Obesity III
Obesogen Assays:
Limitations, Strengths, and New Directions

WAYNE STATE UNIVERSITY

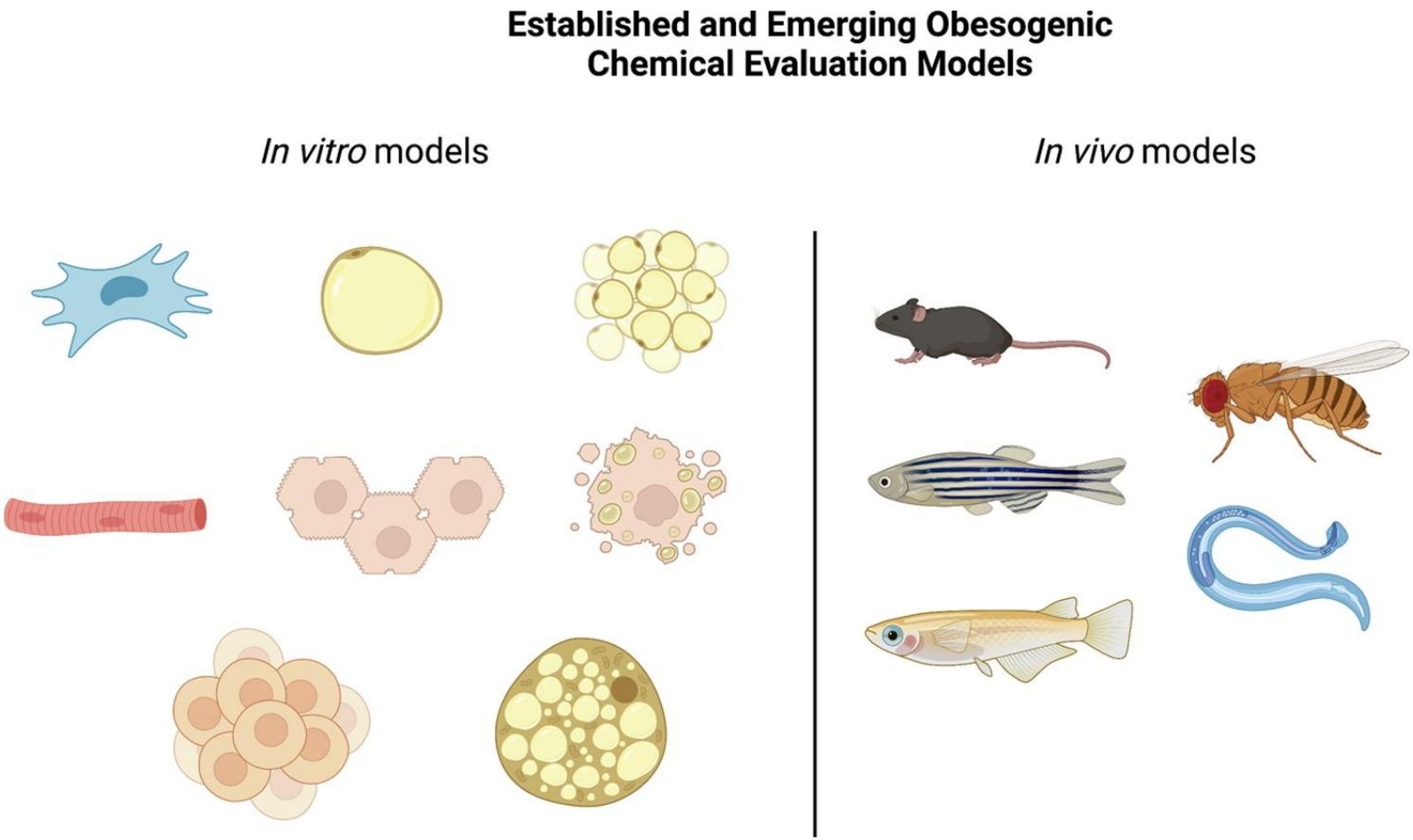
Center for Urban Responses to Environmental Stressors

Chris Kassotis, PhD
Assistant Professor
Wayne State University

@cdkassotis

CHE EDC Strategies Partnership Webinar Series
May 19, 2022

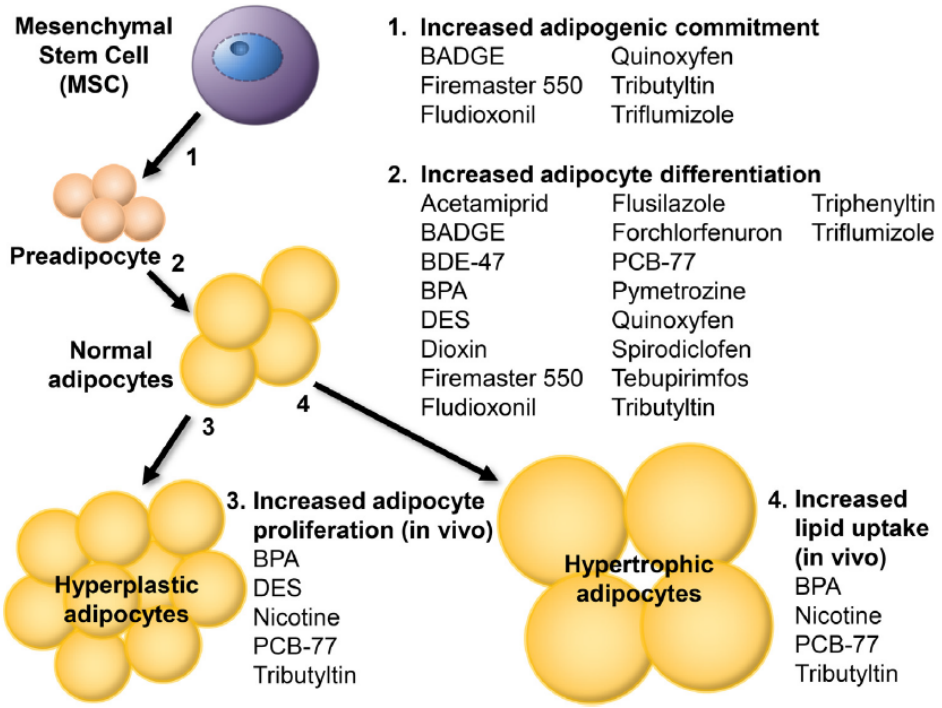
Overview of Obesogen Models



Use of Models in Metabolic Health Toxicity Assessments

- Evaluating causal toxicity of chemicals relies on a combination of *in vitro* and *in vivo* models
  - Need for HTP, reliable *in vitro* models to accurately screen for and prioritize higher order testing
  - Need for reliable *in vivo* models that are cost-effective, have high translation to human health, and are well-validated
- Traditionally, MDC research has relied heavily on rodent-based cell and animal models (3T3-L1)
  - Models used have been broadening over time
    - Increasing use of MSCs and human cell models
    - Increasing use of fish models, particularly zebrafish
    - Increasing use of non-traditional models such as fruit fly, c. elegans

Potential Mechanisms of Metabolic Dysfunction



Heindel et al. 2017, *Repro Tox*

- Numerous potential mechanisms of metabolic disruption:
  - Adipose lineage commitment from MSCs
  - Adipocyte differentiation from precursor committed 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

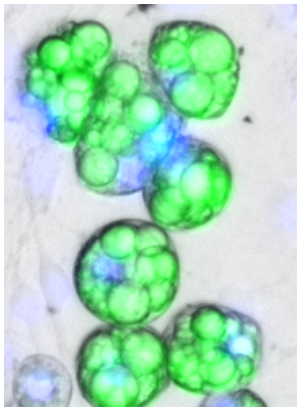
Adipocyte Differentiation Process

Adipocyte commitment

Adipocyte differentiation

Mesenchymal stem cell

Other pathways:
Myoblasts
Osteoblasts
Chondroblasts



Resemble mature white adipocyte

Adipoblast

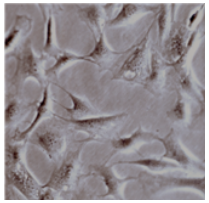
Pref-1

Preadipose cell

A2COL6/pOb24
LPLF A transport
PPARδ

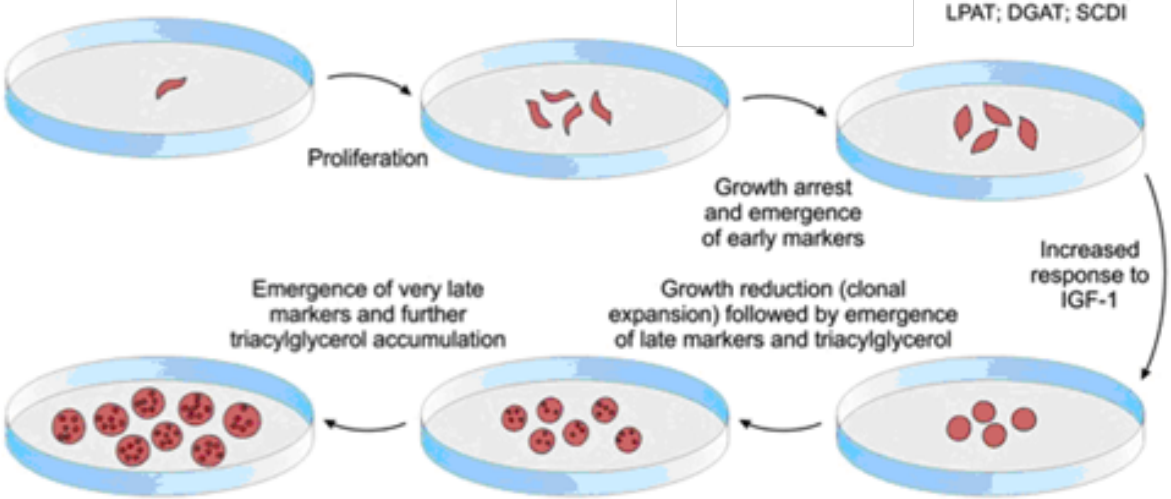
Preadipose cell

C/EBP β/δ
IGF-1
PRAR γ2

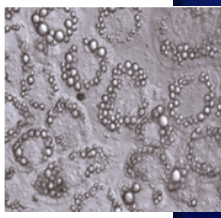


Immature adipose cell

C/EBP γ; GLUT4; β2 AR; β3 AR; ACC FAS; ME; ATP-citrate lyase; GPDH; HSL; ALBP; perilipin; apoE; low Km PDE; GPAT; LPAT; DGAT; SCDI



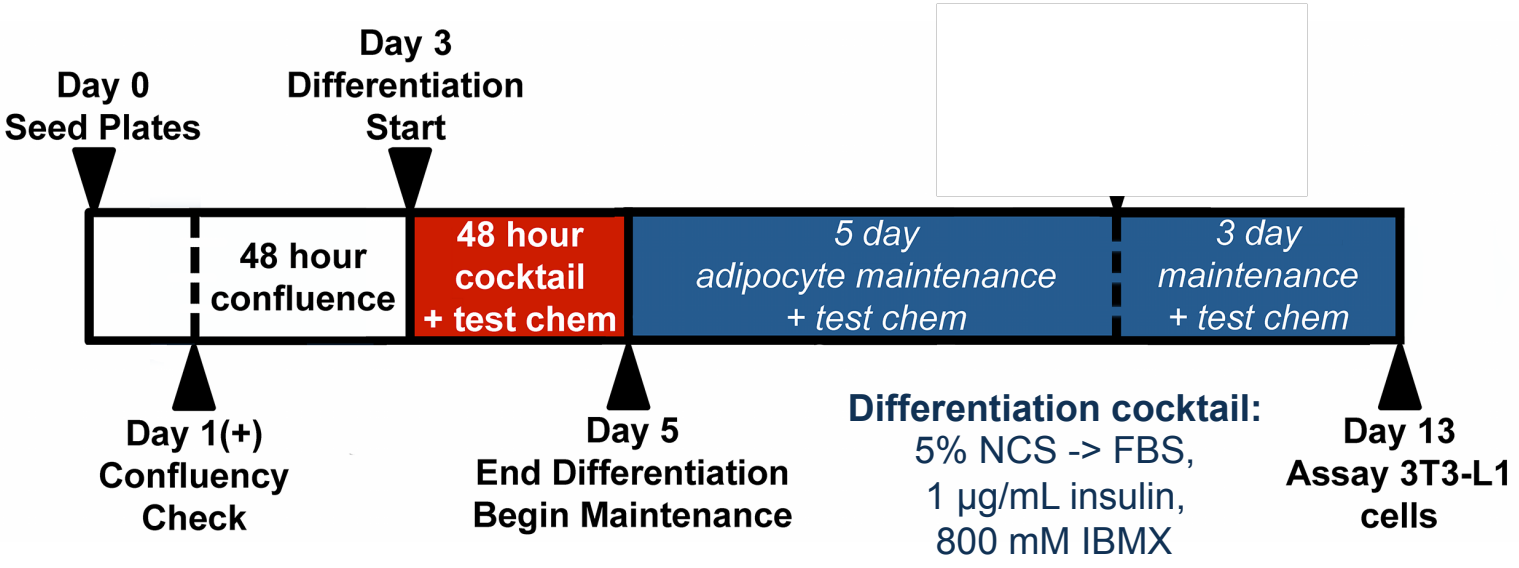
Resemble brown/developing white adipose cell



Nagy et al. 2011, *Mol Med*

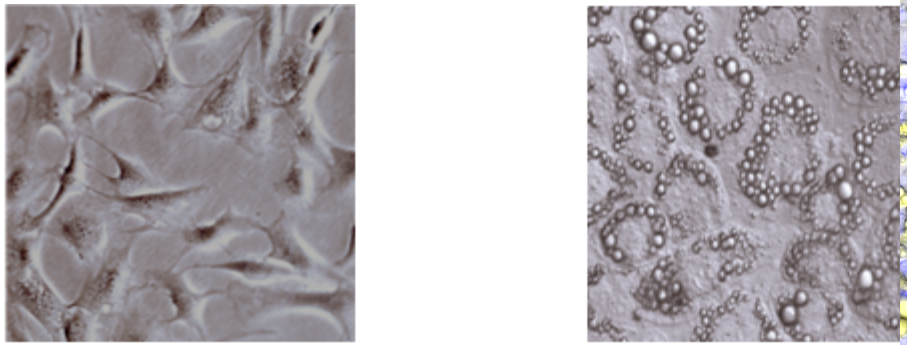
3T3-L1 Pre-adipocyte Adipogenesis Assay

- 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 has proven to be a reliable *in vitro* model for screening metabolic disruption *in vivo*.



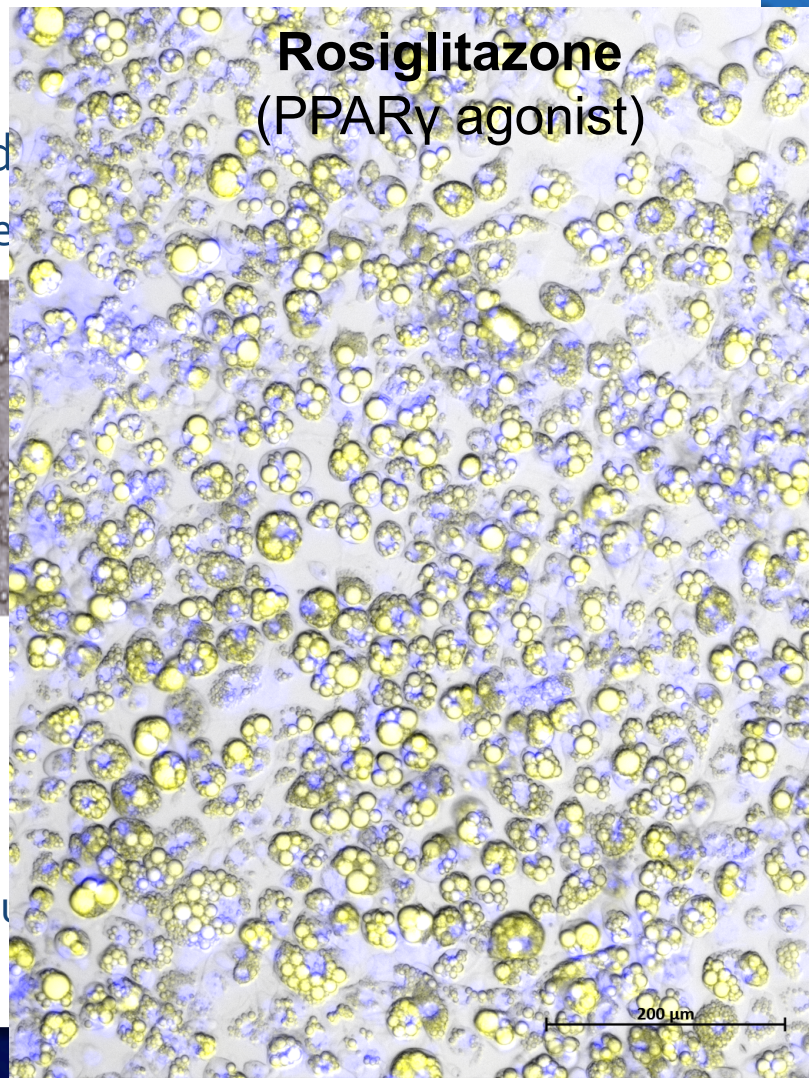
Adipogenesis Assay Measures

- Triglyceride accumulation
  - AdipoRed - hydrophilic fluorescent d
    - Partitions into lipid droplets in the ce



(A) (B)

- Cell proliferation/cytotoxicity
  - NucBlue DNA dye (Hoechst 33342)
    - Partitions into nuclei and fluoresces u DNA



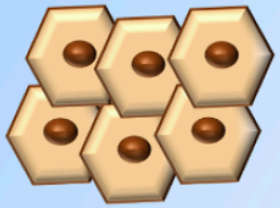
Diversity of Cell Model Utilization

In vitro models for metabolic disruption screening

ADIPOCYTES

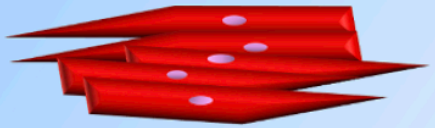
- **Preadipocytes**
  - Proliferation
  - Adipogenesis
- **Mesenchymal Stem cells**
  - Characterization of Obesogens
- **Spheroid adipocyte model**
  - Adipose physiology

LIVER



- **HepaRG**
  - Aldolase B, CYP2E1, CYB3A4
  - CYP1A1, CYP1A2, CYP1B1
- **Primary Human Hepatocyte**
  - Drug metabolism
  - Liver enzyme induction
- **3D cell culture**
  - NASH model

MUSCLE

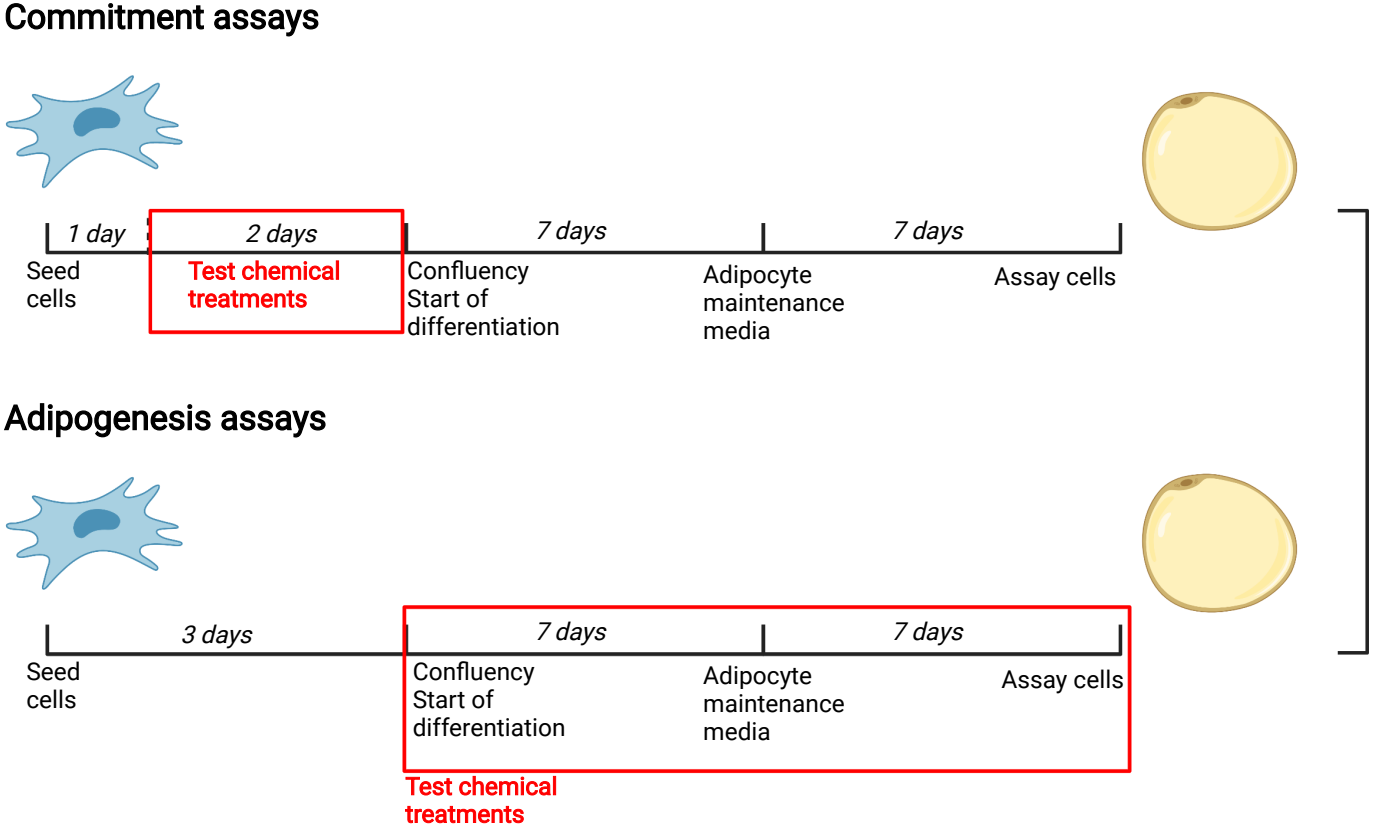


- **C2C12**
  - Insulin signaling
  - Mitochondrial function
  - Protein synthesis
- **L6**
  - IR expression
  - Glucose uptake
  - Insulin Resistance
- **Primary Myoblast**
  - Calcium signaling
  - Resting potential

Growing Reliance on MSCs, Human Cells

- Increasing commercial availability of human MSCs, human pre-adipocytes
  - Less reliance on donors, self-isolation
  - Can source from males/females, lean/obese, diabetic/non, subcutaneous/visceral, etc.
- Ability to examine the interplay of commitment across cell lineages (e.g., bone and adipose, muscle, etc.)
- Increasing utility of liver cell assays to examine TAFLD/NAFLD phenotypes, primary human hepatocytes (despite limitations) have increasing use in drug metabolism
- Limited but increasing evaluation of myogenic differentiation and ability of MDCs to suppress signaling/development

Examination of Adipocyte Lineage Commitment as More Novel Endpoint



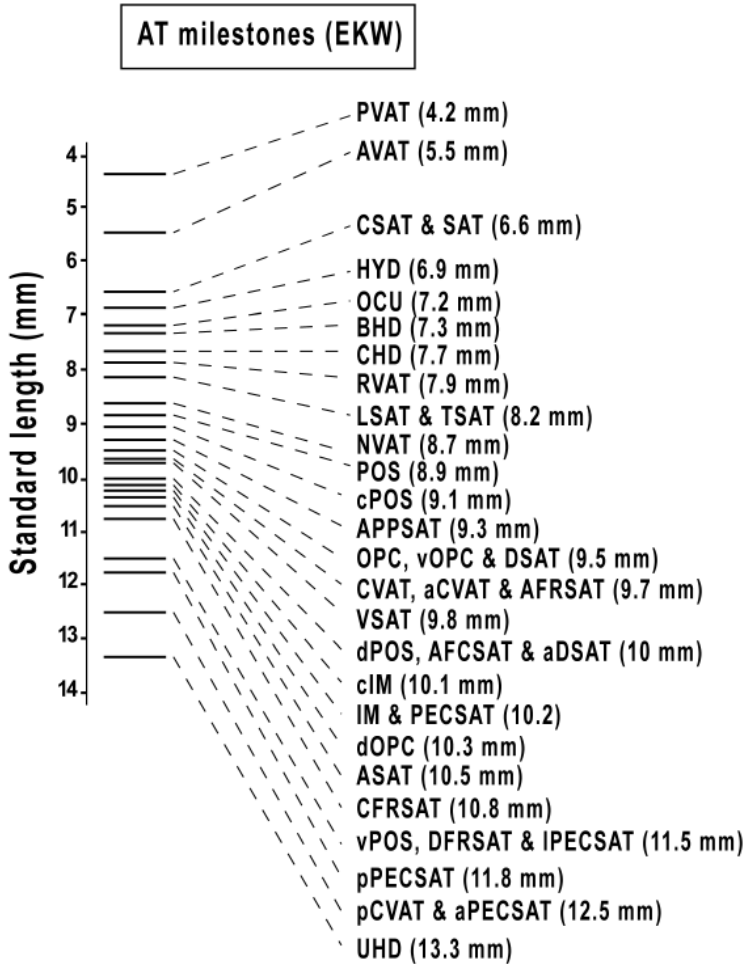
Increasing Diversity of *in vivo Models*

In vivo models for metabolic disruption screening

Models	Advantages	Disadvantages
Zebrafish 	• Rapid development, ease of breeding, transparency • Metabolic organs/tissues are physiologically and anatomically similar to humans • High-resolution fluorescent imaging of total body adipose • Ease of molecular manipulation, wealth of transgenic models	• Moderate flexibility • Moderate translational value
Medaka 	• Genetic sex determination like humans • Rapid development, ease of breeding, transparency • Metabolic organs/tissues are physiologically and anatomically similar to humans • Ease of molecular manipulation, small genome size, high diversity	• Moderate flexibility • Moderate translational value • Less characterization of adipose relative to zebrafish
C. elegans 	• Compounds that modulate fat storage and obesity can be identified • Food intake and energy expenditure can be measured easily • Less regulations governing invertebrate animal use	• Lower conservation of biological pathways with mammals • Lack of specific organs and circulatory systems
D. melanogastor 	• Small size, short generation time, inexpensive and easy breeding • Several discrete organs perform the same as humans • Less regulations governing invertebrate animal use	• Anatomically different from mammals • Lower conservation of many relevant biological pathways with mammals
Rodents 	• Well described model with clear translation to human outcomes • Periconception, pregnancy, parental and offspring, short- and long-term, multi- and trans-generational outcomes can be assessed • Diverse housing materials readily available • Well-characterized & customizable feed options readily available • Inbred and outbred models available to dissect role of genes, environment, and their interactions	• Time consuming and expensive compared to above alternatives, but less so with larger animal models (e.g. porcine, bovine, ovine, and non-human primates). • Ethical issues; regulatory push to reduce use of mammalian vertebrate animal models

Zebrafish as a Metabolic Model

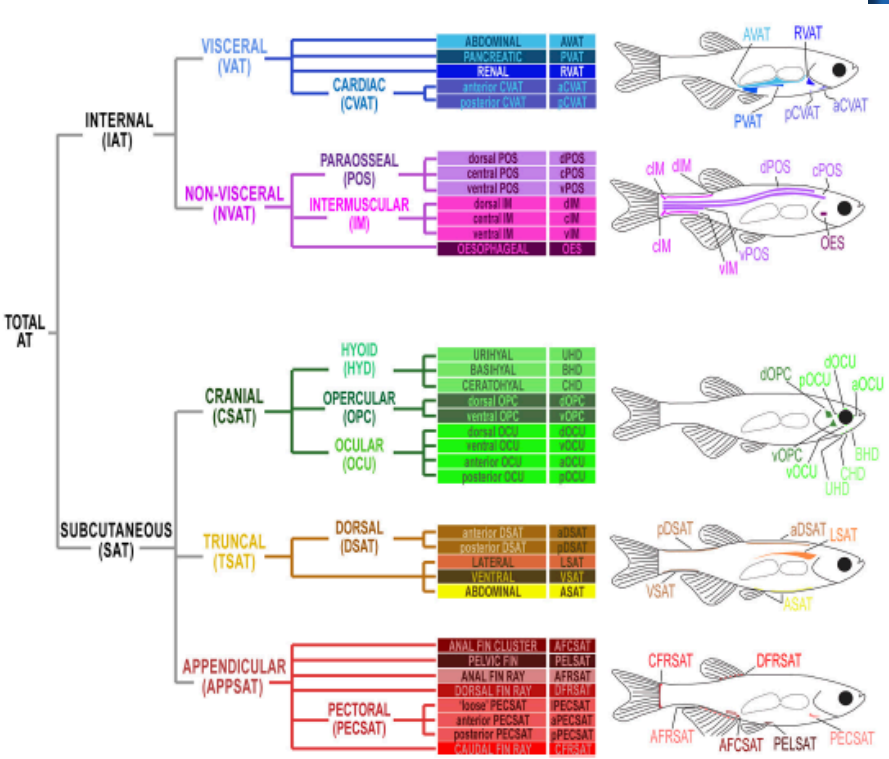
- Measurable adipose/adipocytes appear as early as ~9-12 days of development in zebrafish, originally in the pancreatic and abdominal visceral depots
- 34 anatomically/physiologically/ molecularly distinct adipose depots throughout the body of the fish
  - Clear developmental timeline
- Fish adipose tissue contains a heterogeneous cell population, including adipocyte progenitor cells – similar to mammals
  - Depots separated into subcutaneous, visceral, intramuscular adipose tissues, with characteristics similar to humans
  - Zebrafish do not have brown adipocyte tissue



Minchin and Rawls, 2017 *Dis Mod & Mech*

Zebrafish as a Metabolic Model

- Molecular mechanisms underlying adipocyte and lipid depot development are highly conserved across vertebrates:
  - Genes associated with adipocyte differentiation (*fabp*, *pparg*, *cebpa*), lipolysis (*lipoprotein lipase*), and endocrine function (*leptin*, *adiponectin*, *adipsin*)
  - Energy storage functions and morphology of adipose tissue
- Adipose depots respond to high fat challenge and food withdrawal as you would anticipate
  - organisms utilize the adipose in times of food stress and pack on extra adipose with HFD
- Imaging of whole-animal adipose in mammals is limited, technically challenging, and generally low resolution, whereas imaging in fish is high-resolution and relatively easy



Minchin and Rawls, 2017 *Dis Mod & Mech*

C. Elegans as a Metabolic Model

- Small nematode living in temperature soil environments
- Main regulatory pathways of energy homeostasis shared with mammals
  - Lower conservation of many of these pathways and lack of specific organs
    - Lack PPARg, though express orthologs to PPARa and PPARd
    - No identifiable homolog for leptin
    - No cells specifically designed for lipid storage (i.e., adipocytes)
    - Store lipids primarily in intestinal and epidermal skin-like cells
- BPS, methylmercury, and other MDCs increase lipid deposition, similar to other *in vivo* MDC models

Drosophila melanogaster as a Metabolic Model

- Fruit fly model organism prized for rapid life cycle, large number of offspring per generation, and simpler genetics relative to most vertebrates
- Despite anatomical differences, lots of functional overlap with humans
  - Fat body covers many of the metabolic health functions of both liver and adipose tissue
- DEHP, methylmercury, PFAS have been described to increase weight/adiposity and/or signaling

The Future of Obesogen / MDC Screening

- Need for new/improved standardized testing methods to ID chemicals that disrupt metabolic health through diverse mechanisms.
  - Multiple large-scale EU efforts designed to help address this gap
- Improved understanding and validation of alternative / emerging *in vitro* and *in vivo* obesogen models.
  - Increasing use of other animal models, human *in vitro* models, and 3D/spheroid cell culture techniques
- Predictive modeling may offer some improved utility in screening the myriad chemicals in commerce for MDC properties
  - Need for reliable, reproducible ToxCast and other input data
  - Need for robust understanding of MIEs, contributory mechanisms

Questions?