Combined Effects of Alcohol and Phthalate Exposure on Mammary Gland Development and Breast Cancer

Zelda Galdenzi[1,2], Josephine Park[2,3], Alison O'Neil[2,3], Jerrica Breindel[4], Laverne Melón[1,2]
Department of Biology[1], Department of Neuroscience & Behavior[2], Department of Chemistry[3] Wesleyan University, Department of Biomedical Sciences Quinnipiac University[4]

INTRODUCTION

Alcohol functions as an endocrine disruptor, and has been shown to increase the risk of breast cancer. This risk may be augmented by involuntary exposure to environmental endocrine disrupting chemicals, such as phthalates. Phthalates are a group of endocrine disrupting chemicals used as plasticizers, and are found in a wide range of products including personal-care products, cosmetics and food packaging. Exposure to these compounds during development is associated with precocious puberty and early mammary gland development, both risk factors for breast cancer (1).

We anticipate phthalates such as di(2- ethylhexyl) phthalate (DEHP) may potentiate the effects of the alcohol, due to both of their endocrine disrupting properties. We predict that phthalates cause an increase in progesterone receptors while alcohol acutely increases progesterone synthesis, thus together alter cell signaling that can lead to the increase rates of cancer.

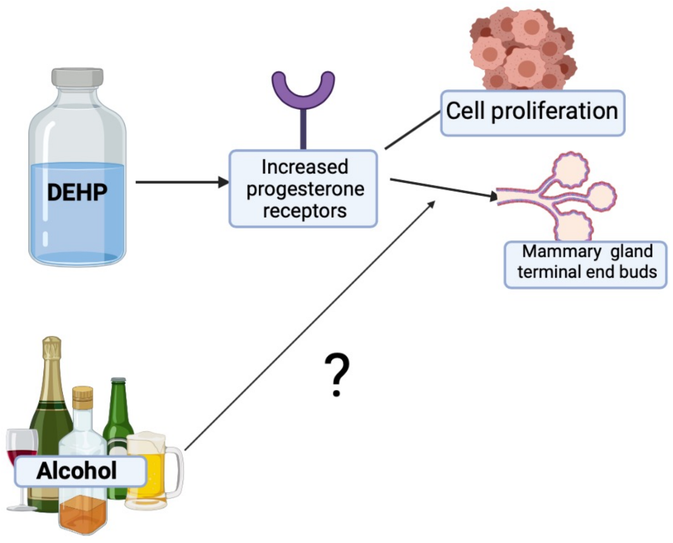
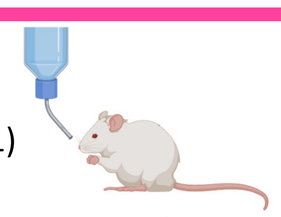


Figure 1. Plasticizers such as DEHP enhance progesterone receptors, and subsequently impact cell proliferation and terminal end bud formation. Alcohol shows the same effects, yet the mechanisms are unknown.

METHODS

In vivo mouse model:



- 7 female C57BL/6J mice used
- Mice were given limited access to ethanol for two weeks (P28-P41) where they voluntarily drank an average of 3.87 - 9.19 g/kg daily
- Whole mounts of mice mammary glands were imaged and analyzed using ImageJ Fiji
  - Terminal End Buds (TEBs) were counted manually
  - Area of gland, branching area, percentage of branching, and number of branch points were calculated
- RNA and protein were extracted from glands using Trizol reagent, and subsequently analyzed

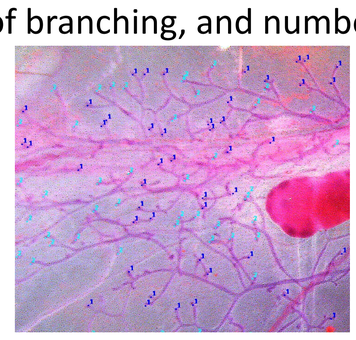
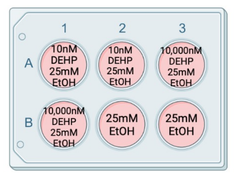
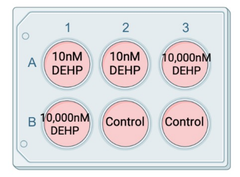


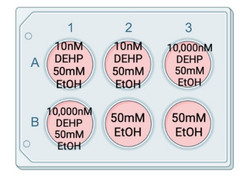
Figure 2. Whole Mount mammary gland image showing labelled terminal end buds

In vitro cell culture model:

- T-47D cells (human breast cancer cell line) used
- Cells exposed to phthalate (DEHP) and ethanol
  - 25mM and 50mM ethanol concentrations
  - 10nm and 10,000nM DEHP concentrations
  - All combinations of above and control tested
- RNA and protein Trizol extracted from cells and analyzed
  - Will look at expression progesterone, progesterone receptors, estrogen, and estrogen receptors, as well as changes in proliferation







RESULTS

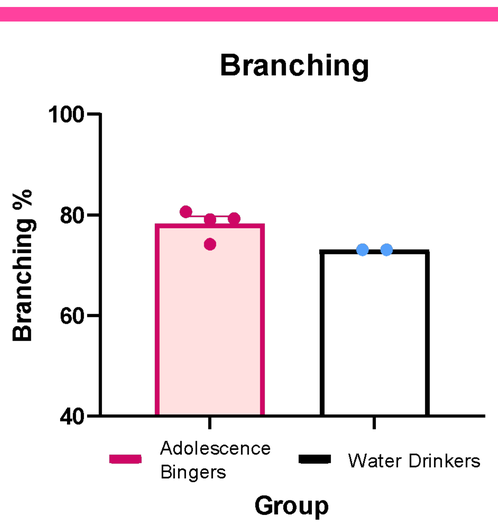


Figure 3. Average percentage of mammary gland branching. No significant differences between the ethanol and water groups were found.

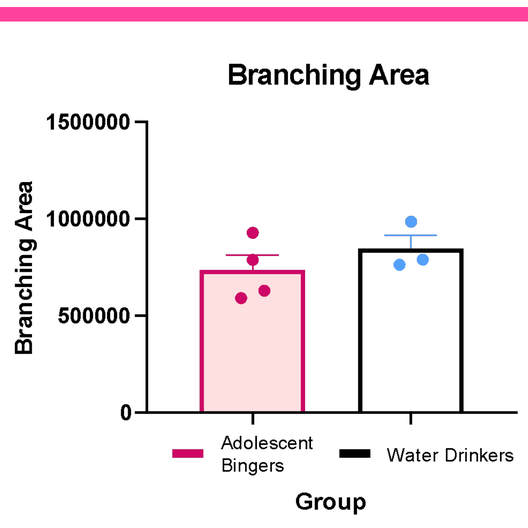


Figure 4. Average branching area in pixels. Marginal differences in branching area were found, with an average of 735,279 pixels for ethanol drinkers and 847,271.3 pixels for water drinkers.

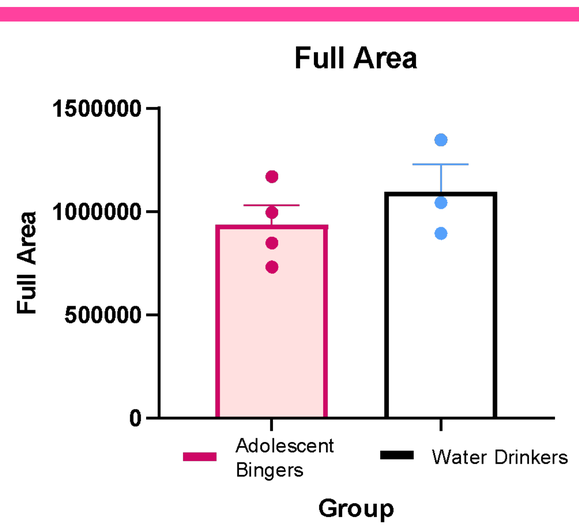


Figure 5. Average total area of gland in pixels. Marginal differences in full area were found, with an average of 937,423.5 pixels for ethanol drinkers and 1,095,864 pixels for water drinkers.

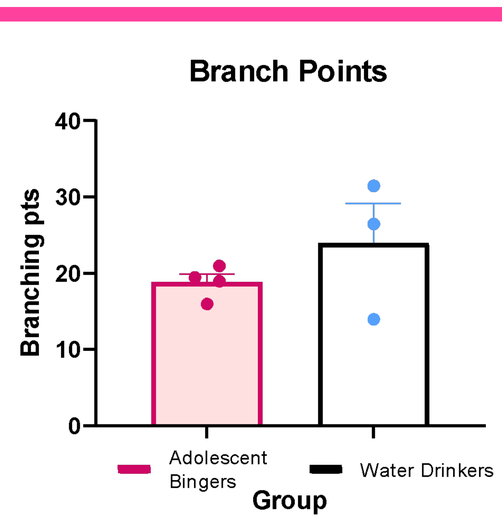


Figure 6. Average number of branch points per gland. Marginal differences in branch points were found, with an average of 18.875 branch points for ethanol drinkers and 24 points for water drinkers.

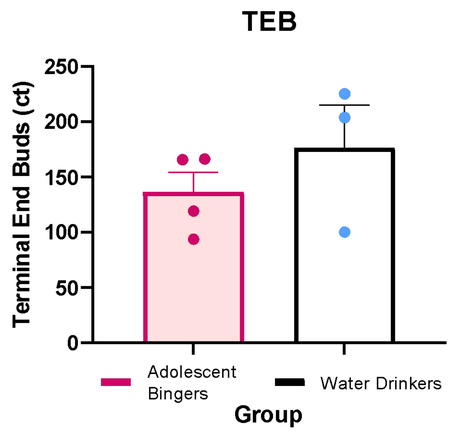


Figure 7. Average number of terminal end buds in the mammary glands of mice exposed to alcohol versus only water. There was no statistically significant effect of alcohol on TEB number, however there was an average of 40 less than the glands of the non-drinking counterparts.

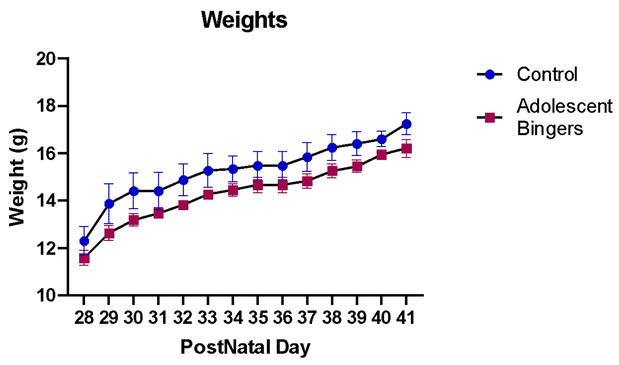


Figure 8. Average mouse weight in grams over the two weeks for drinking. Weight significantly increased with age, but no significant difference between ethanol and water groups.

Figure 9. Average ethanol intake per day per mouse over the two weeks of exposure.

CONCLUSIONS

- Considerable changes in terminal end bud formation for those drinking ethanol compared to those with only water, with less TEBs in the binge drinkers
- Marginal reductions in mammary gland branching and area for binge drinkers
- To follow up these results, we moved to cell culture as a preliminary experiment to test if phthalates potentiate the effects of alcohol
- We observed slight changes in mammary gland branching regulated by progesterone, TEB number regulated by estrogen, and the maturity of the TEBs regulated by both estrogen and progesterone, which indicates both signaling pathways are affected
  - Further work will involve comparing the effects of ethanol and phthalates on progesterone and estrogen signaling in these samples.
- Future experiments include oral exposure of multiple doses of DEHP and DEHP/ethanol combination using mouse model similar to alcohol model used, as well as additional cell culture looking at proliferation, different timing of exposure, and other additional factors.

References:

1. Golestanzadeh M , Riahi R , Kelishadi R . Association of phthalate exposure with precocious and delayed pubertal timing in girls and boys: a systematic review and meta-analysis. Environ Sci Process Impacts. 2020 Apr 29;22(4):873-894. doi: 10.1039/c9em00512a. PMID: 32091510.