Preliminary studies to determine if dietary DHA supplementation is warranted in food insecure pregnant women in Zambia

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Introduction

Background
- Zambia is located in Sub-Saharan Africa
- 540,000 women and 100,000 children under 14 years old in Zambia are both malnourished and HIV positive.
- This can lead to:
  - Impairment of fetal and early childhood development
  - Altered brain structure and impaired cognitive function
  - Increased mortality rates

Docosahexaenoic Acid (DHA)
- DHA is an omega 3 polyunsaturated fatty acid essential for normal brain function and development. It cannot be made by human cells, so they must be obtained by dietary sources.
- DHA comprises about 40% of polyunsaturated fatty acids (PUFA) in the brain and 60% in retina. DHA stimulates phospholipid production and neuronal growth.

Purpose Statement
- To assess a method to reduce blood sample volume and improve storage of samples prior to analysis of DHA levels to assess need for supplementation.
- Survey different formulations of Ready to Use Supplemental Food (RUSF) to determine the source and concentration of DHA that is acceptable for consumption during pregnancy and lactation for food insecure pregnant women in Zambia.

Methods

PHASE 1: Dried Blood Spots
- Conventional approaches for assaying fatty acids use large quantities of blood stored for long periods of time, which is a time consuming process and there is a risk of PUFA oxidation, not allowing for accurate fatty acid profiling.
- Solution: Using dried blood spots as a quick, inexpensive method that uses a smaller sample volume to measure PUFA over time more accurately.
- Ideally, this method would allow the technicians in Zambia to simply spot the blood on a collection paper, then ship the samples to UNL without degradation of DHA by oxidation.

Figure 1. Blood is taken from a finger prick, spotted on filter paper, placed in a plastic bag and sealed for shipment to the UNL Virology and FATTI Labs.

PHASE 2: Ready to Use Supplemental Food (RUSF)
- What is RUSF?
  - A nutritional supplement to the diet, but currently supplied without containing DHA.
  - (e.g) Vitamin A, B, C, E, K, Fat, Protein, Thiamine (B1), Iodine, Cu, Fe, Mg, K, Folic acid etc.
  - Nut butter based: allows for low water content in the sample, increasing its shelf-life, and includes concentrated sources of calories from fat and protein.

PHASE 2: RUSF Survey Samples
- Treatment of RUSF with BHT and EDTA significantly improved retention of DHA, but did not affect PA levels.
- Levels of DHA was significantly diminished after 4 weeks of storage as opposed to PA.
- This data indicate that blood spots on treated papers are useful to minimize sample volume and maximize retention of FA levels prior to analyses, but samples should not be stored more than 2 weeks overall.

Conclusions

PHASE 1: Dried Blood Spots
- Treatment of blood spots with BHT and EDTA improved retention of DHA, but did not affect PA levels.
- More samples could be tested to increase power of the statistical analysis.
- Additional preservatives, and/or higher concentrations could be tested, (e.g.) additional antioxidants, and/or broader range of cheating agents to increase stability over time.

PHASE 2: RUSF Survey Samples
- More participants could bring different or more reliable results on which samples are more palatable than others.
- Tested in the US: It would be best to have the Zambian population taste test the samples as well because of cultural and culinary differences, which is currently being done.

Future Directions
- We have begun testing DHA levels in 100 pregnant women from Zambia to compare to global averages published by the World Health Organization.
- If warranted, a large interventional trial will be conducted supplying RUSF supplemented with high DHA content for malnourished pregnant women living in Zambia.
- Measure DHA levels in blood of pregnant women over time taking RUSF to demonstrate improvements.
- Assess mother’s health and infant development to demonstrate impact of DHA supplementation on pregnancy and developmental outcomes.

Acknowledgments
- We are greatly appreciative of support provided by the NU Food for Health Program.
- I would like to thank the McNair Scholars Program and UNL FATTI Lab for guidance and support in the conduct of this research.

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