The effects of (POPs) on adipose tissue function and inflammation: in vitro and in vivo models and studies in humans

The Interplay between Environmental Exposures and Obesity
NIEHS
March 2-3 2015

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The various functions of the adipose tissue

- Physical function
- Metabolic function
- Endocrine function
- Toxicological function?
Persistent Organic Pollutants (POP)

- Chemical substances which resist biodegradation
- Nutritional contamination primarily
- Highly lipophilic
- *pathologies*: endocrine disruption, immunotoxicity, cancer, metabolic diseases, etc.
- Stockholm convention (2004): 12 POP should be limited
  - Dioxins, Furans
  - PCB (polychlorobiphenyls)
  - Organochlorine pesticides
  - Polybrominated flame retardants
- Tend to accumulate in adipose tissue and liver

*Pelletier et al, Obesity reviews*
The human pathology context
POPs diabetes and metabolic syndrome

- NTP workshop concluded that there was a positive association between organochlorine POPs and type 2 diabetes but the data were not sufficient to establish causality (Taylor et al, EHP, 2013)

- Seveso: 30 years follow up show that exposure to TCDD of women under 12 leads to increased risk of metabolic syndrome but not diabetes or obesity (Warner et al, EHP, 2013)


- Prospective study in the elderly shows that certain POPs may predict occurrence of diabetes several years later (PIVUS study) (Lee DH et al, Diabetes Care, 2011)

- Exposure of rats to POPs combination leads to metabolic disorders and insulin resistance (Ruzzin et al, EHP, 2009)
Adipose Tissue and pollutants
The two sides of the same coin

Consequences of increased adipose tissue mass on:
- uptake and kinetics of pollutants
- toxicity of pollutants

Obesogenic effects of pollutants:
- endocrine disruption (steroids, thyroid hormones, insulin)
- metabolic disruption (PPAR, PXR, LXR, AhR)
- inflammation, oxidative stress
The multiple roles of adipose tissue in Toxicology

Short term protection and long term toxicity
Adipose tissue and POPs: Friend or foe?

Preservation of critical tissues

toxicity:
Cancer
Endocrine disruption
Immunotoxicity
Metabolism…
The adipose tissue: protection function

Correlation between fat mass of animals and acute toxicity of dioxin

\[ \log \text{LD}_{50} = 5.30 \times (TBF) - 3.22 \]

“Survival of the Fattest”??

The obesity paradox in human
Protective effect of fat mass when serum POPs are high

Adjusted hazard Ratio (death)

low PCB
medium PCB
high PCB

Fat mass

<20  20-24  24-28  28-33  >33

Adapted from Hong et al, Int J Obesity, 2012
General approach
Weight loss

Weight loss
Bypass surgery, diet

Increased release?
increased toxicity?

Unique model of internal exposure

Hue, O, Obes surgery, 2006
Adipotox

**Inclusion** of subjects 2007-2008
Obese \((n = 86)\), lean \((n = 23)\)

Follow up 0, 1, 3, 6, 12 months

clinical phenotyping

Tissue phenotyping

Biological determinations (blood, AT)
Population of the adipotox study

- Obese population: 68 women and 18 men, age 18 to 68 years
- Lean population: 20 women and 3 men, age 24 to 57 years
Amounts of POPs and their distribution are similar in visceral and subcutaneous territories.

One biopsy is representative.

17 dioxin et furan congeners
12 PCB- DL (dioxine-like)
7 PCB-ind (indicators)

Distribution is similar among subjects. Small differences between obese and lean.
POPs in obese and lean individuals

**Example: PCB dl**

<table>
<thead>
<tr>
<th></th>
<th>Obese</th>
<th>Lean</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Adipose tissue (per g lipid)</strong></td>
<td><img src="chart1" alt="Bar chart" /></td>
<td><img src="chart2" alt="Bar chart" /></td>
</tr>
<tr>
<td><strong>Serum (per g lipids)</strong></td>
<td><img src="chart3" alt="Bar chart" /></td>
<td><img src="chart4" alt="Bar chart" /></td>
</tr>
</tbody>
</table>

The AT of obese subjects contains 2-3-fold more PCB-dl total than AT of lean subjects.
Serum POPs: quantitative relationship with metabolic phenotype (obese)

Partial correlations adjusted to age: PCB ind* (/ ml)

<table>
<thead>
<tr>
<th>Phenotypes</th>
<th>Rs</th>
<th>P value</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>corpulence</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>-0.44</td>
<td>0.009</td>
<td>fat mass, leptin and adipocyte diameter negatively correlate with serum POPs</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>-0.32</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Leptin (mg/ml)</td>
<td>-0.38</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>Adipocyte diameter</td>
<td>-0.50</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>Lipid parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>total Cholesterol (mM)</td>
<td>0.40</td>
<td>0.003</td>
<td>serum lipids positively correlate with serum POPs</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.39</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>Glucose Homeostasis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOMA-B</td>
<td>-0.30</td>
<td>0.07</td>
<td>Insulin secretion negatively correlates with serum POPs</td>
</tr>
<tr>
<td>Liver parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AST</td>
<td>0.45</td>
<td>0.006</td>
<td>Liver toxicity positively correlates with serum POPs</td>
</tr>
<tr>
<td>ALT</td>
<td>0.40</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>GGT</td>
<td>0.58</td>
<td>0.00025</td>
<td></td>
</tr>
</tbody>
</table>

* Similar results with other PCBs and dioxins
Gene Expression in obese vs. non obese Adipose Tissue

Increase in POP target genes and inflammatory genes in obese subjects AT

There was no clear correlation between gene expression and POP levels due to the high variability of gene expression
Serum POPs following surgery (ng/g blood lipids)

Increase in serum POPs over 1 year expressed per g blood lipids or per ml blood
Release from AT during lipolysis

*p Mannova repeated measures
Decrease of PCBs total body burden, but not of PCDD/F, one year after surgery.
Serum POPs: quantitative kinetic correlation with metabolic phenotypes (during bypass) ex PCB ind (/ml)

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<tr>
<td>corpulence</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>-0.41</td>
<td>0.000008</td>
<td>weight loss and decrease in adipocytes volume positively correlate with serum POPs</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>-0.44</td>
<td>0.000001</td>
<td></td>
</tr>
<tr>
<td>Leptin (mg/ml)</td>
<td>-0.31</td>
<td>0.03</td>
<td>weight loss and decrease in adipocytes volume positively correlate with serum POPs</td>
</tr>
<tr>
<td>Adipocyte diameter</td>
<td>-0.33</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>lipids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Cholesterol (mM)</td>
<td>+ 0.30</td>
<td>0.0004</td>
<td>higher POPs correlate with smaller improvement in serum lipid</td>
</tr>
<tr>
<td>Triglycerides (mM)</td>
<td>+ 0.18</td>
<td>0.000005</td>
<td></td>
</tr>
<tr>
<td>Sensitivity to insulin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOMA- S</td>
<td>+0.49</td>
<td>0.000009</td>
<td>higher POPs positively correlate with insulin sensitivity index</td>
</tr>
<tr>
<td>Adiponectinemia</td>
<td>+ 0.20</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Liver parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GGT (UI/ml)</td>
<td>+ 0.10</td>
<td>0.004</td>
<td>higher POPs correlate with smaller improvement in GGT</td>
</tr>
</tbody>
</table>

linear mixed-effect model evaluation of POPs kinetics and effects (0, 3, 6 12)
Similar results with other POPs

Kim et al, EHP, 2011
Reported weight loss and serum POPs

Higher serum levels of POPs in individuals who reported long term weight loss

Lim et al, Int J Obesity, 2011
The adipose tissue as a POPstat

POPs: Exposure (acute or not) → Chronic release

Protection | Danger

Short term protection and possible long-term toxicity
The multiple roles of adipose tissue in Toxicology

The adipose tissue as a target

Focus on inflammatory effects
Biological anomalies in adipose tissue of obese individuals

**Adipocyte hyperplasia / hypertrophia**

<table>
<thead>
<tr>
<th>Lean</th>
<th>Obese</th>
</tr>
</thead>
</table>

**Immune cells**

- Macrophages (HAM 56)
- Lymphocytes (CD3)
- Neutrophils (CD15)
- Mast cells

**Vessel alterations**

(inflammation & senescence)

**Fibrosis (pericellular)**

- Red picrosirium staining
- 3D Collagen I staining (green)

**Macrophage (M) accumulation**

- CD34
- CD31
- DAPI

Cancello, Diabetes 2010 U872
Liu, Divoux Nature Medicine, 2009
Villaret, Diabetes 2010, U

Courtesy of K Clément
Dioxin action

- Xenobiotic metabolism
- ROS
- Cell Cycle
- Proliferation
- Apoptosis
- Migration plasticity
- Differentiation
- Endocrine disruption
- Cytokines inflammation

TCDD 4h
TCDD 8h
TCDD 48h
The cellular model

**hMADS cells:**
human Multipotent Adipose-Derived Stem cells

Seeded as proliferative cells (preadipocytes)

Differentiate following confluence and treatment with adipogenic agents within 10 days into triglyceride-containing cells (adipocytes)
Large scale microarray gene expression studies in hMADS cells KEGG-based functional profiling of pollutant treated human preadipocytes

TCDD (25 nM) treated preadipocytes

Up-regulation of inflammatory response

PCB126 (1µM) treated preadipocytes

PCB153 (10 µM) treated preadipocytes

Kim et al, EHP, 2013
Pro-inflammatory effects of POPs on hMADS preadipocytes

RT-qPCR assay

Similar data were obtained following differentiation into adipocytes
Effect of an AhR antagonist $\alpha$NF on hMADS preadipocytes

RT-qPCR assay
Effect TCDD treatment (10 µg/kg) on gene expression in epididymal adipose tissue of wild-type and AhR knock out mice

*RT-qPCR assay of several genes*

AhR-dependent induction of cytokines and inflammatory genes in vivo
Effect of TCDD treatment (10 µg/kg) on inflammation in mouse adipose tissue

Increase in adipose tissue inflammatory cells
Other effects of the dioxin receptor AhR on signaling and Adipose Tissue

AhR POP ligands as well as other POPs alter metabolic pathways in other tissues and may induce lipotoxicity

La Merrill et al, EHP, 2013
Higher levels of POPs in metabolically abnormal vs metabolically healthy obese

Table 2. Plasma Concentrations of POPs (pg/mL) in MHO and MAO Participants

<table>
<thead>
<tr>
<th></th>
<th>MHO</th>
<th>MAO</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>36</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Dioxin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OCDD</td>
<td>0.8 (0.2, 3.9)</td>
<td>1.0 (0.2, 4.4)</td>
<td>.161</td>
</tr>
<tr>
<td>PCBs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dioxin-like PCBs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CB 105</td>
<td>9.8 (0.1, 54.5)</td>
<td>16.5 (3.7, 443.4)</td>
<td>.002</td>
</tr>
<tr>
<td>CB 118</td>
<td>57.7 (13.3, 199.7)</td>
<td>86.2 (15.9, 725.0)</td>
<td>.005</td>
</tr>
<tr>
<td>CB 156</td>
<td>26.7 (8.7, 117.6)</td>
<td>43.4 (11.6, 238.8)</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>CB 157</td>
<td>6.4 (1.7, 32.6)</td>
<td>11.0 (2.4, 65.6)</td>
<td>.001</td>
</tr>
<tr>
<td>CB 189</td>
<td>1.5 (0.3, 7.2)</td>
<td>2.6 (0.6, 11.4)</td>
<td>.001</td>
</tr>
<tr>
<td>Sum</td>
<td>98.8 (37.1, 308.3)</td>
<td>151.2 (43.0, 1482.3)</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Non-dioxin-like PCBs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CB 74</td>
<td>49.7 (13.0, 146.0)</td>
<td>62.9 (10.4, 230.8)</td>
<td>.036</td>
</tr>
<tr>
<td>CB 99</td>
<td>23.9 (7.4, 104.1)</td>
<td>46.0 (8.8, 218.1)</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>CB 138</td>
<td>128.8 (56.9, 354.1)</td>
<td>215.6 (40.0, 1243.7)</td>
<td>.001</td>
</tr>
<tr>
<td>CB 153</td>
<td>179.1 (70.5, 463.0)</td>
<td>264.9 (70.2, 1331.6)</td>
<td>.001</td>
</tr>
<tr>
<td>CB 170</td>
<td>55.3 (28.5, 183.2)</td>
<td>85.9 (23.6, 422.5)</td>
<td>.003</td>
</tr>
<tr>
<td>CB 180</td>
<td>176.0 (73.2, 464.2)</td>
<td>221.1 (68.8, 689.8)</td>
<td>.070</td>
</tr>
<tr>
<td>CB 194</td>
<td>24.5 (6.0, 101.2)</td>
<td>33.9 (8.3, 93.9)</td>
<td>.009</td>
</tr>
<tr>
<td>CB 206</td>
<td>7.4 (0.8, 40.2)</td>
<td>10.3 (2.8, 33.8)</td>
<td>.091</td>
</tr>
<tr>
<td>CB 209</td>
<td>4.7 (0.9, 20.4)</td>
<td>5.4 (1.3, 23.8)</td>
<td>.26</td>
</tr>
<tr>
<td>Sum</td>
<td>650.3 (257.3, 1850.6)</td>
<td>908.1 (246.5, 4023.6)</td>
<td>.002</td>
</tr>
<tr>
<td>OPs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trans-nonachlor</td>
<td>66.9 (0.5, 162.1)</td>
<td>118.4 (20.3, 347.3)</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>p,p’-DDE</td>
<td>1495.4 (484.7, 22 175.2)</td>
<td>1893.2 (147.9, 50 573.7)</td>
<td>.66</td>
</tr>
<tr>
<td>BDE</td>
<td>66.0 (15.5, 1071.7)</td>
<td>85.2 (9.4, 1151.2)</td>
<td>.33</td>
</tr>
</tbody>
</table>

Abbreviations: BDE, brominated diphenyl ether; BDE47, 2,2’,4,4’-tetra-bromodiphenyl ether; CB, chlorinated biphenyl; OCDD, octachlorodibenzo-p-dioxin; p,p’-DDE, 2,2-bis (4-chlorophenyl)-1,1-dichloroethene. All data are expressed as median (minimum, maximum).
Complex effects of adipose tissue on dioxin kinetics

In addition to direct effects of pollutants on adipose tissue growth and function, the AT alters the kinetics of these pollutants in such a way as to limit acute toxicity but to increase the risk of chronic toxicity.
The adipose tissue is a complex target of pollutants

pollutants

obesogens

programming, growth

growth
metabolism
inflammation

metabolic disruptors
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