Effects of perinatal exposure to BPA on obesity and metabolic disease later in life

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Bisphenol A: An endocrine disruptor with widespread exposure and multiple effects
Controversy in the literature regarding early BPA exposure and Body Weight.

Weight Gain?

Weight loss?

No Change in BW?
Human studies have reported an association between BPA levels and increased:

- Body weight,
- Waist circumference
- BMI

Also, obesity associated metabolic changes including insulin resistance and diabetes
Questions:

- Does developmental exposure to BPA alter body weight and body composition and contribute to other associated components of metabolic disease?
- What BPA doses cause weight gain and increased fat Mass?
- What is the critical window for BPA exposure?
- Males vs Females?
- Does early BPA exposure interact with a “western diet” in adulthood?
Part 1: Dose Response and Exposure Windows

A. BPA Doses: 0, 0.25, 2.5, 25, and 250 µg BPA/kg BW/day (12-14 litters/treatment group).

B. Exposure Windows:
   1. **Perinatal** (Day 8 of Gestation through Lactational day 16) via osmotic minipumps [P]
   2. **Perinatal + Peripubertal** (additional exposure from PND20-PND35 via drinking water [P+P])

C. Males and Females

D. Internal dose measurements (6 litters/treatment group)
Perinatal Exposure Of CD-1 Mice To BPA

**Treatment:**
(Osmotic pumps)

- **Control (Vehicle only)**
  - 0.25 ug BPA/kg BW/d
  - 2.5 ug BPA/kg BW/d
  - 25 ug BPA/kg BW/d
  - 250 ug BPA/kg BW/d

**Period of Exposure**
- Pump
- Vaginal plug
- E 1
- E 8
- PND 16
- PND 20

**Offspring examined at various time points**
- F1 (Cull to 8)
- Birth
- Wean

**Male & Female Put for mating**

**LOAEL BPA =**
50 mg/kg bw/d

**Tolerable Daily Intake =**
50 ug/kg bw/d
Perinatal + Peripubertal Exposure of CD-1 Mice to BPA

**Treatment:**
- Control (50% DMSO)
- 0.25µg BPA/kg/d
- 2.5µg BPA/kg/d
- 25µg BPA/kg/d
- 250µg BPA/kg/d

**LOAEL BPA:**
- 50 mg/kg bw/d

**Tolerable Daily Intake:**
- 50 ug/kg bw/d

Animals weaned at PND 20
Male Data from Part I: Body Composition Measurements from MRI Suggest Non-Monotonic Dose Response in Males Exposed Perinatally to BPA \((N=12-14/gp)\)
As expected, the fat to lean ratio of males exposed perinatally to BPA showed a similar relationship with the highest Fat:Lean ratio being present in the 25µg males and the lowest present in the control animals.
Body Composition and BW Data from Females Exposed to BPA *Perinatally and Peripubertally* Suggest a Non-Monotonic Dose Response.
Early into data collection for Part 1, The Perinatal and the Perinatal +Peribubertal exposure windows yielded similar results.

However as the animals got older, it became apparent that the 2nd peripubertal exposure exacerbated adverse effects in the females, and not in the males.
Comparison of data from insulin tolerance tests in P and P + P Females at 40 weeks 
weeks of age revealed that P+P females 
were less responsive to insulin and clearly 
more affected than the P females
Data from serum assays suggested more pronounced metabolic issues with P+P females. One example of this is the increase in Serum triglycerides in P+P females that is above the levels measured in P females.

(Mean values for 25µg exposure= 40mg/dl in P females and 70mg/dl in P+P females.)
Analysis of liver extracts revealed a significant increase in Triglycerides in the liver of P+P Females exposed to 25 and 250 µg BPA. The increases observed in P females were not significant.
Is the peripubertal period another critical window for effects of BPA exposure in females?

Methylation analysis in 12 year old girls suggested BPA may affect human health through specific epigenomic modification of genes in relevant pathways throughout pre-adolescent development. (Kim et al. Environmental Health 2013, 12:33)
Summary of Data from Part 1:

1. Differences in Body Weight and Body Composition were influenced by BPA dose and sex.

2. Although the P and P+P groups appeared similar at first, as the animals aged, body composition and metabolic parameters of the P+P females were more affected (including altered glucose homeostasis, increased triglycerides in serum and liver.)

3. Internal BPA doses measured at the CDC in collaboration with Dr. Antonio Calafat, suggest that the doses of BPA were environmentally relevant.
Internal BPA dose was measured at the CDC in collaboration with Dr Antonio Calafat.

For animals exposed to the highest doses of BPA (25 or 250ug BPA/kg BW/day), detectable levels of total BPA were noted in mothers and fetuses on gestational day 18. Total BPA levels were also measurable in P+P animals on PND 32 which was at the end of the peripubertal exposure period. There were no levels of Free BPA above the level of assay detection (0.3 ng/ml) in any of our animals.

These data suggest that levels of BPA exposure in our animals is within the range noted in humans and is environmentally relevant.
Part 2: Examined effects of early BPA exposure combined with a high fat diet (45%) in adulthood

A. Doses: 0, 2.5, 25 ug BPA/kg BW/day (n=16 litters/dose)

B. Exposure Window: Perinatal (Day 8 of Gestation thru Lactation day 16).

C. Diet: Chow (Harlan Teklad 2018) or 45% High Fat Diet (Research Diets)

D. Males and Females
Part 2:

Littermates were matched for body weight and body composition at 8 weeks of age.

One male and one female from each litter was placed on 45% HFD (Research Diets) and one on Chow for the remainder of the study.

![Diagram showing male and female mice with arrows pointing to Chow and HFD diets.]
Measurement of **Body Weight** and **Fat Mass** over time reveals that perinatally exposed males eating a **CHOW DIET** from 8 weeks of age have increased body weight and increased fat mass relative to control males on CHOW DIET.
Measurement of Body Weight and Fat Mass over time reveals that perinatally exposed males eating a High Fat Diet from 8 weeks of age have increased body weight and increased fat mass relative to control males on HFD.
In a review of the **Body Weight** and **Fat Mass** data from the **males** there was no clear evidence of **synergy between BPA exposure and HFD**.

Some evidence of **synergy between BPA and HFD** was observed in **females exposed to 25µg BPA**.
Food Efficiency Data: Grams of Food Consumed /gram of BW gain

Food Efficiency of 18 Week Old P Males, 10 Weeks on Diet

Food Efficiency (g of food/g of BW gain/10 weeks)

- Control
- 2.5 ug
- 25 ug

Chow
High Fat Diet
Assessment of Metabolic Parameters of Male Mice Exposed Perinatally to BPA on Chow or HFD.
Evidence of Altered Glucose Homeostasis in Chow and HFD Males

HFD appears to exacerbate the effects of BPA on glucose homeostasis
Perinatal exposure to BPA resulted in increased fasting insulin levels in chow males when measured at 13-15 weeks of age and when measured at 22 weeks of age.

Glucose levels did not differ across groups.
Perinatal BPA Exposure Promotes Hyperinsulinemia and Hyperglycemia in Mice on a High Fat Diet.

Insulin levels were significantly elevated in BPA exposed animals on a HFD relative to control animals at 13-15 weeks of age. Glucose levels were maintained at a similar level across treatment Groups.

At 22 weeks BPA exposed animals on HFD were hyperglycemic. Insulin levels were similar across treatment groups.
% Hyperglycemic animals at 23 weeks (15 weeks on HFD diet) >250 mg/dl and > 350 mg/dl glucose.
Evidence of Increased Inflammation in *Adipose Tissue* of BPA Exposed Mice and Exacerbation by High Fat Diet
Crown-Like Structures (CLS) in Adipose Tissue are arrangements of Macrophages around the remnant triglyceride of dead adipocytes---often associated with adipose tissue inflammation and systemic insulin resistance.

MAC-2 Immunostaining in Gonadal Fat of CD-1 Male Mice on a Chow Diet
BPA Exposure in General Results in an Increase in Adipose Tissue CLS

Many More Crown Like Structures are seen in BPA exposed Mice on HFD relative to their brothers on Chow diet
BPA Perinatal Exposure Interacts with a HFD to Increase \textit{Adipose Tissue} Macrophage and Inflammatory Cytokine Expression
What about liver???
Measurements of liver extracts revealed significantly increased levels of Cholesterol and NEFA in BPA exposed males relative to control males.

Triglyceride levels were also increased in exposed males although the increase was not statistically significant.
Lipogenic and Adipogenic Gene expression was increased in the livers of chow males exposed to BPA relative to control males.

Also significantly increased in the exposed chow males were expression of genes for cholesterol synthesis.
In Summary, Early BPA Exposure:

- **Increased BW and Fat Mass** – altered body composition
- **Altered parameters of glucose homeostasis**
  - Hyperinsulinemia
  - Insulin resistance
  - HFD exacerbated effects, led to severe hyperglycemia, eventually culminating in a loss of beta cell mass
- **Increased evidence of adipose tissue inflammation**
  - Effect was exacerbated by HFD
In Summary, Early BPA Exposure (cont):

- **Altered Liver Parameters**
  Increased fat accumulation, steatosis
  Increased expression of adipogenic and lipogenic genes
  HFD animals also showed an elevation in inflammatory gene expression

- **Effects of BPA on BW, body composition and elements of Metabolic Disease differ with BPA dose, sex, exposure window, and diet.**
Since the start of this work a growing body of data (but not all data) from studies in mice and rats corroborates our findings that early BPA exposure may act as an obesogen, alter body composition, glucose homeostasis, and may affect liver function.

Important questions remain.
What about Humans?

Some (but not all) epidemiological studies report associations of BPA levels with various parameters of metabolic disease in adults. There is also correlational data in children and teens (Trasande).

To date, there are few reports of a correlation between prenatal/neonatal BPA levels and increased body weights in children—too early?

A recent study reports association of early BPA exposure with increased leptin levels in 9 year old boys (Volberg et al 2013). Valvi et al (2013) reported a correlation between maternal BPA exposure and BMI at age 4.

Other data will follow from current ongoing research in human populations. Accurately documenting BPA exposure will be challenging.
What are the mechanisms through which BPA is working to affect these endpoints?
Early BPA Exposure (Prenatal, Perinatal, Perinatal and Peripubertal)

- Direct Effects on Adipose Tissue, Pancreas, Liver
- Effects on other Endocrine Components (Thyroid, Adrenals)?
- Developing circuits for food intake, metabolism?
- Estrogenic actions?
- Epigenetic changes
- Altered Metabolic Pathways?
- Microbiome?

Widespread Effects: Involvement of Multiple Mechanisms?
Can **Metabolomics** be used to identify metabolic pathways altered by perinatal BPA?

- Metabolomics provides a picture of the small molecule content of a cell or an organism in a particular condition.

- 1H-NMR spectroscopy followed by multivariate statistical analysis is a powerful tool to discriminate the experimental groups according to the dose of exposure.

- Mass spectrometry in tandem with liquid chromatography (LCMS) allows access to a large range of metabolites
[NMR] Metabolic Fingerprints

Overview

Urine, plasma, tissues, cells...

Eventual goal: metabolic networks, (High Resolution MS)

Identification of biomarkers

Glucose
Taurine
Glycine
Glutamate
Lactate
Lysine
Leucine

NMR
Bruker Avance Spectrometer 600 MHz,
CRYO-PROBE
Metabolic Fingerprints (intergroup discrimination?)

Multivariate statistics

NMR Spectra

NMR spectrum bucketing
Data sheet

Daniel Zalko
[NMR] Metabolic Fingerprints

CD1 male mice, PND 21, LIVER : 4-group comparison

\[ R^2 Y = 48.3\%, \quad Q^2 = 0.421 \]

From - Cabaton et al EHP 2013, 121(5):586-93.
CD1 male mice, PND 21, LIVER: 2-group comparisons

From Cabaton et al EHP 2013, 121(5):586-93.
Results of studies thus far:

- Extracts of newborns, and of livers, brains and serum from males and females at PND21, 3 months and 6 months of age were subjected to 1H-NMR spectroscopy. Data was analyzed using Partial Least Square Discriminant Analysis (PLS-DA).

- Perinatal exposure was found to alter the metabolome of exposed offspring at all ages studied, from PND2 to 6 months of age.

- Metabolic Fingerprinting allowed discrimination of experimental groups according to exposure level.

- Variations in glucose, pyruvate, amino acids, and neurotransmitters (including GABA and Glutamate) were among the changes identified suggesting that perinatal BPA exposure can influence global metabolism including alterations in energy metabolism and neurotransmitters.
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