weight loss. This is an initial interim report of baseline tissue concentrations from this study providing data on concentrations of these compounds in fat (visceral and subcutaneous) and liver tissue samples from patients undergoing surgery (bariatric surgery or other abdominal surgery). These are some of the first data on concentrations of brominated dioxins and PBDEs in paired liver and fat samples from human subjects.

Materials and Methods

Patients undergoing Roux-en-y gastric bypass surgery for weight loss and control patients who were undergoing abdominal surgery for non-bariatric reasons were recruited with informed consent for the study, which was approved by National Research Ethics Committee, Yorkshire and Humber – South Yorkshire, UK (REC reference 10/H1304/13). Anthropometric parameters including body mass index were measured at the day of surgery. During surgery, visceral and subcutaneous adipose tissue biopsies, liver biopsy and blood samples were taken.

The method used for the preparation, extraction and analysis of samples has been reported previously[1,2] (Fernandes et al 2004; 2008) and forms part of the CEN method – EN16215:2012 for PCDD/F and PCB analysis.[3] In brief, samples were fortified with 13C-labelled analogues of target compounds and exhaustively extracted using mixed organic solvents. PBDEs and ortho-substituted PCBs/PBBs were separated from non-ortho-substituted PCBs/PBBs, PCDD/Fs and PBDD/Fs by fractionation on activated carbon. The two fractions were further purified using adsorption chromatography on alumina. Analytical measurement was carried out using high resolution gas chromatography-high resolution mass spectrometry (HRGC-HRMS) for all analytes apart from the ortho-substituted PCBs which were analyzed by high resolution gas chromatography-unit resolution mass spectrometry (HRGC-LRMS). The analysis is accredited (UKAS) to ISO 17025 standards, with the inclusion of an in-house reference material and method blanks which were evaluated prior to reporting of sample data and used to determine the limits of detection. Additionally, quality control evaluation for the accompanying data follows the criteria specified for chlorinated dioxins and PCBs (Commission Regulation 252/2012). Not all analytes were measured in all subjects.

Dioxin TEQ concentrations in tissue samples were calculated for chlorinated dioxins, furans, and polychlorinated biphenyls using the World Health Organization 2005 Toxicity Equivalency Factors (WHO TEFs)[4] and for brominated congeners using the WHO interim TEFs.[5] Relationships between concentrations in the different tissue depots (liver, visceral fat, and subcutaneous fat) were assessed and multivariable regressions were conducted to examine the influence of age, gender, and body mass index (BMI) on measured concentrations. For the purposes of statistical analyses, lower bound TEQ estimates (non-detected levels set to zero) were used.

Results and Discussion

Characteristics of the study subjects are presented in Table 1. Patients undergoing bariatric surgery were younger on average than control patients and on average had higher BMIs.

Table 1: Demographics of subjects

	Bariatric	*Control*
N	61	33
Age in yrs, Mean (SD) [Range]	47.9 (12.7) [26 - 83]	68.5 (14.2) [40 - 88]
Gender, % male	36.1	33.3
BMI, Mean (SD) [Range]	47.1 (10.8) [30 - 97.5]	25.3 (4.9) [15.9 - 34]

Tissue concentrations were measured in samples of visceral and subcutaneous fat and in liver biopsies. Because the bariatric and control surgery groups were significantly different in age, had overlapping BMI ranges, and due to the known age-dependent patterns of tissue concentrations for chlorinated TEQ, we combined data from the two groups for further analysis. Average tissue concentrations for a selected set of analytes from the two groups combined are presented in Table 2. Brominated TEQ concentrations were relatively low compared to chlorinated TEQ, constituting less than 5% of adipose tissue TEQ and less than 10% of liver TEQ. The most frequently detected PBDD/F compounds were 2,3,7,8-tetrabromodibenzodioxin, 2,3,7,8-tetrabromodibenzofuran, and 2,3,4,7,8-pentabromodibenzofuran (detailed data not shown). The PBDE compounds presented here are those that were consistently detected in the samples. Of these, BDE 153 was present at the highest concentrations, followed by BDE 47.

Table 2: Tissue concentrations of selected compounds (ng/kg lipid)

	Visceral Fat		Subcutaneous Fat		Liver	
	Mean (SD)	N	Mean (SD)	N	Mean (SD)	N
Chlorinated TEQ[a]	21.1 (12.6)	60	21.2 (13.4)	59	41.2 (35.3)	53
Brominated TEQ[b]	0.84 (0.29)	21	0.85 (0.47)	21	3.88 (5.26)	26
BDE 47	971 (1032)	30	1066 (1197)	28	1616 (1225)	23
BDE 100	357 (297)	30	382 (357)	28	346 (257)	23
BDE 153	3008 (1648)	30	2931 (1615)	28	1834 (675)	23
BDE 183	244 (185)	30	269 (242)	28	222 (152)	23

[a] Includes 29 PCDD/F and PCB congeners. [b] Includes PBDD/Fs and PBB 77, PBB 126, and PBB 169.

We conducted multivariate linear regressions to examine the independent impacts of age, gender, and BMI on measured visceral fat concentrations. Table 3 presents the results of the linear regressions assessing factors influencing visceral fat concentrations. Chlorinated TEQ in visceral fat was significantly positively associated with both age and BMI. In contrast, brominated TEQ compounds showed no significant association with any of the factors considered. BDE 47 was borderline significantly negatively associated with age, while BDE 153 showed a borderline significant negative relationship to BMI. Gender was not a significant factor for any analyte.

Table 3: Results of multivariate linear regression for associations between analyte concentrations in visceral fat samples (ng/kg lipid) and various factors.

	Regression coefficient (Standard error)		
Analyte	Age, yr	BMI, kg/m^2	Gender (F vs. M)
Chlorinated TEQ	0.769 (0.079)**	0.288 (0.107)**	1.877 (1.95)
Brominated TEQ	0.005 (0.005)	-0.0001 (0.0073)	0.04 (0.14)
BDE 47	-23 (12.7)*	2.7 (16.4)	467.6 (355.4)
BDE 100	-4.3 (3.8)	2.5 (4.9)	127.1 (106.5)
BDE 153	1 (20.4)	-52.4 (26.3)*	-441.2 (570.3)
BDE 183	2.1 (2.5)	1 (3.3)	-65.5 (71)

* $p<0.1$; ** $p<0.01$

Subcutaneous fat concentrations were highly correlated with visceral fat concentrations for all analytes (Figure 1). This confirms that concentrations of these compounds in fat depots in the body appear to be

generally in equilibrium, an observation previously made for chlorinated TEQ compounds but not previously demonstrated in humans for PBDD/Fs and PBDEs.

Figure 1: Subcutaneous fat concentrations as a function of visceral fat for chlorinated TEQ, brominated TEQ and BDE 47.

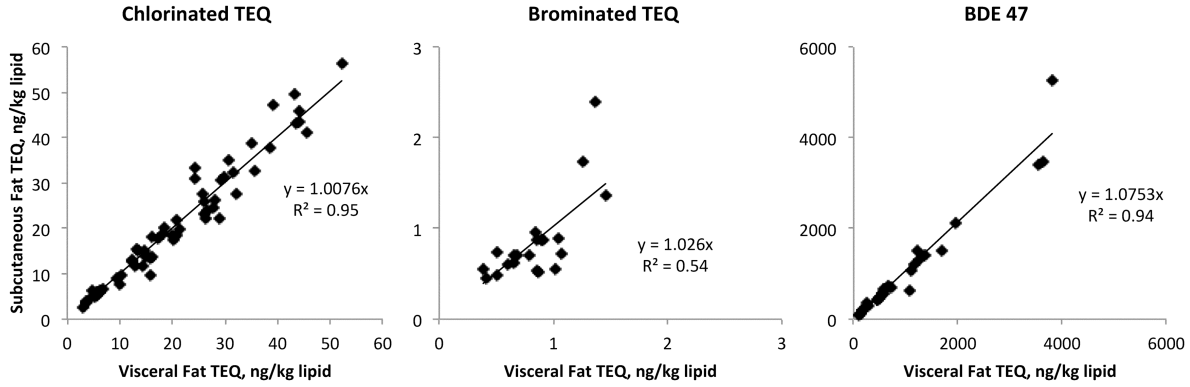
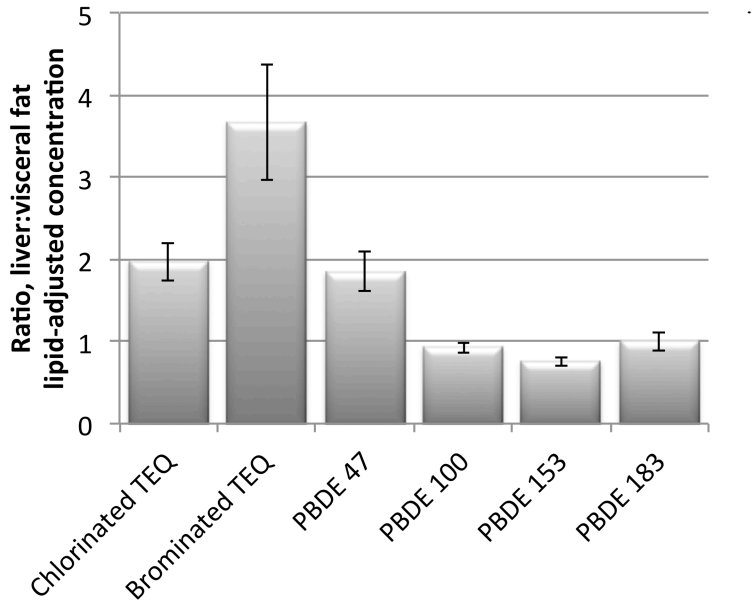


Figure 2: Mean ratio of paired lipid-adjusted liver and visceral fat concentrations. Error bars indicate the standard error on the mean



Chlorinated PCDD/Fs and non-ortho-chlorinated PCBs are known to sequester in liver tissue via binding to the CYP1A2 protein, and also can induce this protein. As a result, lipid-adjusted concentrations in liver can exceed the levels expected due solely to partitioning into lipid in the tissue. The mean ratio of lipid-adjusted liver and visceral fat concentrations of chlorinated TEQ in this study was approximately 2 (Figure 2), suggesting evidence of sequestration of chlorinated TEQ compounds. The ratio of brominated TEQ concentrations was even higher, 3.7, suggesting that these compounds also sequester in human liver, likely also through binding to CYP1A2, as previously demonstrated in rats.[6]

Finally, the data here also suggest sequestration of BDE 47 in human liver, with a mean ratio of lipid-adjusted concentrations of 1.84. Examination of the individual paired ratios shows that of the 21 paired samples,

15 showed ratios of lipid-adjusted hepatic to visceral fat lipid-adjusted concentrations exceeding 1. However, given the modest sample size here, no definitive conclusions can be drawn regarding the possibility of a specific binding mechanism for BDE 47 in human liver.

These data provide for the first time data on concentrations of brominated dioxins and furans as well as PBDEs in paired liver and adipose tissue samples. Based on the provisional TEF values assigned to PBDD/F compounds, these data suggest that brominated dioxins and furans constitute a relatively low fraction of total TEQ. Chlorinated TEQ, but not brominated TEQ or PBDEs, show a positive trend with BMI, with an increase of 4 BMI units associated with an increase in lipid-adjusted TEQ in visceral fat of approximately 1 ng/kg lipid. While the chlorinated TEQ concentrations display a positive trend with age typically observed for these compounds, no such trend is apparent for the brominated TEQ or for PBDE compounds. Two possible reasons may contribute to this observation. First, the notable temporal trend of declining exposure levels with time since the 1970s that has been observed for chlorinated TEQ constituents may not apply to the brominated compounds. Thus, older individuals would not carry an increased body burden due to the higher exposures in earlier time periods. Second, even if a declining trend in exposure exists, if the half-lives of elimination are not as extended for the brominated compounds, differences in exposure levels from earlier time periods would not leave as distinct of a residual signature in tissue concentrations.

Further work with this population will examine changes in tissue concentrations and body burdens over time for these compounds following weight loss.

Acknowledgements

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References

1. Fernandes, A., White, S., D'Silva, K. and Rose, M. (2004) *Talanta*, 63, 1147-1155.
2. Fernandes, A., Dicks, P., Mortimer, D., Gem, M., Smith, F., White, S., and Rose, M. (2008) *Mol. Nutr. Food Res.* 52(2), 238-249.
3. European Commission (2012). Commission Regulation (EU) No 252/2012 of 21 March 2012 laying down methods of sampling and analysis for the official control of levels of dioxins, dioxin- like PCBs and non-dioxin-like PCBs in certain foodstuffs and repealing Regulation (EC) No 1883/2006 *Official Journal of the European Union*, L84/1, 23.3.2012
4. Van den Berg, M., Denison, M.S., Birnbaum, L.S., Devito, M.J., Fiedler, H., Falandysz, J., Rose, M., Schrenk, D., Safe, S., Tohyama, C., Tritscher, A., Tysklind, M., and Peterson, R.E. (2013). *Toxicol Sci.* 133(2), 197-208.
5. Van den Berg, M., Birnbaum, L.S., Denison, M., DeVito, M., Farland, W., Feeley, M., Fiedler, H., Hakansson, H., Hanberg, A., Haws, L., Rose, M., Safe, S., Schrenk, D., Tohyama, C., Tritscher, A., Tuomisto, J., Tysklind, M., Walker, N., and Peterson, R.E. (2006) *Toxicol. Sci.* 93(2), 223-241.
6. Kedderis, L.B., Diliberto, J.J., Linko, P., Goldstein, J.A., Birnbaum, L.S. (1991) *Toxicol Appl Pharmacol.* 111(1), 163-72.