Chemicals in House Dust: Potential Contributors to Metabolic Disorders
Outline

- Metabolic Syndrome & Molecular Mechanisms
  - Metabolic disruptors, adipogenesis
- Diverse Indoor SVOCs as Metabolic Disruptors
- Indoor House Dust Extracts and Metabolic Disruption
Prevalence of Obesity Epidemic in US, Globally

- Currently ~40% of US adult population is obese.
  - ~9% infants/toddlers
  - ~19% of 2-19 year-olds


- Increased comorbidities
  - T2D, CVD, hypertension

- Interventions have produced only modest effects

CDC Data Brief, Oct 2017
Potential Role of Chemicals in Increasing Obesity Rates in Humans

- First posited in 2002, despite decades of experimental evidence.
- Challenges caloric intake, activity, genetics as sufficient factors to explain magnitude/speed of observed trend.
- Summarizes wealth of animal evidence on antibiotics, PCBs, plastics, pharmaceuticals, pesticides, organophosphates, heavy metals, etc.

Normal Hormonal Function

- Estrogen Receptor
- Androgen Receptor
- Glucocorticoid Receptor
- Progesterone Receptor
- Thyroid Receptor

- Sexual Differentiation
- Regulation of Metabolism
- Regulation of Immune Function
- Regulation of Fertility, Pregnancy
- Pubertal Development, Secondary Sex Characteristics
- Organ Development, Maintenance
- Brain Development
- Cell Proliferation, Maintenance
- Muscle/Bone Formation, Maintenance

Regulation of Metabolism, Pubertal Development, Secondary Sex Characteristics
Adipocyte Differentiation Process

Adipocyte commitment
- Mesenchymal stem cell
- Other pathways: Myoblasts, Osteoblasts, Chondroblasts

Adipocyte differentiation
- Preadipose cell
  - A2COL6/pOb24
  - LPLF A transport
  - PPARδ
- Preadipose cell
  - C/EBP β/δ
  - IGF-1
  - PRAR γ2
- Immature adipose cell
  - C/EBP γ; GLUT4; β2 AR; β3 AR; ACC FAS; ME; ATP-citrate lyase; GPDH; HSL; LBP; perilipin; apoE; low Km PDE; GPAT; LPAT; DGAT; SCDI

Emergence of very late markers and further triacylglycerol accumulation
- Growth arrest and emergence of early markers
- Growth reduction (clonal expansion) followed by emergence of late markers and triacylglycerol accumulation
- Increased response to IGF-1

Resemble brown/developing white adipose cell

Nagy et al. 2011, Mol Med
PPARγ-Dependence of Adipocyte Differentiation

Rosen et al. 2000, Genes & Development

Fu et al. 2005, Mol Endo
Potential Mechanisms of Metabolic Dysfunction

Numerous potential mechanisms of metabolic disruption:

- Adipocyte commitment from MSCs
- Adipocyte differentiation from precursor cells
  - Increased pre-adipocyte proliferation
  - Increased lipid uptake
- Shifting energy balance to favor calorie storage
- Altering basal metabolic rate
- Altering hormonal control of appetite and satiety
- Altering brain circuitry that controls food intake, energy expenditure

Heindel et al. 2017, Repro Tox
Diethylstilbestrol and Developmental Exposure

- Synthesized 1938
- Pregnant women, 1940’s-1971
- Growth hormone (livestock), 60’s-70’s
- Adverse health outcomes (children):
  - Vaginal clear cell adenocarcinoma, reproductive tract malformations, infertility, testicular cancer

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**SALES OF DES PILLS AND VAGINAL CANCER INCIDENCE BY YEAR OF DIAGNOSIS**

- **Cases**
- **Sales**

*Source: Melnick et al, 1987 and Market Share Litigation Exhibit*
DES and Metabolic Disruption

- DES promotes triglyceride accumulation in *in vitro* models.
  - Appears to occur through an estrogenic mechanism
  - DES induces adipogenic regulators and markers *in vitro*.

- Gestational & perinatal DES exposure in mice increases body weight throughout life.
  - Increased body fat, altered serum profiles

- Some evidence for increased risk of obesity in adults exposed prenatally (Hatch et al., 2015, others).

Hao et al. 2012, *Tox App Pharm*

Newbold et al. 2007, *Repro Tox*
Indoor SVOCs as Metabolic Disruptors
3T3-L1 Pre-adipocyte Adipogenesis Assay

- Swiss albino mouse embryonic fibroblast cell line – committed pre-adipocytes
- Extensively used over decades to evaluate adipogenesis
  - Mechanisms of adipocyte differentiation well understood
  - This assay, particularly coupled with PPARγ reporter gene assays, has proven reliable for predicting metabolic disruption \textit{in vivo}.

- Limitations:
  - Length of differentiation window: \(~2\) weeks
  - Depends on contact inhibition to arrest cell cycle and differentiate
  - Fails to incorporate other mechanisms of metabolic disruption

Heindel and Schug 2014, \textit{Curr Envir Health Rpt}
Adipogenesis Induction Timeline

Growth Media
Growth Arrest
Clonal Expansion

Day 0
Seed Plates

Day 1(+)
Confluency Check

Day 3
Differentiation Start

48 hour
confluence

48 hour
cocktail + test chem

Day 5
End Differentiation
Begin Maintenance

Differentiation cocktail:
5% NCS -> FBS,
1 µg/mL insulin,
800 mM IBMX

5 day
adipocyte maintenance
+ test chem

+ Test Chem
+FBS/insulin, -IBMX
Morphological changes
Intracellular lipid accumulation

Growth Arrest
Initiate differentiation

Clonal Expansion
+ Test Chem
+ Diff cocktail

+ Diff cocktail
Initiate differentiation

+ Test Chem
+FBS/insulin, -IBMX
Morphological changes
Intracellular lipid accumulation

3 day
maintenance + test chem

Day 13
Assay 3T3-L1 cells
Adipogenesis Assay Measures

- Triglyceride (lipid) accumulation
  - AdipoRed - hydrophilic fluorescent dye (Nile Red)
    - Partitions into lipid droplets in the cells, fluoresces

- Cell proliferation/cytotoxicity
  - NucBlue DNA dye (Hoechst 33342)
    - Partitions into nuclei and fluoresces upon binding DNA
Rosiglitazone Control
Adipogenesis Assay Results

- Triglyceride accumulation per well – total response, normalized to maximal rosiglitazone-induced response.
  - Measure of whether chemical stimulates incorporation of lipids (fat cell size)

- Cell proliferation – DNA content relative to differentiated vehicle control (0.1% DMSO).
  - Measure of whether chemical stimulates increased number of fat cells

- Triglyceride accumulation per cell – response normalized to DNA content, normalized to maximal rosiglitazone-induced response.

Kassotis et al. 2017, ES&T
Brominated and Organophosphate FRs Promote Substantial Fat Cell Development

Kassotis et al. 2017, ES&T
Several Phthalates and Pesticides Promote Significant Fat Cell Development

Kassotis et al. 2017, ES&T
Perfluorinated Compounds and Parabens Induce Limited Fat Cell Development

**Perfluoroalkyl Substances (PFASs)**

A. Triglyceride Accumulation per Well
   - % Triglyceride Accumulation vs. Concentration (M)

B. Cell Proliferation/Cytotoxicity
   - % DNA Content Relative to Vehicle vs. Concentration (M)
   - 8.2 FTAc, 8.2 FTOH, 6.2 FTOH

C. Triglyceride Accumulation per Cell
   - % Norm. Triglyceride Accum. vs. Concentration (M)

**Phenols**

G. Triglyceride Accumulation per Well
   - % Triglyceride Accumulation vs. Concentration (M)

H. Cell Proliferation/Cytotoxicity
   - % DNA Content Relative to Vehicle vs. Concentration (M)
   - 2,4,6-TBP, Tridosan

I. Triglyceride Accumulation per Cell
   - % Norm. Triglyceride Accum. vs. Concentration (M)
Seven of 11 extracts exhibited significant triglyceride accumulation.

Nine of 11 extracts exhibited significant pre-adipocyte proliferation.

One of 11 extracts exhibited no significant adipogenic activity.
Summary of Findings

- >2/3 of the semi-volatile indoor contaminants were active in promoting triglyceride accumulation and/or pre-adipocyte proliferation.
- PFRs exhibit much greater activity than the majority of the BFRs.
  - BFRs are generally weakly adipogenic, though novel BFRs (TBPH, TBBPA, TDBPIC) are more active than legacy PBDEs.
- Pyraclostrobin, TBPDP, and DBP were the most active chemicals tested.
  - Several appear to exhibit effects through mechanisms other than PPARγ
- PFASs, phenols, parabens exhibited minimal activity overall.
  - Previous work found moderate activity for bisphenols (TBBPA, BPAF, etc.)

- Many house dust extracts exhibited significant triglyceride accumulation and pre-adipocyte proliferation at < 10 μg.
- Children estimated to consume ~50 mg of dust each day (EPA)
Future Directions, Next Steps

- Assessing activity of larger numbers of house dust samples.
  - CIE cohort (n=~150), TESIE cohort (n=~200)

- Assessing mechanisms through which house dust promotes fat cell development.
  - Causative constituent chemicals
  - Causative molecular pathways

- Assessing relationships between house dust driven fat cell development and metabolic health outcomes in residents.
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