Fundamental Principles of Conducting Mouse Obesity Studies

(from Lale Ozcan and adapted in part from Hasty & Gutierrez, Endocrinology 155:12-14, 2013)

- **Background strain:** even slight genetic drift could impact the results obtained. Thus, the use of littermate controls is ideal.

  For example, if we have *Camk2g*$^{fl/fl}$ mice and we want to have macrophage specific CaMKII deficient mice, the proper breeding pairs should be: *Camk2g*$^{fl/fl}$ crossed with *Camk2g*$^{fl/fl}$ *LysMCre*$^{+/-}$. That way all the (male) mice can be used in experiments: 50% experimental cre-fl/fl, 50% control mice fl/fl.

- **Mouse fighting:** For mice bred in our lab, place mice in the same cage after weaning. Do not place older mice in same cage if they were not in then same cage after weaning. After receiving DIO mice from outside vendors, split the 5 mice into one cage of 3 and one cage of 2. Observe the mice closely, because sometimes the vendors place males that were originally housed in different cages in the same cage. If you do observe fighting marks on mice, you should rid the cage of the mouse who has no scars, *i.e.*, the dominant mouse.

- **Gut microbiota:** co-house experimental and control mice, and use outside-vendor mice after a set number of days (at least 7-10) of housing at CUMC.

- **Weight:** matching the experimental and control groups for body weight at the beginning of the study is critical. Even small differences at baseline can become exaggerated over time for reasons related to inherent susceptibility to weight gain. Also, sometimes mice in certain cages gain less weight than mice in other cages, and this needs to be noted and considered when interpreting the data. In addition, when working with *ob/ob* mice, match for the blood glucose before the experiment.

- **Single abnormal event:** a single abnormal event, *e.g.*, cage flooding, can stunt the growth of mice and influence their metabolic phenotype for their entire lives. Also, make sure that cages do not run out of food--even fasting the mice for 5-6 hours changes a lot of metabolic events.

- **Number of mice per cage:** mice housed individually may not thrive as well as those that are group housed, but with too many mice per cage, several of the mice may not do as well. Three mice per cage may be ideal.

- **Litter #:** mice from first litters of a dam may have different metabolic characteristics than those born to multiparous dams

- **Food/diet studies:** change DIO diet twice each week to avoid spoilage, and make sure that the metal top is also changed—this is very important when working with diets that have high fat content. The control and experimental animals should receive the diet at the same time and should be sacrificed at the same time. Animal facility temperature and humidity changes according to seasons, and this may alter mouse feeding habits.

- **FBG/insulin assay:** fast mice for 5-6 hrs before drawing blood. At the start of a fast, make sure the entire cage is changed--do not simply get rid of the food, because there are food particles inside the bedding and on the metal top. Quickly measure the blood glucose of all the mice and then continue with the blood collection for the insulin assay. This practice will help avoid falsely elevated blood glucose levels resulting from the “stress”.

- **Mouse handling:** any mouse manipulation that causes stress before a particular assay, such as during phlebotomy, could influence the data