

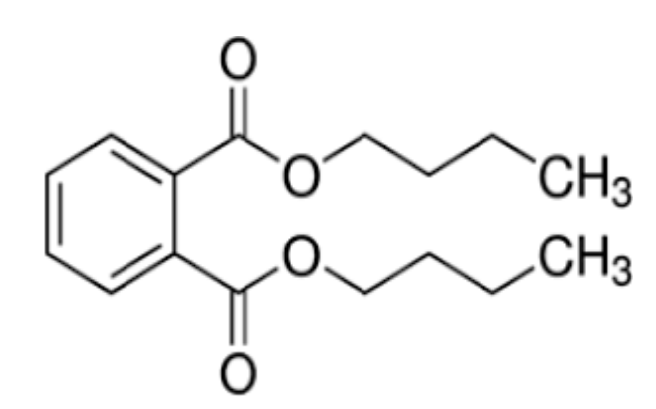
# Exposure to a Phthalate Mixture Alters the Fatty Acid Profile in Mouse Ovarian Antral Follicles

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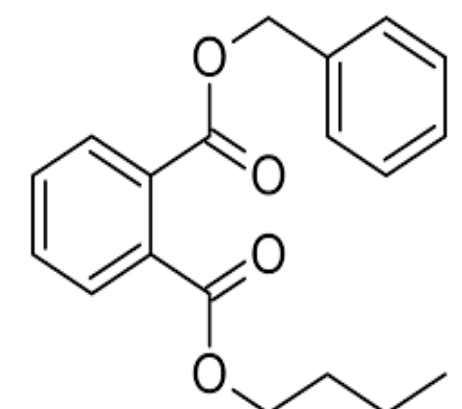
Pharmacology & Toxicology<sup>1</sup>, School of Nutritional Sciences and Wellness<sup>2</sup>, Environmental Health Transformative Research Undergraduate Experience<sup>3</sup>, School of Animal & Comparative Biomedical Sciences<sup>4</sup>, Clinical and Translational Sciences<sup>5</sup>, University of Arizona Cancer Center<sup>6</sup>, BIO5 Institute<sup>7</sup>, Southwest Environmental Health Sciences Center, The University of Arizona<sup>8</sup>

## Introduction

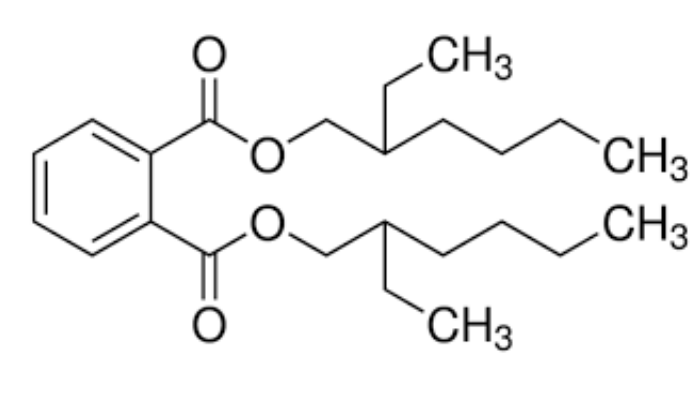
- 1 in 5 women are infertile in United States (CDC)
- Endocrine Disruptors, natural or synthetic chemicals that mimic or interfere with the endocrine system, are a threat to female fertility
- Phthalates, a group of endocrine disrupting chemicals, are associated with several negative reproductive outcomes in females
- Dibutyl phthalate (DBP), benzyl butyl phthalate (BBP), and di-2-ethylhexyl phthalate (DEHP) have an extensive presence in the environment and consumer products including personal care products, medical equipment, and the coating of pharmaceuticals



DBP

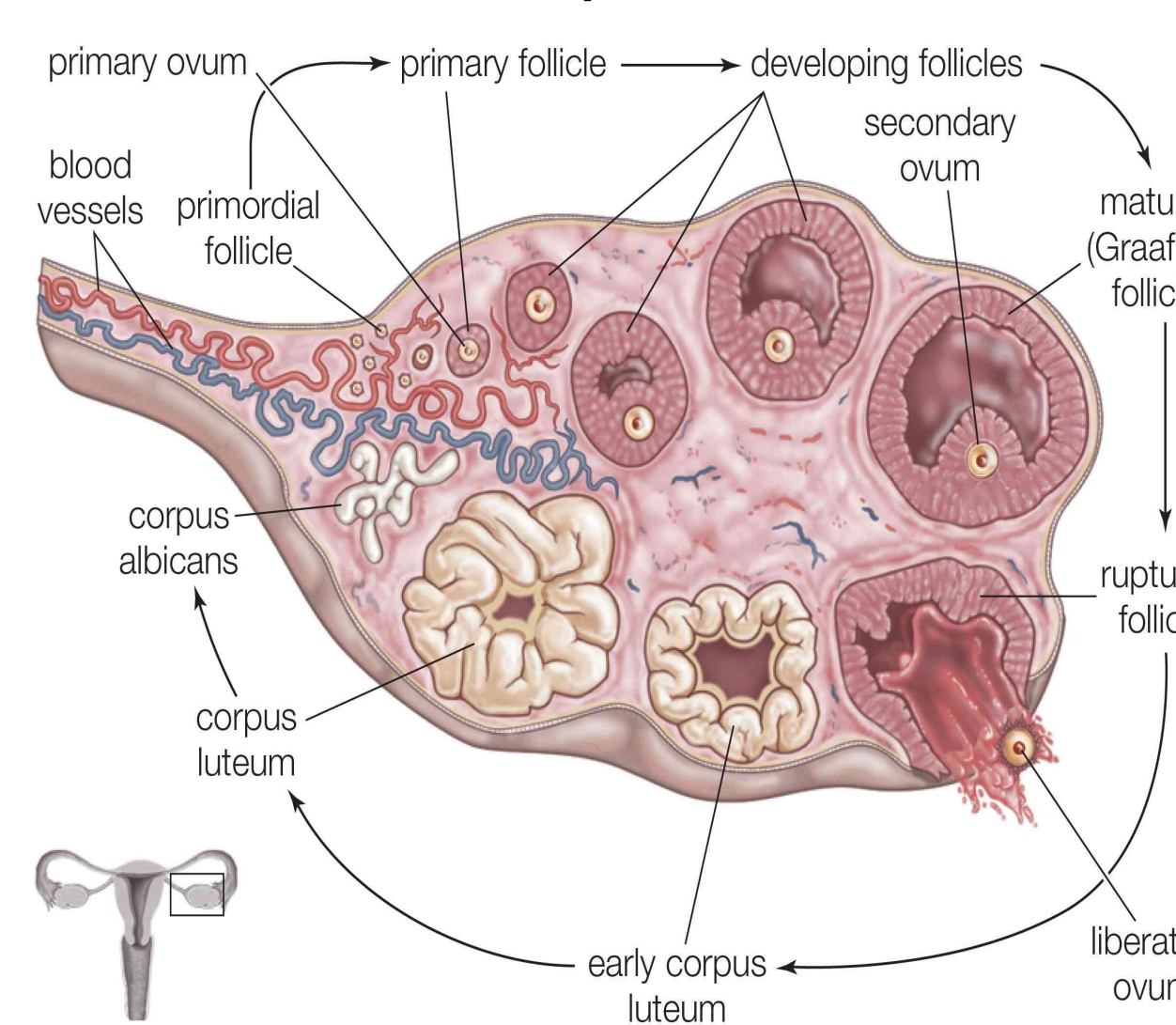


BBP



DEHP

- Folliculogenesis is the maturation of an immature primordial follicle to a mature antral follicle
- Antral follicles house the oocyte and play a crucial role in ovarian steroidogenesis and ovulation
- DBP, BBP, and DEHP have been reported to inhibit in vitro antral follicle growth and ovulation rates in mice. They have also been associated with reduced antral follicle counts in women
- Little is known on the effects of a human relevant mixture of DBP, BBP, and DEHP on the female reproductive system & specifically antral follicles in vivo



## Objective

The objective of this study was to identify the effects of a human-relevant mixture of DBP, BBP, and DEHP on ovarian antral follicle fatty acid profiles.

## Methods



Saponification → Derivatization → Methyl esterification → GC-FID → Analysis of Total FA

## Rationale

A Phthalate Mixture Exerts Differences on Antral Follicle Protein Profiles

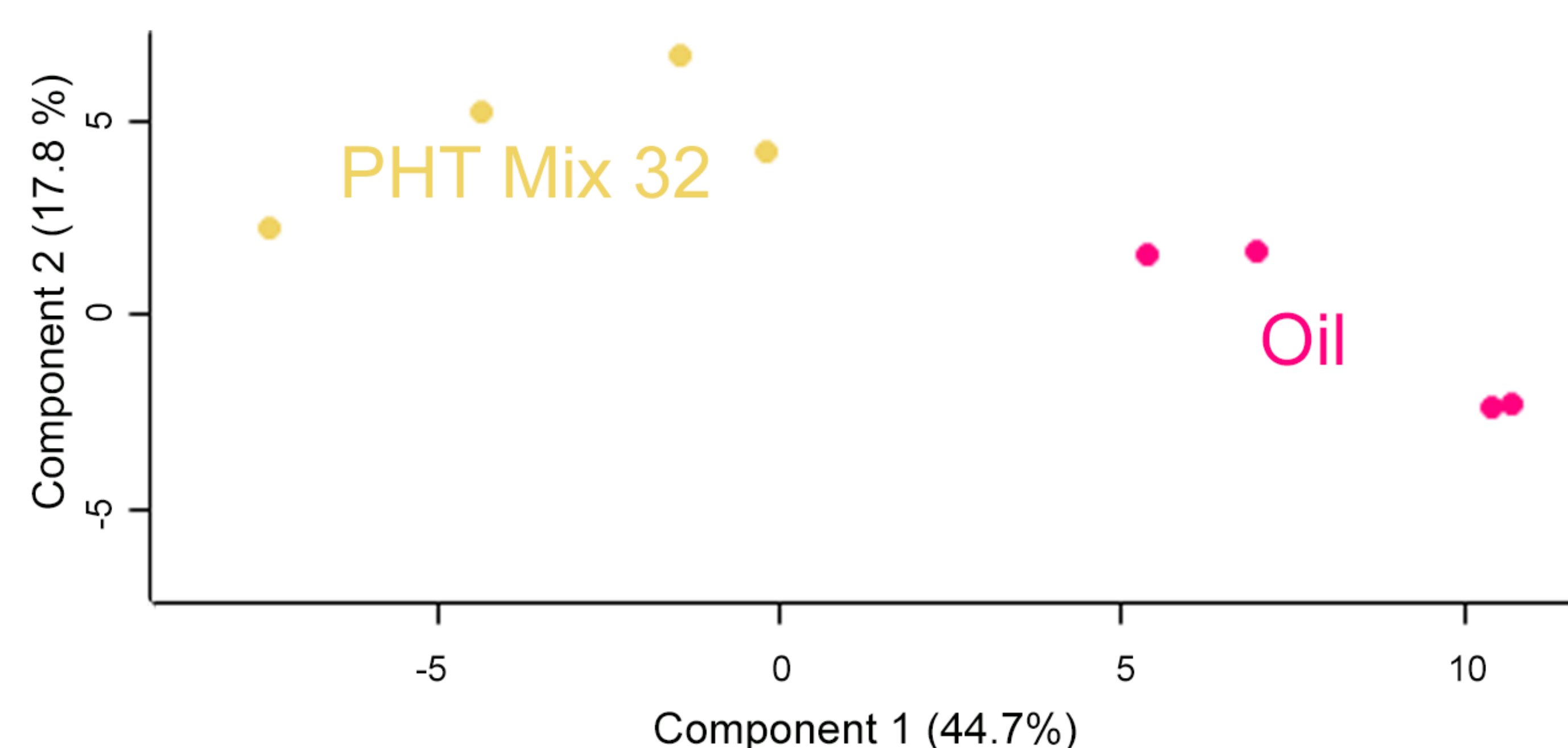


Figure 1: Principal component analysis: control (Oil) differentiated from the phthalate mixture (PHT 32) along component 1 with 44.7% variance and along component 2 with 17.8% variance.

## Rationale

Exposure to a Human Relevant Mixture of DBP, BBP, and DEHP Downregulated Proteins in Lipid and Fatty Acid Processing

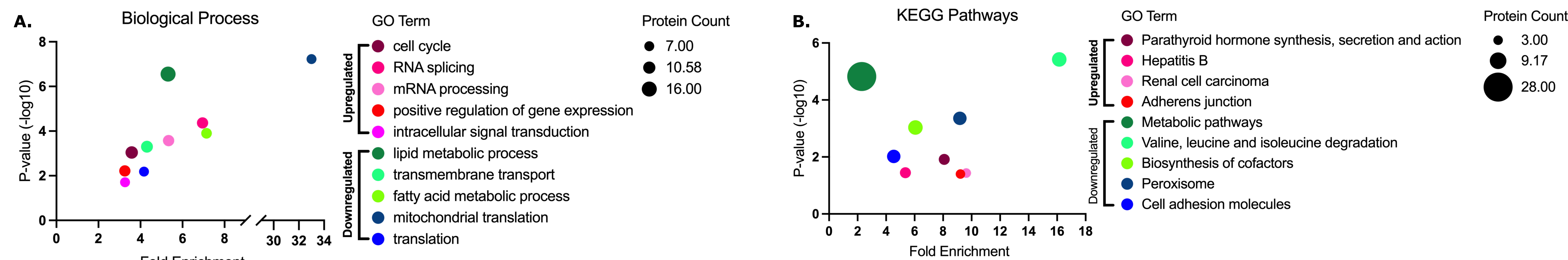


Figure 2: Proteins found to have significant changes in abundance were subjected to gene ontology analysis using DAVID. Classification of significantly overrepresented biological processes associated with upregulated (pink) and downregulated (blue) proteins in Oil vs PHT 32 (A). Classification of significantly overrepresented KEGG pathways associated with upregulated (pink) and downregulated (blue) proteins in Oil vs PHT 32 (B). Fold enrichment vs significance along with the number of proteins identified for each biological process is graphically displayed.

## Results

Exposure to a Human Relevant Mixture of DBP, BBP, and DEHP Altered the Composition of Fatty Acids in Antral Follicles

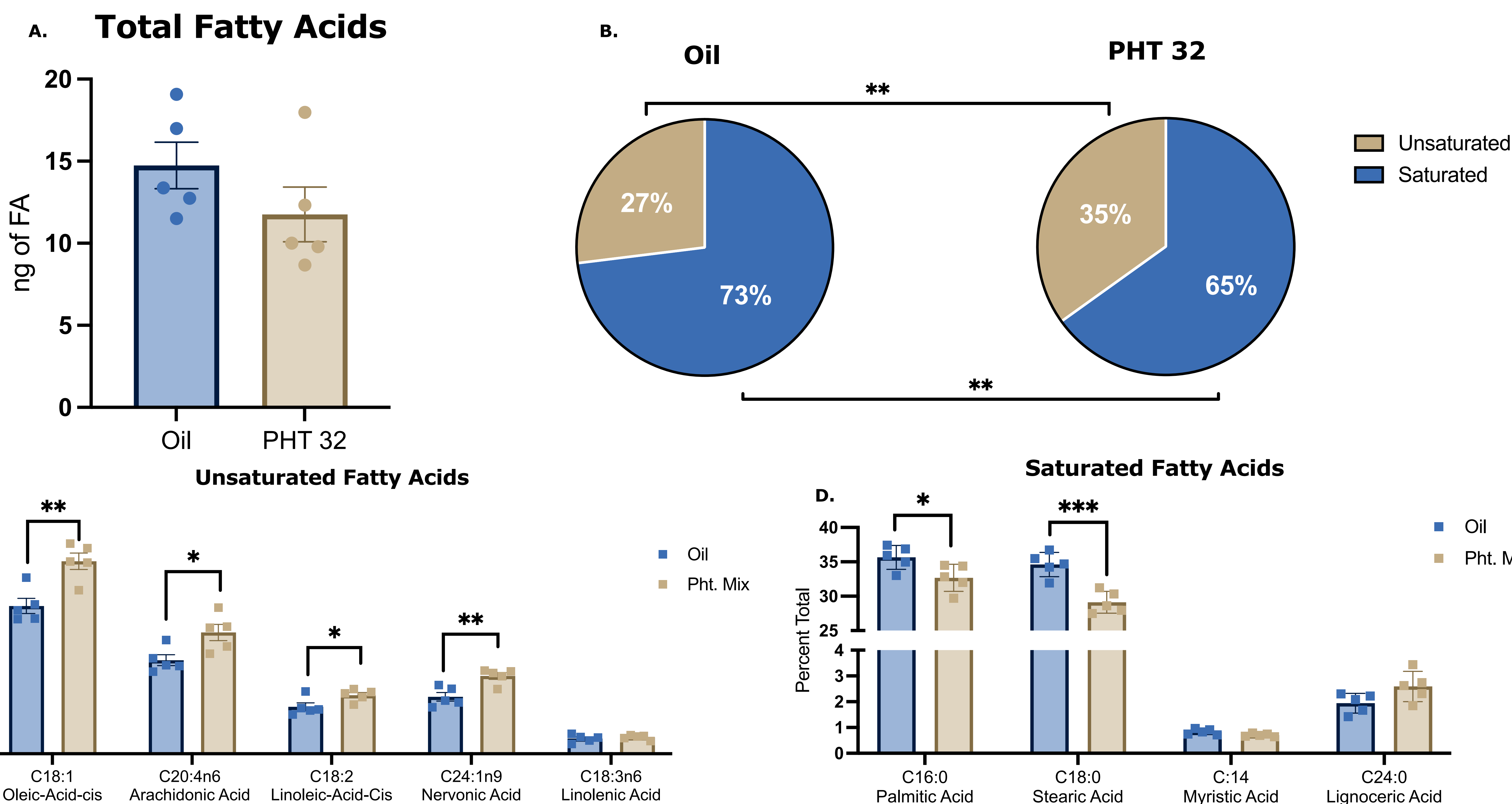


Figure 3: Effect of phthalate exposure on individual fatty acid percentages within antral follicles. Antral follicles were collected and subjected to Fatty Acid Methyl Ester (FAME) profiling. No significant difference was observed in the quantity of fatty acids from antral follicles (A). A significant increase in percent total of unsaturated and a significant decrease in percent total of saturated fatty acids was observed (B). A significant increase in percent total of C18:1, C40:4n6, C18:2, and C24:1n9 was observed (C). A significant decrease in percent total of C16:0 and C18:0 was observed (D). Data is presented as mean relative to control ± SEM and parts of whole. Asterisks (\*\*), (\*), and (\*) indicate statistical differences (P<0.001), (P<0.01), and (P<0.05) respectively.

## Summary

Exposure to a human relevant mixture of DBP, BBP, and DEHP:

- Altered the Abundance of Antral Follicle Proteins**
  - 194 proteins: 99 downregulated and 95 upregulated
  - Fatty acid processes associated with downregulated proteins
- Dysregulated Composition of Fatty Acids in Antral Follicles**
  - Decrease in percent total of Saturated Fatty Acids
  - Increase in percent total of Unsaturated Fatty Acids

## Conclusion

These results, combined with the proteomic profile, suggest that a human relevant mixture of DBP, BBP, and DEHP causes negative reproductive outcomes by targeting fatty acid metabolic processes within the antral follicle.

## Future Research

- Investigate Free Fatty Acid Profiles**
  - Is this a release issue?
- Evaluate overall Lipid Regulation**
  - Cholesterol Levels
- Explore pathways of Interest**
  - Supported by proteomic data

## Acknowledgements

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