THE INFLUENCE OF MODERATE AND HIGH FORMULA DOCOSAHEXANOIC ACID ON TERM BABOON NEONATE TISSUE COMPOSITION AND CLINICAL PARAMETERS

A Dissertation
Presented to the Faculty of the Graduate School of Cornell University
In Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

by
Andrea T. Hsieh
August 2006
THE INFLUENCE OF MODERATE AND HIGH FORMULA DOCOSAHEXAENOIC ACID ON TERM BABOON NEONATE TISSUE COMPOSITION AND CLINICAL PARAMETERS

Andrea T. Hsieh, Ph. D.
Cornell University 2006

Long-chain polyunsaturated fatty acids (LCPUFA) are indispensable for normal infant growth and development. Docosahexaenoic acid (DHA, 22:6n-3) and arachidonic acid (ARA, 20:4n-6) are LCPUFA that play a critical role in central nervous system development. During the brain growth spurt, rapid accumulation of LCPUFA occurs in the brain and retina. Currently, insufficient evidence exists to determine optimal levels of dietary LCPUFA required during the perinatal period.

In the context of a safety and efficacy study of dietary LCPUFA in baboon neonates, we examined the influence of medium and high levels of formula DHA levels on tissue fatty acid composition and hematological and clinical chemistry measures. Infant formulas were fed from birth to 12 weeks of age: Control (C, no DHA/ARA); 1× LCPUFA (L, 0.32%DHA/0.64%ARA); 3× LCPUFA (L3, 0.96%DHA/0.64%ARA).

At 12 weeks, tissue DHA levels were more sensitive to dietary manipulations than ARA. While DHA in the cerebral cortex increased with higher concentrations of DHA, no differences between L and L3 were detected in the basal ganglia and limbic system. These findings indicate that current levels of LCPUFA in infant formula are not sufficient to optimize DHA levels in the developing cortex.

RBC, hematocrit, hemoglobin, and red blood cell distribution width (RDW) were significantly elevated by formula DHA and ARA. All erythrocyte values were
within accepted normal ranges for infant baboons and no differences were detectable at 12 weeks. These data provide the first indication that dietary LCPUFA may influence hematopoiesis during the first weeks of life and mitigate the precipitous decline in red cell values associated with neonatal anemia.

All clinical chemistry parameters were normal up to 12 weeks of age. Many of the trends observed were similar to those documented in human infant development. No negative effects on growth measures, hematological or clinical assessments were observed between formula groups. These results suggest that levels of DHA higher than presently included in US infant formulas enhance cerebral cortex DHA and may provide additional benefits by improving erythropoiesis. They also provide a basis for interpretation of parallel human infant studies currently underway.
BIOGRAPHICAL SKETCH

Andrea T. Hsieh was born on October 21, 1978 in Elmhurst, Illinois. She was the oldest of four children and spent her childhood and adolescent years in the sprawling suburbs of Chicago. She attended the University of Illinois Urbana-Champaign and received a Bachelors of Science in Biochemistry. In 2000, she moved to Ithaca, NY and joined the Division of Nutritional Sciences at Cornell University.
ACKNOWLEDGMENTS

To my advisor, Dr. J. Thomas Brenna- thank you for your tremendous guidance, infinite patience, endless humor and confidence in me. A special thanks for the numerous trips to Rudy’s BBQ during the SFBR experience.

I would like to express my sincere appreciation to the members of my committee, Dr. Steven S. Robertson, Dr. Linda M. Nowak, and Dr. Barbara J. Strupp for valuable discussions during initial stages of this work.

I acknowledge Dr. Peter Nathanielsz and Dr. Natalia Schlabritz-Loutsevitch for their continued support and gracious hospitality during my time at the Southwest Foundation for Biomedical Research. A heartfelt thanks goes to Dr. Cun Li and Dongbin Xie for their assistance, encouragement and kindness in San Antonio, TX.

An enormous thank you to members of the Brenna group, especially Pete Lawrence, Carolyn Tschanz, Dr. Srisatish Devapatla, Rinat Ran-Ressler, Gavin Sacks, Bruce Pan, Kumar Kothapalli, Behzad Varamini and my favorite undergraduates Evan Walther and Tara Kelly for all the lovely times in the basement of Savage Hall. My appreciation also goes to Françoise Vermeylen for her statistical knowledge and endurance for answering SAS questions.

I would like to acknowledge past and present Nutritional Science students, especially Anne LeBourg, Nkosi and Mdu Mbuya, Dave Buress, Natalie Nicod, Krista Kauppinen, Samantha Morley, Li Ling Lee, Christina Nyhus, Sera Young, Tim Sontag and Greg Jackson for your invaluable friendships and gorges Ithaca memories.

Thanks to Sara Gulbrandsen, Ivana Lukaska, Jennie Jiang, Peg Smith and Sara Wettling for the wonderful Ithaca visits and adventures deep in the heart of Texas. A very special thank you goes to my favorite Romanian mathematician, Radu Murugesu for a fabulous final year at Cornell.
Last but not least, I would like to thank my parents, Joyce and Victor for their unconditional support and everlasting love. Thanks to my siblings Philip, Roger and Emilie for all the trips to Ithaca, the Underground Railroad escapade and the 12+ hour car ride with the mischievous Babycat.
TABLE OF CONTENTS

CHAPTER 1. INTRODUCTION

1.1 BACKGROUND 1
1.2 METABOLISM OF n-3 AND n-6 FATTY ACIDS 1
1.3 ESSENTIAL FATTY ACID DEFICIENCY 6
1.4 ESSENTIAL FATTY ACID CONVERSION TO LCPUFA 8
1.5 LCPUFA SYNTHESIS IN NEONATES 8
1.6 LCPUFA DURING THE PERINATAL PERIOD 9
1.7 NHP MODELS FOR HUMAN INFANT DEVELOPMENT AND FA METABOLISM 12
1.8 INFLUENCE OF LCPUFA ON INFANT NEURODEVELOPMENT AND GROWTH 13
1.9 LCPUFA IN HUMAN MILK AND INFANT FORMULA 18
1.10 SUMMARY 22
1.11 REFERENCES 23

CHAPTER 2. THE INFLUENCE OF MODERATE AND HIGH LEVELS OF LONG CHAIN POLYUNSATURATED FATTY ACID (LCPUFA) SUPPLEMENTATION ON BABOON NEONATE TISSUE FATTY ACIDS

2.1 ABSTRACT 30
2.2 INTRODUCTION 31
2.3 MATERIALS AND METHODS 33
   2.3.1 Animals 33
   2.3.2 Diets 33
   2.3.3 Growth 34
   2.3.4. Sampling 34
2.3.5 Fatty Acid Analyses 36
2.3.6 Statistics 36

2.4 RESULTS
2.4.1 Formula Consumption and Growth 37
2.4.2 RBC and Plasma Fatty Acids 37
2.4.3 Liver and Heart Fatty Acids 38
2.4.4 Retina Fatty Acids 38
2.4.5 Central Nervous System Fatty Acids 39

2.5 DISCUSSION 51
2.6 CONCLUSION 54
2.7 ACKNOWLEDGEMENTS 55
2.8 REFERENCES 56

CHAPTER 3. FORMULA DOCOSAHEXAENOIC ACID AND ARACHIDONIC ACID IMPROVES POSTNATAL HEMOGLOBIN AND RELATED INDICES IN TERM BABOON NEONATES

3.1 ABSTRACT 59
3.2 INTRODUCTION 60
3.3 MATERIALS AND METHODS
3.3.1 Animals and Diets 62
3.3.2 Blood Sampling 63
3.3.3 Hematology 63
3.3.4 Statistics 64
3.4 RESULTS AND DISCUSSION 64
3.5 CONCLUSION 74
3.6 ACKNOWLEDGEMENTS 75
3.7 REFERENCES 76
LIST OF FIGURES

1.1 Metabolic pathways for the conversion of linoleic acid and \( \alpha \)-linolenic acid into LCPUFA. 3

1.2 Conversion of linoleic acid to arachidonic acid. 4

2.1 Summary of baboon neonate formula consumption and growth. 47

2.2 Baboon neonate FA concentrations at 12 weeks of age. 49

3.1 Regression analysis calculation for RBC, Hemoglobin, Hematocrit and RDW. 65

3.2 Regression analysis calculation for MCV, MCH and MCHC. 69

4.1 Clinical chemistry parameters influenced by dietary LCPUFA. 88
LIST OF TABLES

1.1 Common dietary sources of n-6 and n-3 fatty acids. 3
1.2 Target LCPUFA concentrations in US commercial term infant formulas. 21
2.1 Characteristics of baboon neonate groups. 34
2.2 Nutrient content of Enfamil® LIPIL® 35
2.3 Liver, RBC, plasma and heart FA composition. 41
2.4 Cerebral cortex precentral gyrus and frontal lobes and retina FA composition. 43
2.5 Basal ganglia (globus pallidus, putamen, caudate) and amygdala FA composition. 44
2.6 Superior and inferior colliculi FA composition. 46
3.1 Characteristics of baboon neonate groups. 63
4.1 Characteristics of baboon neonate groups. 84
4.2 Changes in clinical chemistry parameters for baboon neonates at 6 and 12 weeks of age. 88
4.3 Ontogeny of white cell parameters for baboon neonates from 2 to 12 weeks of age. 90
LIST OF ABBREVIATIONS

ALA, \( \alpha \)-linolenic acid (18:3n-3)
ARA, arachidonic acid (20:4n-6)
C, Control formula: DHA (0%w/w), ARA (0%w/w)
CBC, complete blood count
CNS, central nervous system
DHA, docosahexaenoic acid (22:6n-3)
DHGLA, dihomo-\( \gamma \)-linolenic acid (20:3n-6)
DPA, docosapentaenoic acid (22:5n-3, 22:5n-6)
EDTA, ethylenediaminetetraacetic acid
EFA, essential fatty acid
EPA, eicosapentaenoic acid (20:5n-3)
EPO, erythropoietin
ERG, electroretinography
FA, fatty acid
FAME, fatty acid methyl ester
GC, gas chromatography
GI, gastrointestinal tract
GLA \( \gamma \)-linolenic acid (18:3n-6)
IUGR, intrauterine growth restriction
L, LCPUFA intermediate formula: DHA (0.32%), ARA (0.64%)
L3, LCPUFA high formula: DHA (0.96%), ARA (0.64%)
LA, linoleic acid (18:2n-6)
LCPUFA, (\( \geq 20 \) carbons) long-chain polyunsaturated fatty acids
MCV, mean cell volume
MCH, mean cell hemoglobin
MCHC, mean cell hemoglobin concentrations
MDI, Mental Development Index
MPV, mean platelet volumes
MS, mass spectrometry
MUFA, monounsaturated fatty acids
NEC, necrotizing enterocolitis
NHP, non-human primate
PDI, Psychomotor Development Index
RBC, red blood cells
RDW, red blood cell distribution width
SID, Bayley Scales of Infant Development
SFA, saturated fatty acids
VEP, visual evoked potential
VLBW, very low-birth weight
WBC, white blood cell
w/w, weight ratio of FA to total FA