Obesity-associated immune environment changes in the endometrium as potential contributors to cancer development

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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 EC in the U.S. parallels the rise of obesity prevalence, and nearly 20% of women diagnosed with EC are obese or overweight.
- 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.

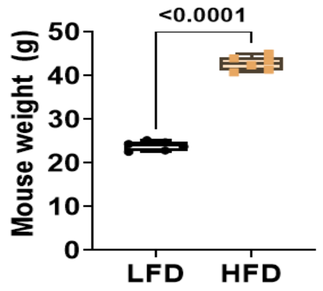

Fig. 1. Mean mass of mice increased 1.79-fold in HFD group.

Examination of estrous cycling revealed possible dysregulation in HFD mice.

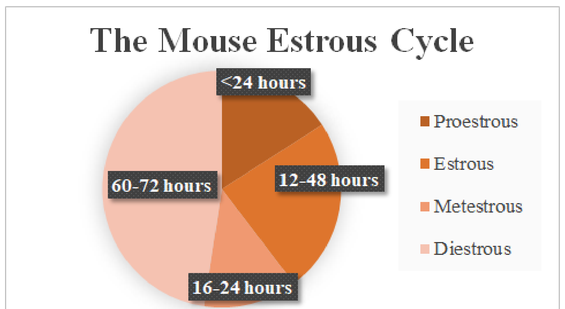

Fig. 2. Stages of normal mouse estrous cycle and duration of each stage in hours.

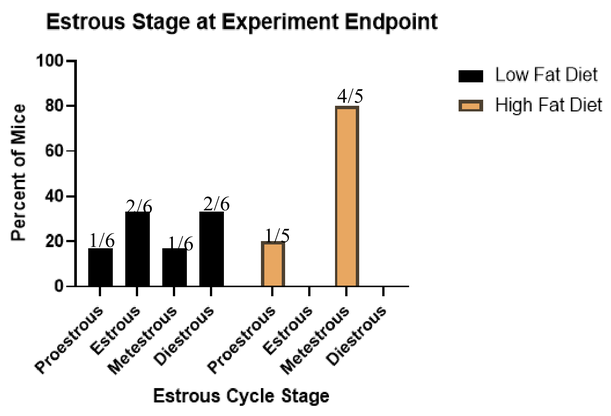

Fig. 3. Percent mice in each estrous stage, within each treatment group, evaluated at end-of-life.

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

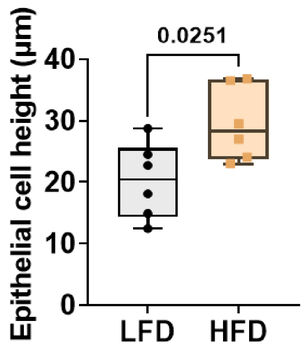

Fig. 4. Average measurements of lumen epithelial cell height significantly increased 1.49-fold in the HFD group.

Innate immune cell characterization in mouse uterine samples:

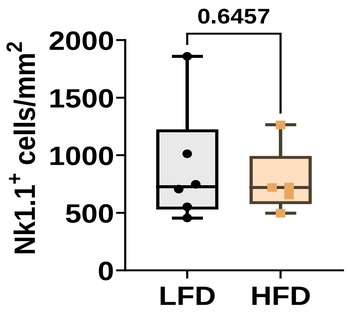

Fig. 5. Infiltration of NK1.1+ cells was not significantly different between diet groups.

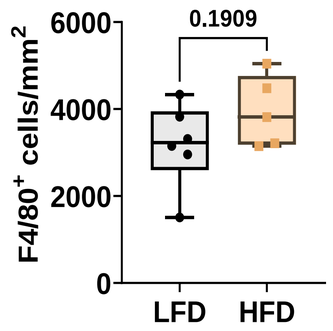

Fig. 6. Macrophage infiltration was slightly elevated in the HFD group; however, it was not significantly different between the groups .

Functional characterization of immune cells in mouse uterine samples:

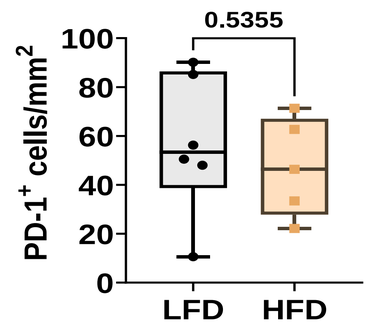

Fig. 7. PD-1+ cells were not significantly different between diet groups.

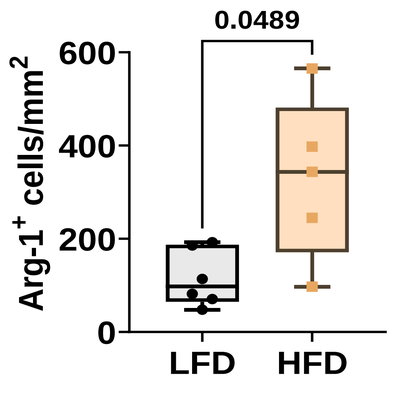

Fig. 8. Arg-1+ cells significantly increased 2.86-fold in the HFD group, as compared to the LFD group.

Conclusion

- 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

1) Onstad et al, J of Clinical Oncology 2016; 34(35): 4225-4229.
2) Vanderstraeten et al, J of Reproductive Immunology 2014; 109(2015): 7-16.
3) Pardall, Semin Oncol. 2015 August; 42(4): 523–538.
4) Saltiel and Olefsky, J of Clinical Investigation; 127(1): 1-4.
5) Med Sci Monit. 2016; 22: 1601-1608.