Introduction

- Endometrial cancer (EC) occurs in the lining of the uterus and is the most common reproductive cancer in the U.S.
- It is expected that there will be 66,570 new EC cases and 12,940 EC-related deaths in the next year in the U.S.
- The rise of obesity and carcinogenesis demonstrated about its effects on the immune environment and its contribution to EC development.
- While the long-term effects of high fat diet (HFD) on reproductive hormones and estrous cycle have been well-documented in mice, there is still not much known about its effects on the immune environment and its contribution to EC development.
- Prior studies suggest that obesity triggers the release of pro-inflammatory cytokines (IL-6, IL-8, IL-10, TGF-B). Chronic inflammation has been associated with decreased anti-tumor response and an increase in tumor cell proliferation and survival.
- While the long-term effects of high fat diet (HFD) on reproductive hormones and estrous cycle have been well-documented in mice, there is still not much known about its effects on the immune environment and its contribution to EC development.

Methods

- Starting from 8 weeks of age, C57BL/6J mice were fed a low-fat diet (10% LFD, n=20) or a high-fat diet (60% HFD, n=20) for 12 months.
- Initial assessment started with 6 LFD mice and 5 HFD mice.
- Hematoxylin and eosin staining was performed to assess estrous cycling by veterinary pathologist (n=11).
- Uterine 4 µm cross-sections were mounted into slides and used for immunohistochemistry (IHC). Immune cell markers (NK1.1, F4/80) and functional markers (PD1, Arginase-1) were investigated.
- Using the Aperio system by Leica Biosystems, immune infiltrates were quantified, and lumen epithelial cell height was measured.
- The Shapiro-Wilk test was used to test normality. To test for differences, an unpaired 2-sample t-test or a Mann-Whitney test was used.

Results

At 12 months, consumption of 60% HFD significantly increased weight of C57BL/6J mice compared to 10% LFD.

Examination of estrous cycling revealed possible dysregulation in HFD mice.

Fig. 1. Mean mass of mice increased 1.79-fold in HFD group.

Fig. 2. Stages of normal mouse estrous cycle and duration of each stage in hours.

Epithelial cell height, which is predominantly regulated by estrogen/progesterone balance, increased in the HFD group.

Fig. 4. Average measurements of lumen epithelial cell height significantly increased 1.49-fold in the HFD group.

Fig. 5. Infiltration of NK1.1+ cells was not significantly different between diet groups.

Fig. 6. Macrophage infiltration was slightly elevated in the HFD group; however, it was not significantly different between the groups.

Fig. 7. PD-1+ cells were not significantly different between diet groups.

Fig. 8. Arg-1+ cells significantly increased 2.86-fold in the HFD group, as compared to the LFD group.

Conclusions

- At 12 months old, mice in the HFD group had a 1.79-fold increase in weight compared to the LFD treatment group.
- Long-term HFD consumption (12 months) resulted in possible dysregulation of the estrous cycle, as evident by the disproportionate number of mice found in metestrous cycle (4/5).
- Lumen epithelial height increased 1.49-fold in the HFD group, which could be associated with an increase in estrogen levels.
- Evaluation of NK cell infiltration showed no significant difference between diet groups.
- While not statistically significant, there was a trend of increasing macrophage infiltration in the HFD group.
- Functional assessment showed a 2.86-fold increase in Arg-1 producing cells. Arg-1 is known to inhibit T-cell and NK cell function.
- Our study supports the link between obesity and carcinogenesis demonstrated by the increase of Arg-1 that leads to immune cell dysfunction that could contribute to EC carcinogenesis.

Future Implications

- Future studies will expand immune characterization to all mice in the study.
- Based on these results, we will prioritize characterization of Arg-1-producing cells (e.g., neutrophils, macrophages) and immune signaling that induces chemotaxis of these cells.
- We will also investigate possible drivers of inflammation and carcinogenesis in obesity such as IL-6, IL-10, and IL-1α.

References