



Objectives

Obesity is associated with increased risk and mortality from endometrial cancer (EC). Research shows that omega-3 polyunsaturated fatty acids (PUFAs) have activity against obesity-driven cancers; this has not been investigated in EC. We assessed the effect of omega-3 PUFAs on cell proliferation and tumor growth in endometrial EC cell lines and an LKB1^{fl/fl}p53^{fl/fl} mouse model.

Methods

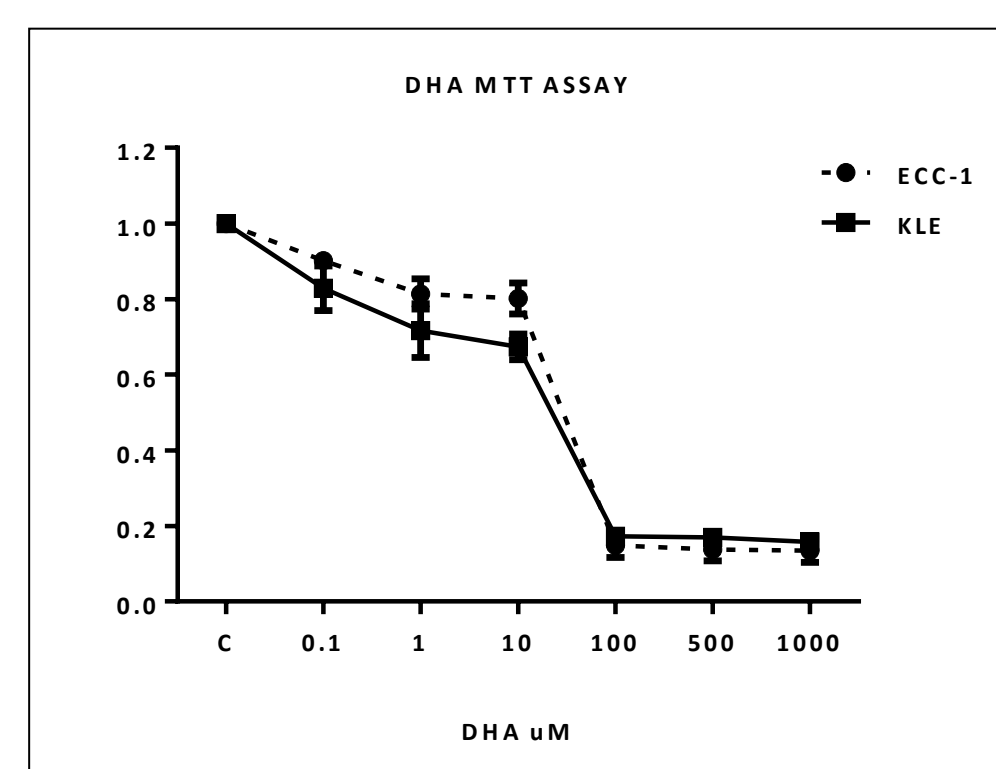
The EC cell lines ECC-1 and KLE were exposed to varying concentrations of docosahexaenoic acid (DHA), an omega-3 PUFA. Cell proliferation was assessed by MTT assay, apoptosis by Annexin V-FITC assay, and reactive oxygen species (ROS) by dichloro-dihydro-fluorescein diacetate (DCFH-DA) assay. Western immunoblotting was performed to assess trends in anti-apoptotic proteins BCL-2 and MCL-1 and cellular stress proteins PERK, Bip, and PDI. LKB1^{fl/fl}p53^{fl/fl} mice were fed either a low-fat (lean) or high-fat (obese) diet and then treated with placebo or DHA. Immunohistochemistry (IHC) was performed on treated and untreated tumors to assess proliferation and apoptosis. Metabolomics identified the effects of DHA in the tumors.

References

- Onstad, Michaela A., Rosemarie E. Schmandt, and Karen H. Lu. 2016. "Addressing the Role of Obesity in Endometrial Cancer Risk, Prevention, and Treatment." *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology* 34 (35): 4225–30.
- Manni, Andrea, Karam El-Bayoumy, and Henry Thompson. 2017. "Docosahexaenoic Acid in Combination with Dietary Energy Restriction for Reducing the Risk of Obesity Related Breast Cancer." *International Journal of Molecular Sciences* 19 (1). <https://doi.org/10.3390/ijms19010028>.

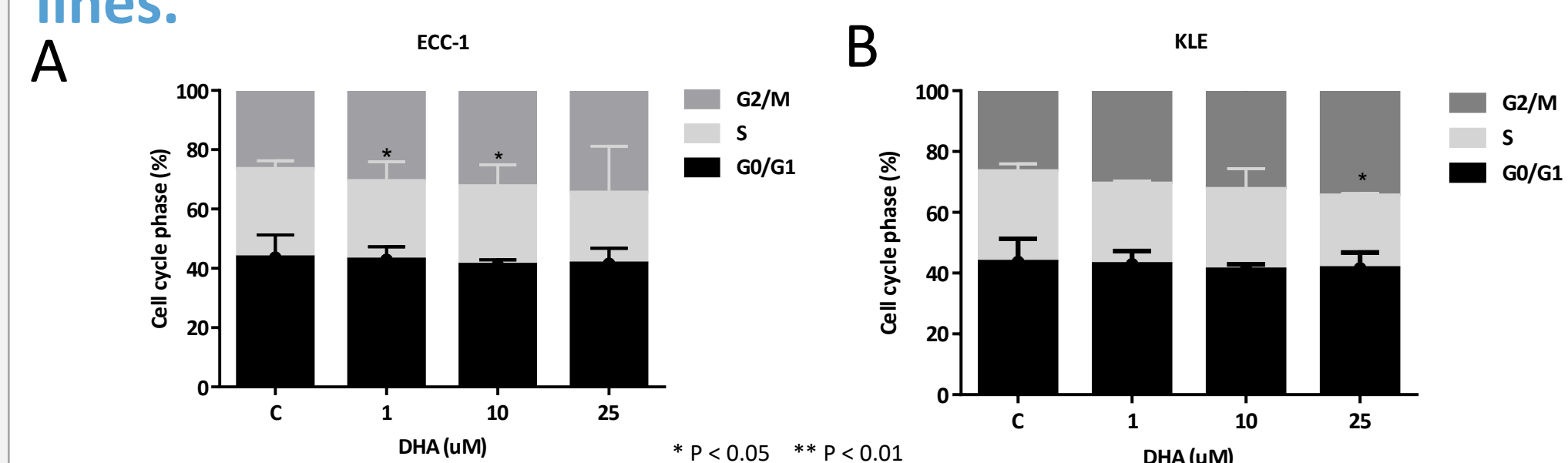
Results

Figure 1: DHA inhibits cancer cell proliferation in both cell lines.



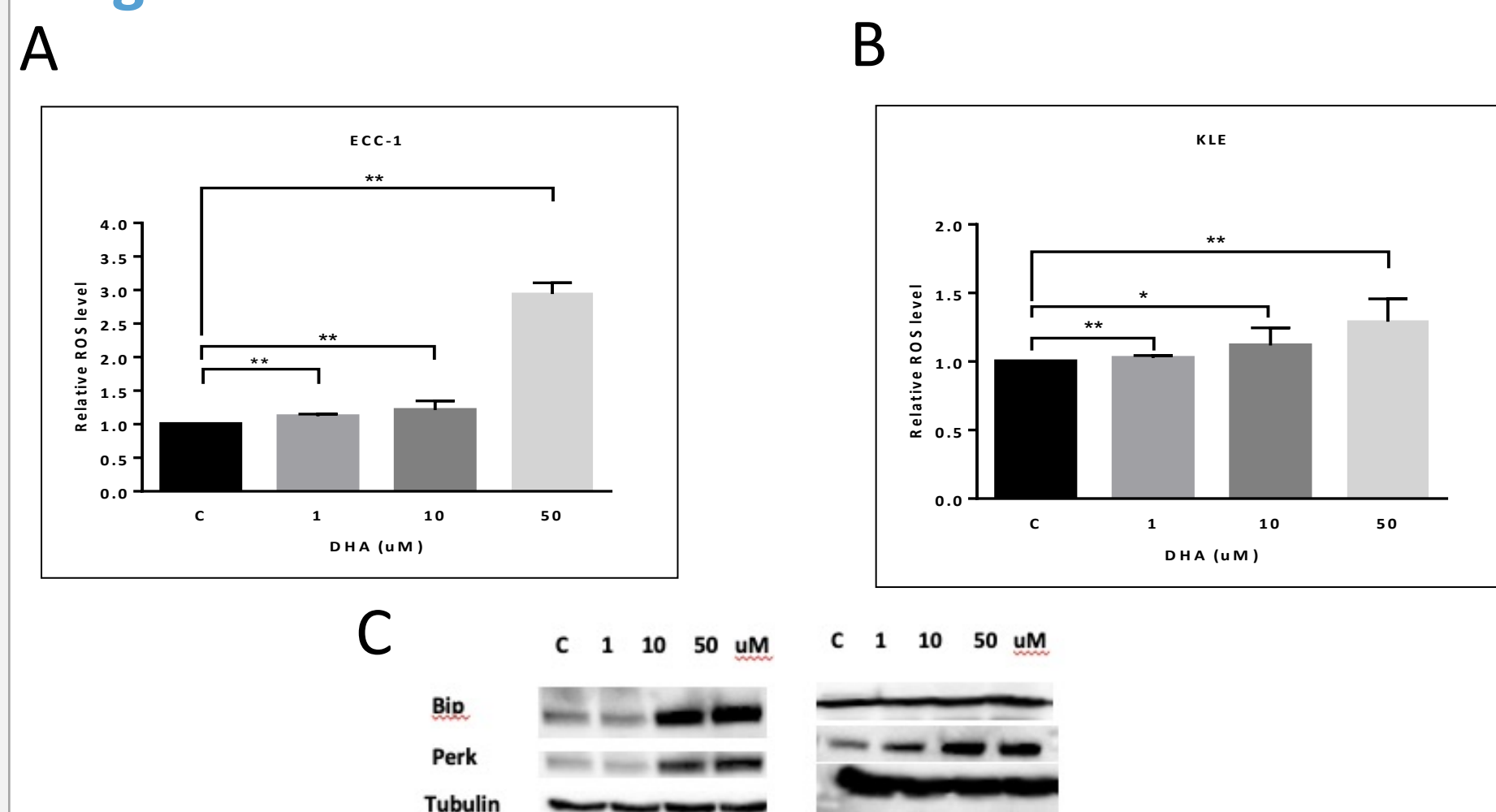
ECC-1 and KLE were treated for 72 hours with varying concentrations of DHA. Both cell lines demonstrated dose-dependent inhibition of cell proliferation with an IC-50 of approximately 50uM.

Figure 2: DHA induces G2 cell-cycle arrest in both cell lines.



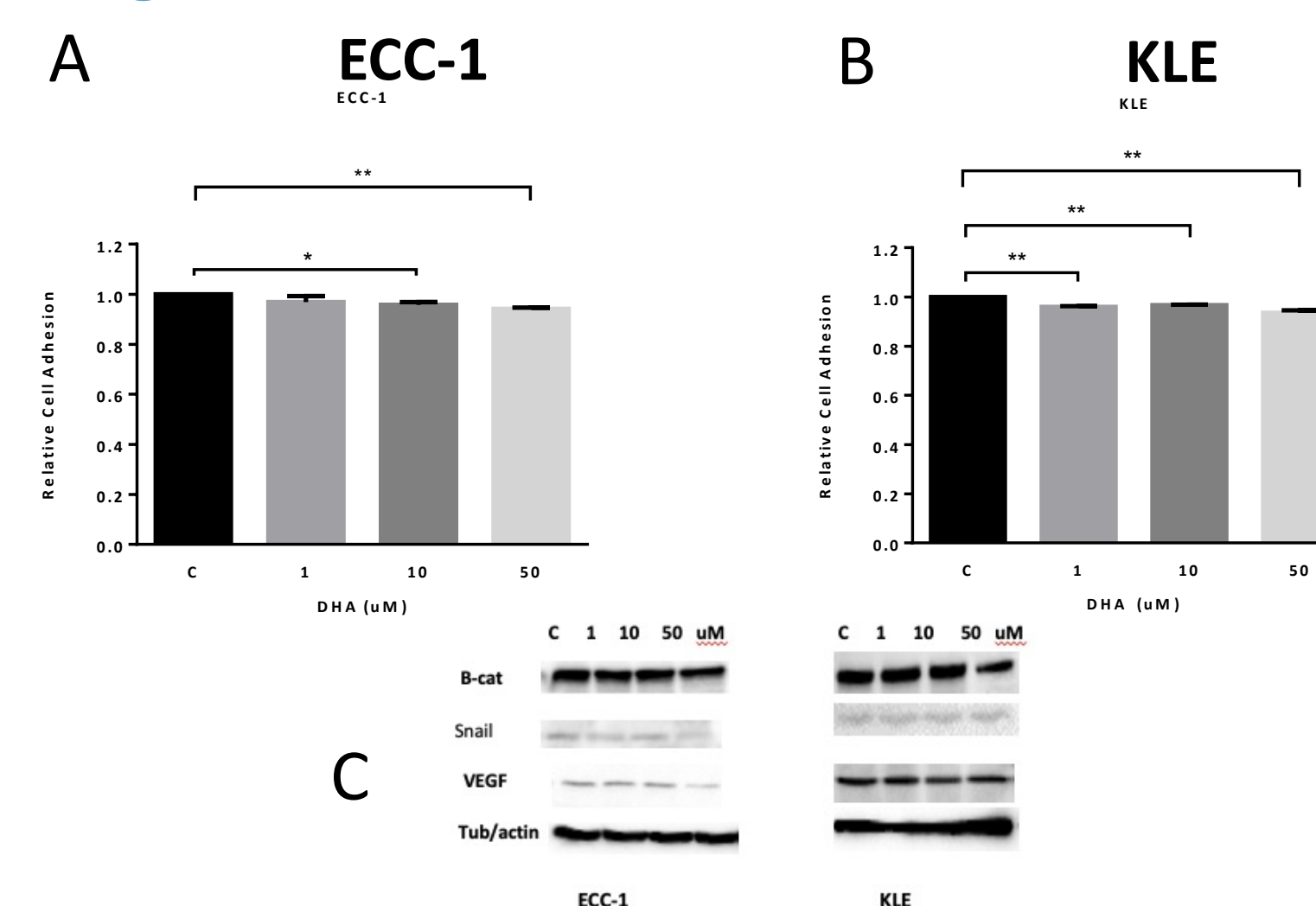
ECC-1 (A) and KLE (B) cells treated for 24 hours with DHA demonstrated cell cycle arrest in G2 phase in a dose-dependent manner.

Figure 3: DHA causes cellular stress in both cell lines.



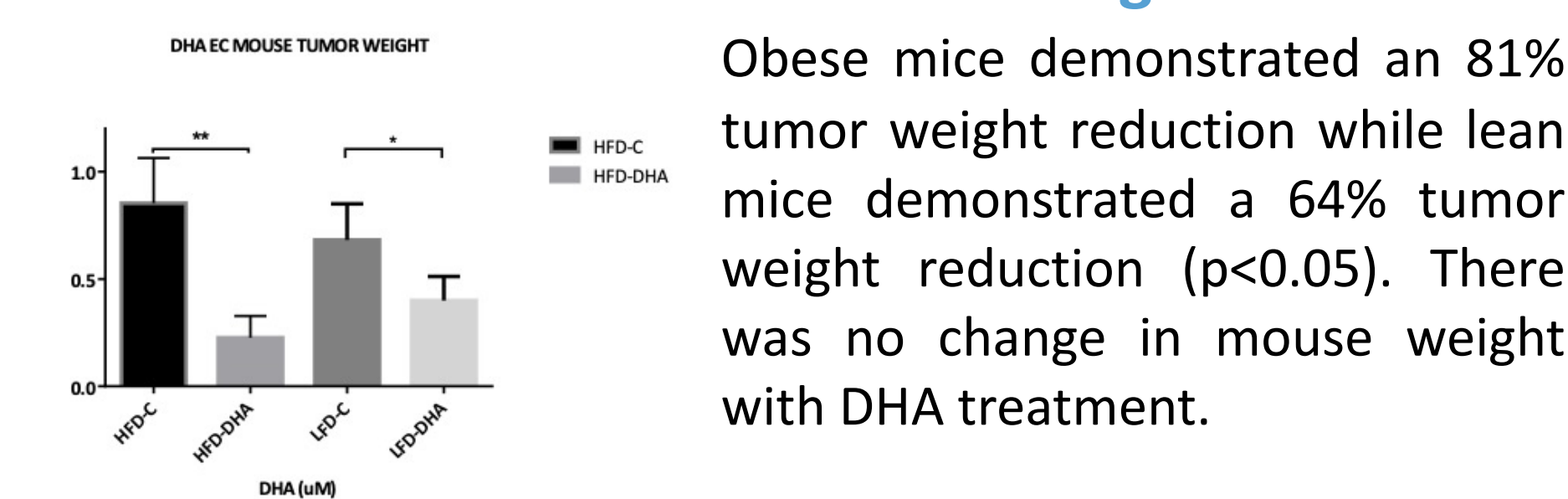
ECC-1 (A) and KLE (B) cells treated for 18 hours with DHA showed increasing reactive oxygen species in a dose-dependent manner. Western immunoblotting showed increased expression of Bip and Perk in both cell lines (C).

Figure 4: DHA treatment inhibits cell adhesion.



Treatment with DHA diminished cell adhesion in a dose-dependent manner (A and B). Confirmatory western blot assays demonstrated decreased expression of the adhesion proteins B-catenin, Snail, and VEG-F in both cell lines (C).

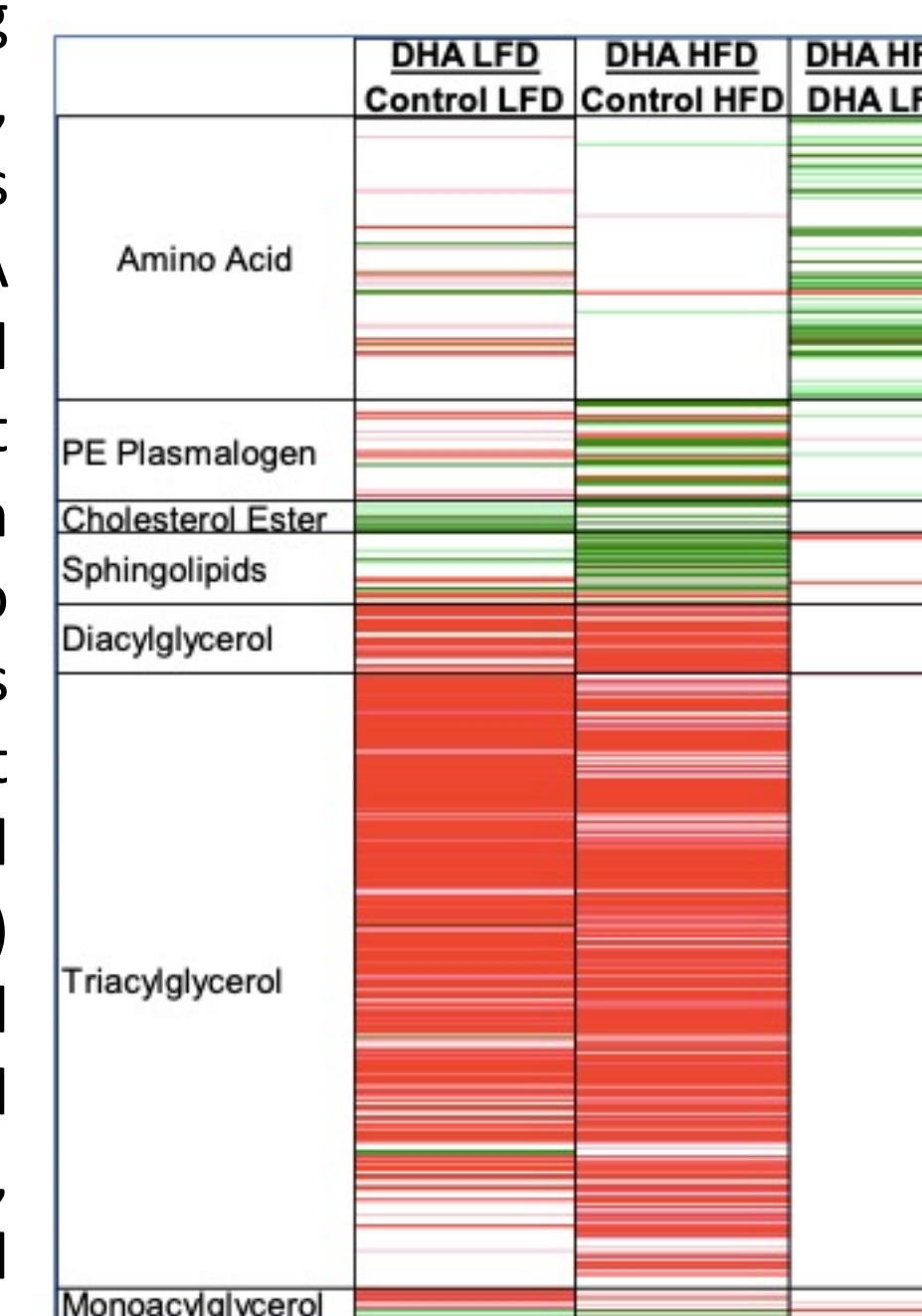
Figure 5: Obese and lean mice treated with DHA demonstrated decreased tumor weight.



Obese mice demonstrated an 81% tumor weight reduction while lean mice demonstrated a 64% tumor weight reduction (p<0.05). There was no change in mouse weight with DHA treatment.

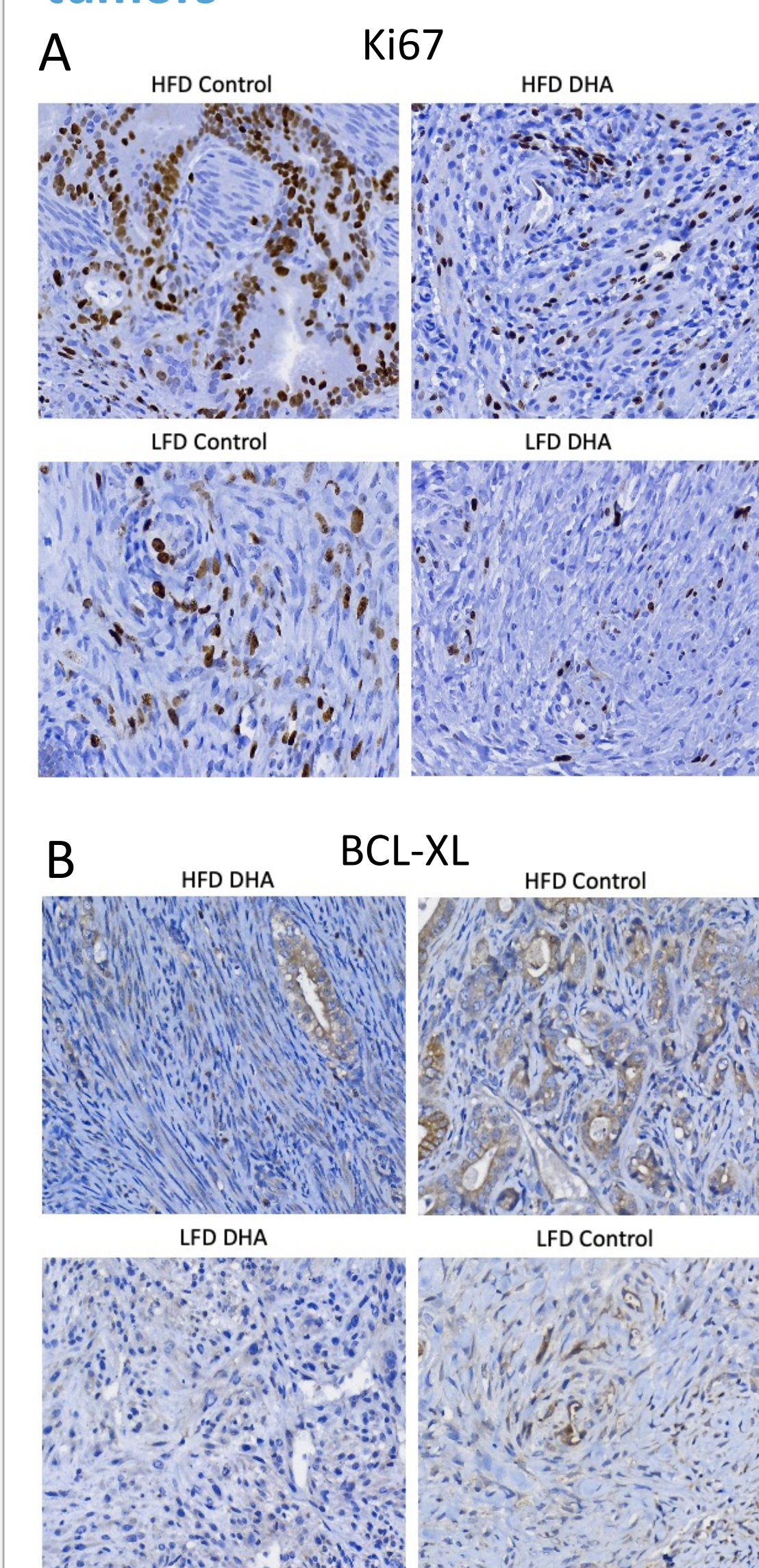
Figure 6: Metabolomic data in obese and lean mice

Metabolomic profiling revealed that total monoacyl-, diacyl- and triacyl-glycerols were increased with DHA treatment in both obese and lean mice, suggesting that lipids were diverted from membrane biosynthesis to energy storage. Differences were found in DHA's effect when comparing obese and lean mice, including (1) decreases in lipid biosynthesis and amino acid metabolism in obese mice, and (2) increases in fatty acid desaturase activity in lean mice.



Results, cont.

Figure 7: Ki67 and BCL-XL Immunohistochemistry in DHA-treated and control mouse tumors



IHC showed decreased Ki67 (A) expression and increased BCL-XL (B) expression in both groups.

Conclusions

- DHA robustly inhibited EC proliferation and tumor growth in vitro and in vivo
- Omega-3 PUFA supplementation may be a promising dietary strategy for endometrial cancer prevention and treatment.