3c. Research Design and Methods

Introduction. We found that TBT exposure increased fat storage in F3 and F4 animals derived from F0 dams treated with TBT throughout pregnancy,[20] or pregnancy and lactation.[19] We inferred that TBT exposure lead to changes in epigenetic regulation of gene expression in tissues critical for energy balance, such as fat[19] and liver. Based on our published and preliminary studies, *we hypothesize that TBT exposure modifies the epigenome across multiple generations, sensitizing animals to weight gain and that this "thrifty phenotype" is revealed or exacerbated by increased dietary fat*. The proposed experiments will identify what functional and epigenomic changes are elicited by TBT exposure leading to altered gene expression before and after exposure to TWD in mature adipocytes and in hepatocytes. This will reveal key epigenomic changes mediating how the interactions between TBT and diet modify energy balance in TBT-exposed mice.

- ***Aim 1*** *will study interactions between TBT and TWD to alter lipid accumulation in WAT and liver, identifying transcriptomal and epigenomic changes linked to the thrifty phenotype.*
- ***Aim 2*** *will investigate the effects of TBT and TWD on fat mobilization during dietary restriction, identifying key transcriptomal changes and revealing whether WAT is healthy and responds to normal signals.*

It is unclear how obesogens such as TBT interact with Western-style diets to alter fat storage and mobilization. Despite considerable efforts, the US population has been unable to reduce its obesity. We showed that *in utero* exposure to the obesogen TBT increases lipid storage and fat depot size,[37] biases MSC fate toward the adipogenic lineage[61], causes fatty liver, and that these phenotypes can be transmitted through subsequent generations.[19-20] Understanding how and to what extent TWD interacts with obesogen exposure to modulate obesity and the response to fasting may help to understand and prevent the continuing rise in obesity trends.[38]

Specific Aim 1: How does TBT exposure exacerbate the effects of TWD?

Rationale and hypothesis: We showed that exposure of pregnant F0 mice to TBT biases MSCs toward the adipogenic lineage, increases white adipocyte size and number, and produces fatty liver in F1-F3 generations.[20] We extended these results in a new transgenerational experiment, showing that F4 males descended from F0 TBT-treated females gained substantially more WAT after HFD challenge and remained fatter than controls after returning to LFD.[19] Epigenomic analyses revealed that altered expression of important metabolic genes in the TBT group was linked with blocks with iso-directional changes in DNA methylation (isoDMBs) comprising coordinated hyper- or hypo-methylation of DNA.[19] Importantly, isoDMBs are up to several millions of base pairs in size involving multiple genes,[19] suggesting large-scale epigenetic alterations reminiscent of the topologically associated domains (TADs). On the other hand, our attempts to identify TBT exposure-induced, differentially methylated regions corresponding to promoters or enhancers have been largely unsuccessful.[19] *We hypothesize that exposing pregnant F0 dams to TBT throughout pregnancy elicits epigenomic changes in metabolic tissues such as WAT and liver that are revealed or exacerbated by TWD challenge*. We will analyze metabolic responses of control and TBT groups to TWD challenge to identify transcriptomal and epigenomic changes in WAT and hepatocytes that sensitize these animals to increased adiposity (**Fig 1A,B**).[19] Since the obesity phenotype is much stronger in F3/F4[19-20] than in F1 animals,[20-21, 61] we must analyze all 4 generations.

Methods:

Experiments:

Expected results:

Potential pitfalls and alternative approaches to overcome pitfalls: