2. Specific Aims

Obesity adds more than $200 billion annually to health care costs in the US. 39.8% of US adults are now obese with the burden falling disproportionately on blacks (46.8%), Hispanics (47%) and on women (41.1% overall), particularly black (54.8%) and Hispanic (50.6%) women.[38] 18.5% of US youth are obese by the age of 19.[38] The current clinical paradigm for obesity is one of energy intake versus energy expenditure,[39] with clinical management of obesity focused on diet and exercise.[25] While diet and exercise are clearly important factors in obesity, particularly the energy dense Western dietary pattern, they do not fully account for the obesity epidemic. US adults were 2.3 kg/m^2 higher in BMI in 2006 than in 1988, even at comparable caloric intake and energy expenditure and despite leisure time physical activity increasing by 47% in males and 120% in females over the same period.[13] 83% of patients who lost large amounts of weight soon regained it, implying the existence of a pro-obesity metabolic programming that is superimposed on the effects of diet and exercise.[27, 63]

Emerging research links developmental exposure to endocrine-disrupting chemicals (EDCs) to the obesity epidemic.[44] These "obesogens" promote adiposity by increasing fat cell number, size, or by interfering with hormonal regulation of metabolism, appetite, and satiety.[51] People with the highest levels of perfluorinated EDCs in their blood had lower resting metabolic rates and regained lost weight faster after dieting than those with the lowest levels.[70] We showed that exposure of pregnant F0 mouse dams to the EDC tributyltin (TBT) at environmentally relevant (nM) levels biased the mesenchymal stem cell (MSC) compartment towards the adipose lineage in F1 offspring.[61] We then found that treatment of pregnant F0 dams with TBT increased lipid accumulation in liver, testis, and adipose depots (even on a normal chow diet) in F1, F2, and F3 generations.[20] Lastly, male F4 descendants of F0 TBT-treated dams exhibited a large increase in fat storage compared with controls when fed with a diet containing modestly increased fat content; this fat persisted after return to normal chow and the exposed animals were resistant to fasting-induced fat loss, mimicking the human situation.[19]

Two key questions in the field are how transgenerational programming of obesity is carried through the germline across generations and what changes have been elicited in somatic tissues in directly and transgenerationally exposed animals that promote obesity when dietary fat is increased. The first is studied in our ongoing mPI award 2 R01 ES023316; the current proposal addresses the second. We found that fat in F4 male descendants of TBT treated dams showed persistent DNA hypomethylation in regions encompassing important metabolic genes such as the *Lep* gene, increased leptin mRNA expression, elevated plasma leptin levels, and that these hypomethylated regions in fat were less accessible in sperm chromatin of F3/F4 males. We proposed that these animals exhibited a transgenerational "thrifty phenotype" caused by altered chromatin structure and accessibility.[19] *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*. We propose the following specific aims to test this hypothesis:

Aim 1: How does TBT exposure exacerbate the effects of "Total Western Diet" leading to weight gain? Human exposure to obesogenic chemicals and Western-style diets is historically recent but now encompasses multiple generations. Little is known about how interactions between diet and obesogen exposures can impact obesity. Combining our TBT-induced transgenerational, high fat diet-initiated mouse obesity model and a chemically-defined, human relevant, "Total Western Diet" for rodents,[45] **Aim 1** dissects molecular mechanisms underlying transgenerational obesity mediated by interactions between diet and obesogen exposure. We will use a multi-omic approach, including Hi-C sequencing, ATAC-seq, RNA-seq, ChIP-seq, and DNA methylation analysis to link changes in the epigenome and transcriptome induced by TBT exposure in white adipose tissue (WAT) and liver with the effects on obesity elicited by the Total Western Diet (TWD) in F1-F4 generations.

Aim 2: How does TBT exposure make animals resistant to fat loss? Fetal exposure to EDCs can impact how blood glucose status is interpreted by the endocrine system, predisposing animals to obesity and diabetes.[44] Ancestral TBT exposure inhibited fasting-induced fat mobilization in F1-F4 male mice; this effect was exacerbated by increased dietary fat.[19] **Aim 2** addresses whether WAT of the affected animals aberrantly responds to metabolic signals, is inflamed, whether the animals are leptin resistant systematically or in a cell type-specific manner, and what transcriptomal signatures underlie these changes. This aim is very relevant for the exploration of new preventive and potentially therapeutic approaches to combat the obesity epidemic.

Impact: Mounting evidence implicating environmental factors in obesity,[17, 44, 53, 66] underscores the importance of determining how obesogens may interact with Western diets to promote obesity. The proposed research will reveal which molecular mechanisms may underlie the effects of obesogens and how a Western dietary pattern interacts with obesogen exposure to predispose toward fat gain and promote the transgenerational programming of obesity. This will greatly inform the thinking of clinicians and the public in understanding individual susceptibility to obesity and how best it may be treated and prevented in individuals.