EFFECT OF FISH OIL OMEGA-3 FATTY ACIDS ON REDUCTION OF DEPRESSIVE SYMPTOMS AMONG HIV-SEROPOSITIVE PREGNANT WOMEN

ROSE OKOYO OPIYO
A80/82301/11

A THESIS SUBMITTED IN FULL FULFILLMENT FOR THE AWARD OF THE DOCTOR OF PHILOSOPHY IN NUTRITION IN THE DEPARTMENT OF FOOD TECHNOLOGY AND NUTRITION, UNIVERSITY OF NAIROBI

August 2015

DECLARATION OF ORIGINALITY

Name of student:	Rose Okoyo Opiyo
Registration Number:	A80/82301/11
College:	Agriculture and Veterinary Sciences
Faculty:	Agriculture
Department:	Food Technology and Nutrition
Course:	PhD (Nutrition)
Title of Research work:	*Effect of fish oil omega-3 fatty acids on reduction of depressive symptoms among HIV-seropositive pregnant women*

DECLARATION

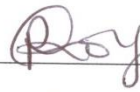
1. I understand what Plagiarism is and I am aware of the University's policy in this regard;

2. I declare that this Thesis is my original work and has not been submitted elsewhere for examination, award of a degree or publication. Where other people's work or my own work has been used, this has been properly acknowledged and referenced in accordance with the University of Nairobi's requirements;

3. I have not sought or used the services of any professional agencies to produce this work;

4. I have not allowed, and shall not allow anyone to copy my work with the intention of passing it off as his/her own work;

5. I understand that any false claim in respect of this work shall result in disciplinary action, in accordance with University Plagiarism Policy.

Signed 

Date 17-8-2015.

APPROVAL

This thesis has been submitted for examination with our approval as university supervisors

Signed_____ Date 20/8/2015

Prof. Wambui Kogi-Makau, BEd, MSc, PhD,
Associate Professor,
Department of Food Technology and Nutrition,
University of Nairobi.

Signed_____ Date 18/08/2015

Prof. Kamau, R. Koigi MBChB, MMed,
Associate Professor,
Department of Obstetrics and Gynaecology,
University of Nairobi,

Signed_____ Date 18/8/2015

Dr. Obondo Anne B.A, MSW, DipPsy, PhD.,
Senior Lecturer,
Department of Psychiatry,
University of Nairobi,

Signed_____ Date 18/8/15

Prof. Ogoyi, Dorington, O., BSc, MSc, PhD.
Associate Professor,
Department of Biochemistry and Biotechnology
Technical University of Kenya

iii

DEDICATION

To my mother, Calsina Rebba Nasike, who during my childhood, always reminded me of the value of education, and to all HIV-positive pregnant women, whose depressive symptoms are hardly noticed. You all gave me the motivation to complete this research.

ACKNOWLEDGEMENT

I wish to express my sincere appreciation to my academic supervisors: Prof. Wambui Kogi-Makau, Prof. Koigi Kamau, Dr. Anne Obondo and Prof. Dorington Ogoyi for their knowledge and patience in supporting me in this work. They all tirelessly guided me through the development of the study protocol, study design, data collection and reviewed the thesis write-ups. I am also greatly indebted to the Consortium for Advanced Research Training in Africa (CARTA), jointly led by the African Population and Health Research Center and the University of Witwatersrand, for facilitating my PhD training and professional development through series of Joint Admission Seminars (JAS) and a short course on clinical trials. The JAS trainings gave me the opportunity to acquire special research skills and knowledge in critical thinking, analysis, reading and writing from international public health experts and from my CARTA PhD Cohort-1 fellows. I wish to acknowledge the financial support received from the University of Nairobi and CARTA for my PhD training and research. I also wish to acknowledge the support received from Innovix Pharma Inc, California for the trial products, fish oil omega-3 and soybean oil soft gels.

I wish to thank the health facility staff from Riruta, Mathare North, Kariobangi North and Kayole-II Sub-district Hospital for allowing me to conduct the research in their facilities. I also wish to thank Angella, Catherine, Wanja, Kirika and Kennedy for their support in data collection. I acknowledge the support received from Francis for randomization, Merab for packaging and allocation concealment, Director for Center for Biotechnology and Bioinformatics (CEBIB), UoN, for laboratory support in preparation of cheek cell samples, Public Health and Toxicology Laboratory personnel: Nderitu, Ken and Gitau for GC analysis, Mugo for computation of nutrient values and Kennedy for data entry. I also wish to acknowledge my Data and Safety Monitoring Board members, Dr. Mathai, Mr. Nyabola, Dr. Mbuya and Dr. Suwirakwenda for their guidance and support during the clinical trial implementation. I appreciate the statistical support from CARTA facilitators (Prof. Levin, Prof. Chirwa, Prof. Eriksson and Prof. Petzold) and cohort-1fellows (Kennedy, Peter and Babatunde). I also thank Dr. Okalebo for cross-checking my statistical analysis, the Director of School of Public Health, for his support and understanding in completion of this work. And to the study participants, thank you for your consent and patience in this study.

TABLE OF CONTENTS

List of Tables

LIST OF FIGURES

LIST OF ACRONYMS

AA	Arachidonic acid
AIDS	Acquired immunodeficiency syndrome
ALA	Alpha-linolenic acid
ANC	Antenatal care
ANCOVA	Analysis of covariance
ANOVA	Analysis of Variance
ART	Antiretroviral therapy
ARV	Antiretroviral
BDI	Beck Depression Inventory
BDI-II	Beck Depression Inventory, Second Edition
BMI	Body Mass Index
CD4	Cluster of differentiation 4
CDC	Centre for Disease Control
CES-D	Centre for Epidemiologic Studies Depression Scale
CI	Confidence Intervals
CNS	Central Nervous System
CONSORT	Consolidated Standards of Reporting Trials
DALYs	Disability Adjusted Life Years
DGLA	Dihomo-gamma linoleic acid
DHA	Docosahexaenoic acid
DHEA	Dehhydroepiandrosterone
DPA	Docosapentaenoic acid
DRI	Dietary Reference Intakes
DSMB	Data Safety and Monitoring Board
EACCR	East African Consortium for Clinical Research
EFAs	Essential Fatty Acids
EAR	Estimated Average Requirements
EPA	Eicosapentaenoic acid
EPDS	Edinburgh Postnatal Depression Scale
ETA	Eicosatetraenoic acid
ERC	Ethics Review Committee
FAMES	Fatty acid methyl esters
FAO	Food and Agricultural Organization
FID	Flame Ionization Detector
GC	Gas chromatography
GC-MS	Gas Chromatography-Mass Spectrometry
GLA	Gamma Linoleic acid
GCP	Good Clinical Practices
HAART	Highly Active Antiretroviral Therapy
HAM-D	Hamilton Rating Scale for Depression
HIV	Human immunodeficiency virus
HPLC	High-performance Liquid Chromatography
IQR	Interquartile Range
ICIPE	International Centre of Insect Physiology and Ecology
JAS	Joint Advanced Seminars
KAIS	Kenya Aids Indicator Survey
KII	Key informant Interviews
KNBS	Kenya National Bureau of Statistics
KNH/U oN–ERC	Kenyatta National Hospital/University of Nairobi Ethical Review Committee

kPa	Kilopascal
KSh.	Kenyan Shillings
LA	Linoleic Acid
LC	Long Chain
LoD	Limit of Detection
LoQ	Limit of Quantification
M2M	Mother-to-Mother
MOH	Ministry of Health
MUAC	Mid-Upper arm circumference
NAP	National Academic Press
NASCOP	National AIDS and Sexually Transmitted Diseases Control Program
NCST	National Council for Science and Technology
NICE	National Institute for health and Clinical Excellence
OR	Odds Ratio
PASW	Predictive Analysis Software
PHD	Doctor of Philosophy
PMTCT	Prevention of mother-to-child transmission
PUFAs	Polyunsaturated fatty acids
RCT	Randomized Controlled Trial
RDAs	Recommended Dietary Allowances
RR	Relative Risk
SA	Stearidonic acid
SAD	Seasonal Affective Disorder
SAE	Serious adverse events
SD	Standard Deviations
SOP	Standard operating procedures
SPSS	Statistical Package for Social Sciences
TG	Triglycerides
TLC	Thin-layer chromatography
UNAIDS	Joint United Nations Program on HIV/AIDS
UNICEF	United Nations Children's Fund
WHO	World Health Organization
X^2	Chi-square

DEFINITION OF OPERATIONAL TERMS

Compliance and retention in the study: In this study, compliance was defined as the extent to which participants took the study intervention dosage of one soft gel, three times a day: morning, lunch time at mid-day and in the evening after dinner. Retention on the other hand was defined as the extent to which each enrolled participant remained in the study for the eight-week study period from week-zero at enrolment to week-eight at exit from the study.

Depression: In this study, depression was defined using a cut-off point of 14 or more scores on the Beck Depression Inventory Second Edition (BDI-II) Scale.

Effect of Intervention: The smallest difference (effect size) in mean depression scores between intervention and control group detectable with power 80% in this study is 20%.

HIV-seropositive: In this study, human immunodeficiency virus (HIV) seropositive refers to study subjects with known positive HIV antibody test results and are on antiretroviral (ARV) therapy. The cut-off point for Cluster of differentiation 4 (CD4) cell count for inclusion in this study was CD4 cells not more than 500 cells/µl which was the World Health Organization (WHO) thresh-hold for antiretroviral therapy (ARV) initiation during the study planning period in 2011.

Fish oil omega-3 fatty acid intervention arm: The intervention group in this study received three soft gels of fish oil omega-3 fatty acid per day, each containing more Eicosapentaenoic acid (EPA) of 0.715 grams than Docosahexaenoic acid (DHA) of 0.340 grams. The total daily intake of omega-3 fatty acids from the three fish oil soft

gels was 3.17grams/day (EPA=2.15 grams/day; DHA=1.02 grams/day) for each participant.

Control group: The control group in this study took three soft gels of soybean oil per day, each containing saturated fatty acids (0.178 grams), monounsaturated fatty acids (0.299 grams) and polyunsaturated fatty acids (0.985 grams) with traces of EPA (0.115 grams). Their total EPA intake was 0.345 grams/day.

Pregnant women: These were women who had completed their first trimester and were on their 12^{th} week of pregnancy at the time of recruitment into the study.

ABSTRACT

Background: Depression in HIV-infected pregnant women is a public health problem due to its negative effects on both maternal and child health, and, on adherence to HIV/AIDS medication regimens. Evidences suggest that nutrient deficiencies may further enhance the depressive illness and that fish oil omega-3 fatty acids may alleviate the depressive illness.

Objective: The study aimed at assessing the effect of fish oil omega-3 EPA-rich supplements on BDI-II depressive symptom scores among HIV-seropositive pregnant women.

Methods: This study was an interventional randomized controlled trial with two parallel groups of fish oil omega-3 as intervention and soybean oil as control. It was double-blinded to participants and those administering the interventions including the principal investigator. Participants were HIV-positive pregnant women enrolled in Prevention of mother-to-child transmission programs and attending antenatal clinics at Nairobi city council's Riruta Health Centre, Mathare North Health Centre, Kariobangi North Health Centre and Kayole-II Sub-district Hospital. Recruitment was from health records of HIV-positive pregnant women. The study inclusion criteria were CD4 cell count of not more than 500 cells/µl, second trimester of pregnancy at 14 to 27 weeks, and participation consent. In addition, all participants had at least mild depression according to Beck Depression Inventory Second Edition (BDI-II) scale. Standardized individual questionnaires were used to collect data on participants' demographic, socio-economic, health and HIV-related characteristics. Dietary intake data was collected using a food-frequency checklist and 24-hour dietary recall methods. Daily nutrient consumption values were computed from food composition databases. Recommended daily allowances for pregnant women were

used to compute dietary adequacy. Omega-3 EPA and DHA cellular levels were determined from cheek cell samples by gas chromatography method. Change in BDI-II depressive symptom scores was computed as post-intervention BDI-II scores (at end of study) minus baseline BDI-II scores (at week 0).

Data analysis: Participants' characteristics data (age, gestational age, HIV status, marital status, parity, education, employment, knowledge of serostatus before pregnancy, HIV status disclosure to anyone, support group meetings attendance and stressful life events experienced) were summarised as median and inter-quartile ranges and proportions. Data analysis followed per-protocol analysis method with participants who completed the 8-week trial included in the analysis of covariance statistical model with fish oil as the main effect and participants' baseline characteristics and nutrient adequacy as covariates in change in BDI-II depressive symptom score outcome. The presence of interaction between covariates was tested.

Results: The study recruited 282 participants and randomized 109 to receive fish oil group and 107 to receive soybean oil group. Most participants had mild to moderate depressive symptoms with BDI-II scores of Median (IQR): 20(16-25) in experimental group and 21(17-25) in control group. Baseline attributes were all similar in both study groups. Completion rate was 78.9% (n=86) in experimental group and 89.7% (n=96) in control group. Dietary nutrient intake was below the estimated average requirements for pregnant women for all nutrients under investigation in more than 60.0% of participants in both groups except for vitamin C (baseline: Fish oil = 56.9%, Soybean = 55.1%); week-8: Fish oil: 44.2%, soybean oil: 40.6%) and vitamin B1 (week-8: Fish oil: 46.5%, soybean oil: 42.7%) and zinc (week-8: Fish oil: 44.2%, soybean oil: 46.9%). Poor concentration of omega-3 EPA and DHA fatty acids in both groups was also noted, with no significant difference between the fish oil

experimental group and soybean oil control group at baseline (EPA (z=0.32; p=0.74) and DHA (z=-0.78;p=0.43)), and after intervention at week-8, EPA (z=0.61; p=0.54) and DHA (z=-1.70; p=0.09)). The participants in both groups had mild to severe BDI-II depressive symptoms (Fish oil: mild=43.1%, moderate=42.2%, severe=14.7%; soybean oil: mild=43.0%, 44.8%, 12.1%) before randomization. The intervention effect, all baseline attributes held constant, was not statistically significant at week-4 (0.14 (95% CI: -1.51 – 1.78), p=0.87) and week-8 (0.85 (95% CI: -0.73 – 2.44), p=0.29). The change in BDI-II scores was significantly associated with baseline BDI-II scores, -0.87 (95% CI: -1.02 - -0.72; p=0.000) parity status -2.23(95% CI: -4.38 – -0.09, p=0.04) assuming all other covariates were held constant.

Conclusion: Fish oil omega-3 EPA-rich supplementation with a daily dosage of 3.17 grams (EPA=2.15 grams; DHA=1.02 grams) is not effective in reduction of depressive symptoms among HIV-infected pregnant women with mild, moderate and severe depression symptoms. The fish oil omega-3 supplements are however welll tolerated, with no adverse side effects among the HIV-infected pregnant women. Severity of depressive symptoms at baseline and maternal parity status can significantly cause a reduction in change in depressive symptoms severity in an 8-week intervention period. This study recommends inclusion of routine screening for depression among HIV-infected pregnant women for timely management of women with severe depressive symptoms. A more focused nutrition assessment, counseling and support for this vulnerable population at the antenatal care is also recommended. Future research on fish oil omega-3 and depression in HIV-infected pregnant women should focus on either moderately depressed or severely depressed women separately.

CHAPTER 1: INTRODUCTION

1.1 Background information

1.1.1 Omega-3 fatty acids

Omega-3 fatty acids are essential nutrients for health, that regulate basic physiological functions in the body such as electrical excitability of the heart and brain cells, inflammation, immune response, cell signalling, blood pressure as well as cancer and cell death. During pregnancy these nutrients support normal growth, development, maturation and functioning of foetal organ systems, particularly the brain and eyes (Hornstra 2003; Innis and Friesen 2008). These fatty acids occur naturally as polyunsaturated fatty acids, and consist of short chain alpha-linolenic acid (ALA, C18:3n-3) and long chain eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3) (Erasmus 2007). The long chain omega-3 fatty acids have 20 or more carbon atoms on the chemical structure (Figure 1).

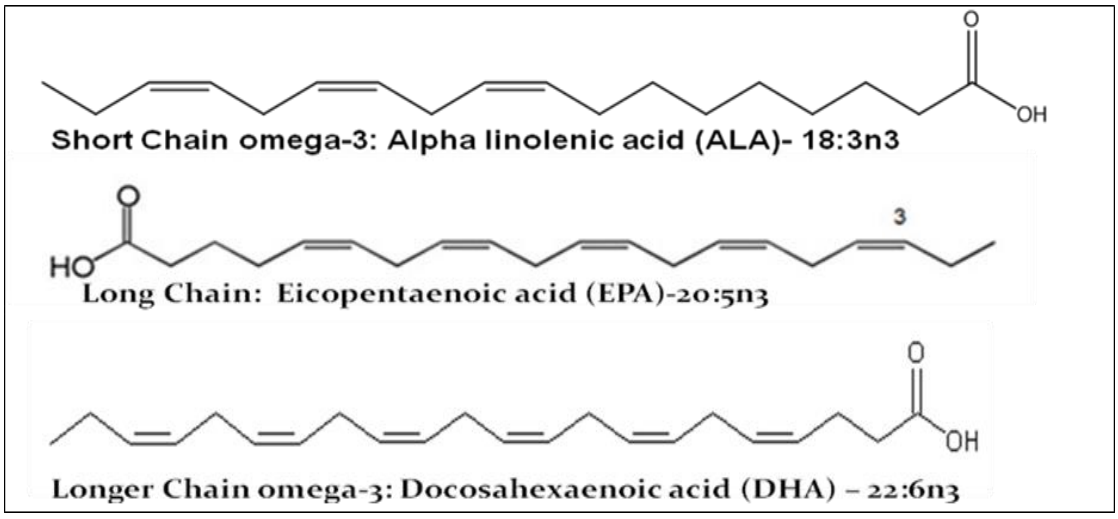


Figure 1– Chemical structure of omega-3 fatty acids

Naturally, long chain omega-3 EPA and DHA fatty acids are synthesized in the chloroplasts of marine and fresh-water algae and other plants. These are in turn consumed by fish, (Stoll 2001), mainly salmon, mackerel, sardine, tuna, herring,

swordfish, trout and tilapia (Flock et al. 2013). Freshwater trout, tilapia, silver fish and Nile Perch also contain substantial amount of long chain omega-3 fatty acids (Masa et al. 2011). The shorter chain omega-3, the ALA, found in soybean, green leafy vegetables, certain seed oils (flaxseed, linseed, canola, chia) and nuts is the main dietary source of omega-3 (Erasmus 2007; Turchini et al. 2011). The metabolic conversion of ALA to EPA and DHA fatty acids may be slowed down by high consumption of dietary omega-6 fatty acids from vegetable cooking oils, edible seeds, soybean, soybean oil, salad dressings, peanut butter due to metabolic competition with ALA omega-3 fatty acids for the same desaturation enzymes (Burdge and Wootton 2002; Emken et al. 1994; Grosso et al. 2014; Stoll 2001) as seen in Figure 2.

Recommendations on the daily nutrient intake values for omega-3 fatty acids in healthy adults vary from 200 to 500 milligrams of EPA plus DHA per day (Flock et al. 2013; Food and Agriculture Organization of the United Nations 2010; Kris-Etherton et al. 2009; Opperman 2013). Achieving these dietary recommendations through regular consumption of oily fish or plant-based omega-3 fatty acid sources an intake of 50 to 60 grams of oily sea fish per day (Flock et al. 2013). This may not be feasible depending on access to the fish, the type of fish and preparation method of the fish which determines the levels of EPA and DHA methods (Chetty et al. 1989; Hibbeln et al. 2006; Moradi et al. 2011; Opperman 2013).

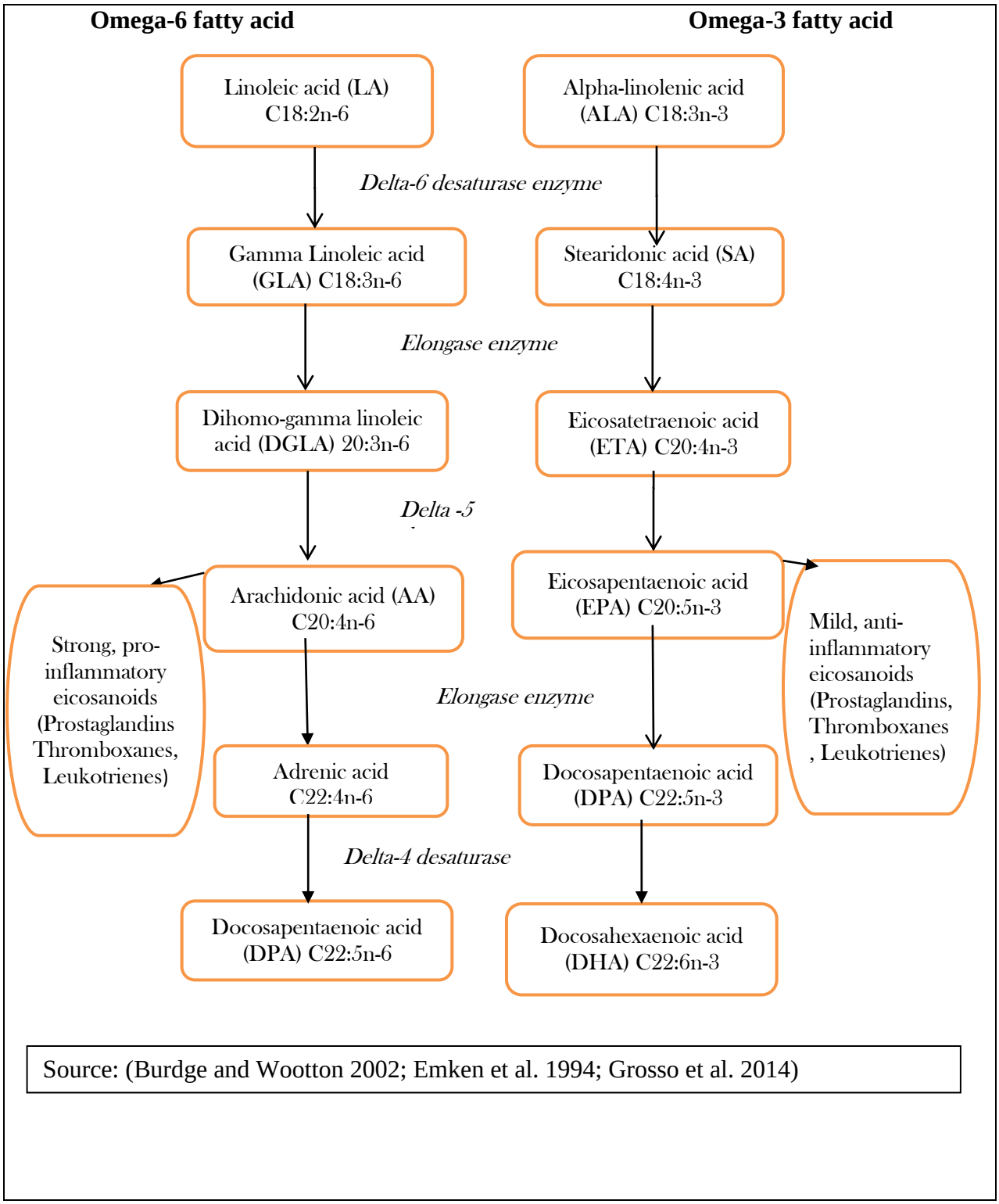


Figure 2- Metabolic pathways for omega-6 and omega-3 fatty acids

The alternative, convenient ways of ensuring regular intake of omega-3 EPA and DHA are use of fortified foods or fish oil nutritional supplements. The supplements are available in the market as either re-esterified triglycerides or ethyl-esters

formulations. The ethyl-esters formulations are the most commonly accessed fish oil omega-3 supplements. However, their digestion and absorption is low and depends on the fat content of the meal due to reduced activity of pancreatic lipase enzyme which hydrolyses the ingested omega-3 into free fatty acids. The ethyl-esters formulations of omega-3 contain an ethanol molecule which is more resistant to the hydrolysis than the triglycerides and phospholipids found in natural fats and oils (Opperman 2013).

1.1.2 Omega-3 fatty acids and depression

Apart from its positive contribution to general health and foetal organ systems development, there is evidence that long chain omega-3 fatty acids found in fish can alleviate symptoms of depression (Lesperance et al. 2011; Panagiotakos et al. 2010; Puri et al. 2001; Su et al. 2008). There is however no evidence on the health benefits of ALA on depressive symptoms except for its role a s aprecursor for EPA and DHA (Turchini et al. 2011). The omega-3 DHA has been recognized as an essential structural component of the cell membranes in the central nervous system (CNS). Some evidence however suggest that omega-3 EPA may be more beneficial in reduction of depression than DHA, based on its anti-inflammatory effects of the metabolic products of eicosanoids and its oxidized derivatives as well as its efficacy at reducing the inflammatory cytokines (Grosso et al. 2014; Logan 2004). These metabolic products of EPA are important in controlling the cellular inflammation in the CNS that may manifest as depressive symptoms. In HIV infection, opportunistic infections in CNS may affect the patients cognitive, motor and behavioral functioning, and, manifest as psychiatric disturbances including depression (Dubé et al. 2005). Evidence on fish oil omega-3 and HIV infection further suggest that omega-3 fatty acids may increase the CD4 cell count (de Luis et al. 2005b), thus improving the body's immunity to infections.

1.1.3 Statement of the research problem

Globally, it is known that HIV-infected pregnant women are prone to depression and that fish oil omega-3 may reduce the depression. However, screening for depression is not part of the routine healthcare package for HIV-infected pregnant women in most countries. Furthermore, the effects of omega-3 fatty acids in reduction of depressive symptoms among this vulnerable population have not been established. Yet, about 50% of HIV-infected pregnant women are depressed (Kapetanovic et al. 2009; Kwalombota 2002; Manikkam and Burns 2012; Smith Fawzi et al. 2007). Hence, there is an existence of knowledge gap on whether fish oil omega-3 reduces depressive symptoms among HIV-infected pregnant women.

1.1.4 Rationale and policy relevance of the study

Depression is an important public health problem affecting about 350 million people globally, and the burden is 50% higher in females than males (WHO 2008). In Kenya, mental disorders account for 25% of out-patients in both public and private health facilities (WHO 2012). The HIV infection in pregnancy is a global challenge with an annual prevalence of more than two million. About 90% of the global burden of HIV infection in pregnancy is found in sub-Saharan Africa (World Health Organization/UNAIDS/UNICEF 2007). According to National AIDS and STI Control Programme (2014), in 2012, 6.3% of pregnant women in Kenya had HIV/AIDS.

While depression is common in pregnancy and HIV infection is also a factor that induces depression, a combination of the two may cause severe depression effects hitherto unaddressed. Among HIV positive pregnant women, therefore, depression is a public health problem due to its negative effects on both maternal and child health

and on adherence to HIV/AIDS medication regimens (Cook et al. 2002; Smith Fawzi et al. 2007), Evidence suggests that long chain omega-3 EPA and DHA fatty acids found in fish and fish oil can alleviate depressive symptoms, but dietary intake of these nutrients is mainly through their pre-cursor, plant-based short chain ALA omega-3 fatty acids found in soybean, green leafy vegetables and edible oils (flaxseed, linseed, canola, chia). However, the conversion of short chain ALA in adults to long chain omega-3 EPA and DHA fatty acids is slow. The conversion may be further reduced in HIV-infected pregnant women due to alteration and reduced activity of pancreatic lipase enzyme responsible for breakdown of fats in the body (Manfredi et al. 2004). The pre-formed long chain omega-3 EPA and DHA fatty acids must therefore be consumed directly in the diet or through fish oil supplements for optimal health.

1.1.5 Conceptual framework

Stressful life events such as rejection by partners, other family conflicts, death in the family and other traumatic events (Petersen 2013) could cause hormonal imbalance, which, in the process can increase the stress hormone, cortisol, causing depression (Kammerer et al. 2006a) as seen in Figure 3.

Both pregnancy and HIV infection status are accompanied by high nutrient demand. However, inadequate and inappropriate dietary intake of omega-3 fatty acids and high intake of saturated fats are nutritional challenges faced by women (Singh et al. 2009; Steyn et al. 2012b). This is likely to contribute to nutrient deficiencies, including omega-3 fatty acids which might precipitate the occurrence of depression in pregnant women (Bodnar and Wisner 2005) unless the nutrient deficit is met through dietary intake of omega-3 rich foods or supplementation. Other nutrient that have also been

linked to depression include folate, vitamin B-12, calcium, iron, selenium, zinc, and omega-3 fatty acids (Lespérance et al. 2010; Leung and Kaplan 2009; Logan 2004; Smith Fawzi et al. 2007). This study focused on the relationship between omega-3 fatty acids and depressive symptoms (Figure 3). The link between other nutrients and depression, including factors contributing to nutrient deficiencies, were not the focus of this study. Other nutrients that may be vital for brain-health were only investigated as potential confounders for the effect of fish oil omega-3 on reduction of depression. The expected long-term outcome of this study is policy adoption of corrective measures to relieve depressive symptoms among HIV infected pregnant women with the aim of reducing maternal depression and improving the quality of life for both the mother and child. The policy implications of this study were however not within the immediate scope of this study.

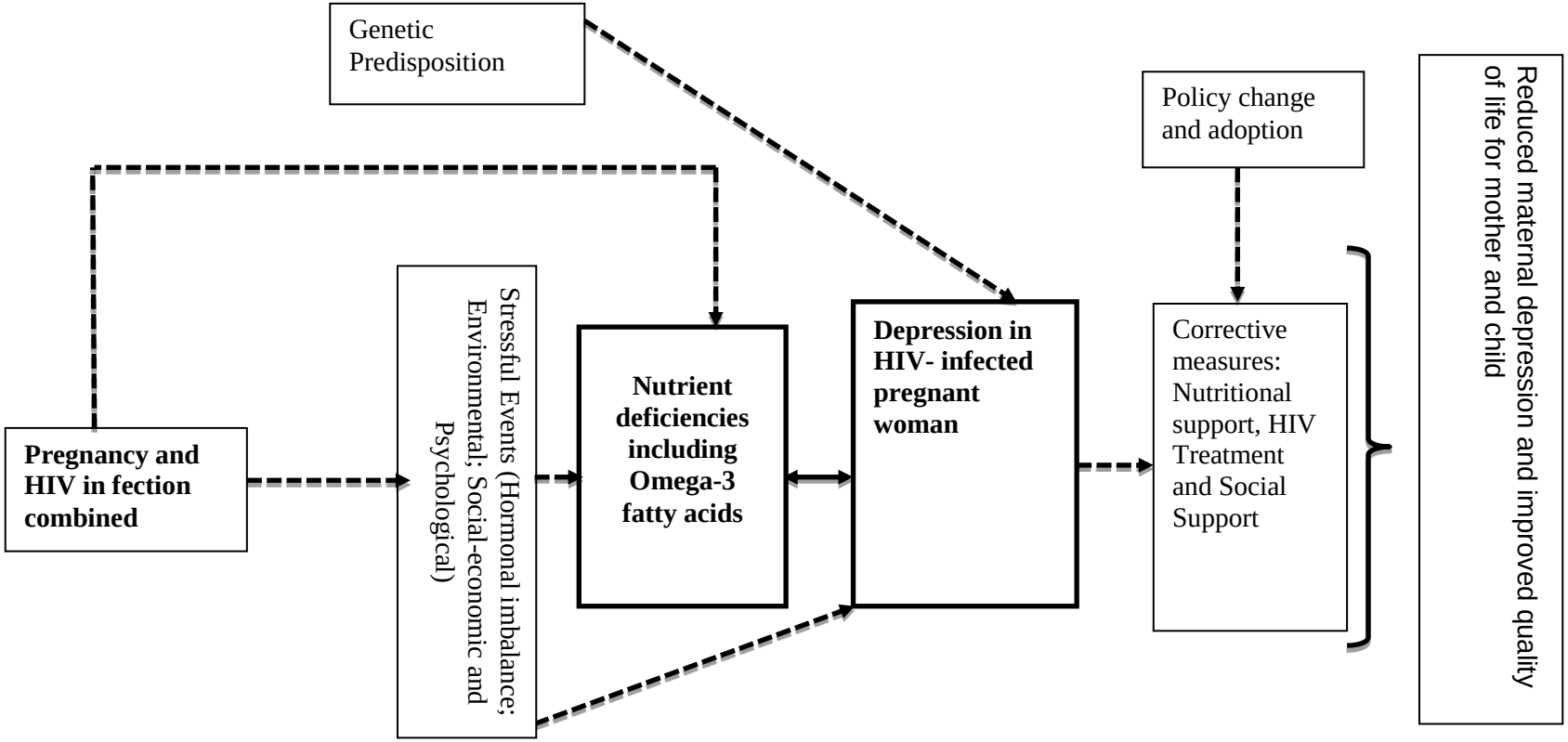


Figure 3 – Research conceptual framework

1.2 Research question, hypothesis and objectives

1.2.1 Research question

Does fish oil omega-3 EPA-rich supplement reduce depressive symptoms among HIV-seropositive pregnant women?

1.2.2 Research hypothesis

The research hypothesis for this study was derived from the evidence that omega-3 can alleviate symptoms of depression (Freeman et al. 2008; Lesperance et al. 2011; Panagiotakos et al. 2010; Puri et al. 2001; Rees et al. 2009; Su et al. 2008). Sources of omega-3 fatty acids also influenced the designing of the study. Whereas fish oil contains long chain omega-3 EPA and DHA fatty acids, plant-based edible oils like soybean contain short chain omega-3 ALA which must be metabolized after consumption to EPA and DHA in the body.

If omega-3 EPA fatty acid is the most important compound in alleviating depressive symptoms (Freeman 2008; Rees et al. 2009; Su et al. 2008), then depressed individuals taking fish oil omega-3 supplements should exprience a change in severity of their depressive symptom condition. A 50.0% reduction in depressive symptom scores after 8 weeks of supplementation with fish oil (EPA=2.2 g/day; DHA=1.2 g/day) had been earlier reported (Su et al. 2008). This study therefore hypothesized that there is a difference in the magnitude of change in depressive symptom scores of at least 20% as measured by Beck Depression Inventory Second Edition (BDI-II) scale among HIV-seropositive pregnant women with depressive symptoms taking fish oil omega-3 EPA-rich supplements than the control group taking soybean oil soft gels whose ALA are minimally metabolized to EPA (Burdge and Wootton 2002; Pawlosky et al. 2001). The null hypothesis of this study was that there is no difference in the magnitude of change in BDI-II scores between HIV–

seropositive pregnant women with depressive symptoms taking fish oil omega-3 EPA-rich supplements and the control group taking soybean oil soft gels.

1.2.3 Research objective

The main objective of the study was to assess the effect of fish oil omega-3 EPA-rich supplements on BDI-II depressive symptom scores among HIV-seropositive pregnant women. Specific objectives of the study were:

1. To assess dietary intake correlates of BDI-II depressive symptom scores among HIV-seropositive pregnant women;
2. To determine the effect of fish oil omega-3 EPA-rich supplements on the omega-3 EPA and DHA fatty acid status among HIV-seropositive pregnant women;
3. To assess the BDI-II depressive symptom scores and levels among HIV-seropositive pregnant women;
4. To determine the effect of fish oil omega-3 EPA-rich supplements on change in BDI-II depressive symptom scores among HIV-seropositive pregnant women

1.2.4 Study variables

The primary outcome variable of the study was the change in depressive symptom scores between baseline scores and the scores after eight weeks as measured by BDI-II scale. The secondary outcome variable was the change in omega-3 EPA and DHA fatty acid levels in cheek cell samples between the baseline and after eight weeks. The main explanatory variable was the intervention with fish oil omega-3 EPA-rich supplement. The secondary explanatory variables were: age of participants, gestation weeks, parity status, marital status, education level, occupation, household income, types of food consumed, estimated omega-3 nutrient levels from dietary intake, changes in pregnancy weight, mid-upper arm circumference (MUAC), CD4 cell count, disclosure of HIV status to non-health facility

workers, blood pressure, recent stressful events, duration since status known, attendance to support group meetings, the decision to have a child and compliance to routine pregnancy and HIV medications as well as to the study intervention. The next chapter presents a deeper view of the published literature on omega-3, its health benefits, and its possible therapeutic use for alleviating depressive symptoms among vulnerable groups with a focus on pregnant women and HIV-infected individuals.

1.2.5 Summary of thesis outline

In chapter two, a review of existing evidence and knowledge, including methodological issues on fish oil omega-3 and depression research with a focus on pregnant women and HIV-infection is presented. Chapter three outlines the research design and methodology in this study. In chapter four, the study research findings are presented by specific research objectives with an introductory section on the characteristics of study participants. The discussions on key findings by specific objectives and overall objective are presented in chapter five. Chapter six contains the conclusions and suggestions for policy and future research. All cited publications cited in this thesis are in the refence chapter at the end of the thesis. Any other relevant material used in this study are found in the appendix at the end of this thesis.

CHAPTER 2: LITERATURE REVIEW

2.1 History of research on role of omega-3 fatty acids in health

Research on the role of omega-3 fatty acids in health dates back to 1929 when it was found to promote growth and prevent inflammation of the skin in rats (Burr and Burr 1929; Holman 1998). The essentiality of long-chain Polyunsaturated fatty acids (PUFA) for human health however emerged in 1970's when the first total parental nutrition which was fat-free was found to induce the essential fatty acid deficiencies among infants with volvulus, a bowel obstruction, at birth (Paulsrud et al. 1972). Further research in 1970s on the health status of modern-hunter-gatherer Inuit Eskimos demonstrated that the low incidences of heart dieases and arthiritis were related to the staple diet of fatty sea fish and fish eating marine mammals rich in long chain omega-3 fatty acids, EPA and DHA (Stoll 2001).

The possibility that depressive symptoms may be alleviated by long chain omega-3 supplementation was first suggested in the late 1990s when Edwards and colleagues (1998) established that deficiency of these fatty acids was significantly associated with severity of depression. In the last decade, research, awareness and use of omega-3 supplements has increased compared to the previous years. The therapeutic mechanisms and active compounds of omega-3 on depression however still appear inconclusive.

2.2 Depression in pregnancy and HIV infection

Depression is a common mental disorder, characterized by sadness, loss of interest or pleasure, feelings of guilt or low self-worth, disturbed sleep or appetite, feelings of tiredness, and poor concentration. It is therefore not a single disease, but a syndrome encompassing a spectrum of symptoms with multiple causes (Leung and Kaplan 2009;

World Health Organization/UNAIDS/UNICEF 2007). As a public health problem, depression affects about 350 million people globally (Marcus et al. 2012), and the burden is 50% higher in females than males (WHO 2008).

During pregnancy, women may experience at least one episode of minor or major depression referred to as perinatal or maternal depression. This is often under-diagnosed, undetected and missed out due to lack of screening (Santoro and Peabody 2010) since screening for depression is not routine in antenatal care (ANC). Studies that have screened for depression in pregnancy indicate that 20%-40% of pregnant women are depressed (Goodman and Tyer-Viola 2010; Kaaya et al. 2010; Marcus et al. 2004).

Depression is also common among HIV infected individuals (Cook et al. 2002; Cruess et al. 2005; Dilley et al. 1985; Holland and Tross 1985; Ickovics et al. 2001; Morrison and Lift 1990). Therefore, whereas depression is common in pregnancy, HIV infection is also a factor that induces the depression. The depression symptoms in HIV-infected pregnant women may be due to the effects of pregnancy, HIV-infection or a combination of both pregnancy and HIV infection. Some symptoms of depression such as fatigue, mood disorders, changes in appetite, or changes in sleeping pattern have been known to overlap with the symptoms present in pregnancy, HIV infection or due to side effects of HIV medication (Lipps et al. 2010a; Psaros et al. 2009).

Among HIV-positive pregnant women in Sub-Sharan Africa, Smith Fawzi and colleagues (2007) reported a prevalence of elevated depressive symptoms of 42.4% in Tanzania, Manikkam and Burns (2012) established a depression prevalence of 38.5% in KwaZulu-Natal, Rochat and colleagues (2006) reported a prevalence of 47% from rural South Africa and Kwalombota (2002) reported a high prevalence of major depression of 85% from

Zambia. This evidence suggests that depression in HIV-infected pregnant women is a public health problem. It negatively impacts on the progression of HIV disease (Cruess et al. 2005; Ickovics et al. 2001) and adversely affect the quality of life and adherence to HIV/AIDS medication regimens (Cook et al. 2002).

2.3 Risk factors of depression in pregnancy

The risk factors of depression in pregnancy include stressful life events including environmental or socio-economic factors (Lancaster et al. 2010; Marcus et al. 2004) genetic predisposition and hormonal imbalance greater than usually experienced in pregnancy (Kammerer et al. 2006a; Leung and Kaplan 2009; Thase 2011; Warner et al. 1996), as well as nutrient deficiencies (Leung and Kaplan 2009). During pregnancy, production of serotonin, the "good mood" neurotransmitter is also reduced as oestrogen hormone is needed to maintain the pregnancy. Other risk factors for depression during pregnancy are maternal anxiety and previous history of depression (Lancaster et al. 2010).

The imbalance in the sex hormones, estrogen and progesterone which occurs in pregnancy may leads to an increased production of the stress hormone, cortisol to help the mother cope with stresses of pregnancy and delivery, hence normal increase in cortisol level is necessary in pregnancy and delivery. The hormonal imbalance which is greater than usually experienced in pregnancy, increases stress hormone, cortisol, causing depression (Kammerer et al. 2006b). The cortisol levels are higher in late than in early pregnancy (Obel et al. 2005). Higher levels of cortisol above normal for pregnancy can also lead to reduced levels of dehydroepiandrosterone (DHEA) hormone. The DHEA hormone buffers the negative effects of cortisol. Thus depression during pregnancy may be associated with the raised cortisol at pregnancy (Kammerer et al. 2006b).

2.4 Dietary intake as risk factors of depression in pregnant women

Inadequate and inappropriate dietary intakes resulting from socio-economic and environmental factors can lead to depression in pregnancy through reduced maternal nutrient stores including omega-3 fatty acids. Existing research evidence links depressive symptoms to folic acid and vitamin B12 deficiencies (Coppen and Bolander-Gouaille 2005) calcium, iron, selenium, zinc, and omega-3 fatty acids (Lespérance et al. 2010; Leung and Kaplan 2009; Smith Fawzi et al. 2007). Antioxidant vitamins (C and E) and minerals (zinc, selenium and manganese among others), are required to protect the nervous membrane from free radicals and enhance bioavailability of omega-3 fatty acids (Bourre 2004; Logan 2004; Singh et al. 2009). Studies have also linked reduced levels of omega-3 fatty acids to depression in pregnancy and after delivery (Marcus et al. 2004; Rees et al. 2008; Su et al. 2008).

Among pregnant women, inadequate and inappropriate dietary intake has been reported globally. For example, in the United States of America (Giddens et al. 2000), Canada (Denomme et al. 2005), Europe (Mouratidou et al. 2006; Pinto et al. 2009), Australia (Blumfield et al. 2011), India (Singh et al. 2009) and Africa (Steyn et al. 2012a) studies indicate that micronutrients and omega-3 fatty acid intake values are below the recommended levels for normal healthy life. However, in pregnancy and human immunodeficiency virus (HIV) infection, nutrients' demand is higher than normal because of the increased need by the developing foetus in the context of nutrient deficiencies that result from reduced food intake, impaired nutrient absorption and altered metabolism due to the HIV status (Piwoz and Preble 2000). This high demand of nutrients in the context of deficiency is likely to impact negatively on the growing foetus and health status of the woman, including her mental health.

The main methods for assessment of individual present or recent food intake include keeping records, 24-hour recall and food frequency questionnaires (Food and Agriculture Organization of the United 2009). Where quantities of nutrients consumed are required, a food composition database or table is often required to convert the dietary intake into nutrient levels. Variations however exist in the list of foods and nutrient content of foods in the food composition tables due to real differences in nutrient content of the food or methods of data collection and analysis (Charrondiere et al. 2013; Greenfield and Southgate 2003). Food items from different regions may contain different nutrient levels partly due to environmental and ecological differences as well as production and processing methods, hence the need for region-specific food composition databases.

2.5 Role of omega-3 fatty acids in depressive disorders

Depression has been associated with reduced levels of omega-3 EPA and DHA fatty acids. Previously, it was believed that it is the DHA that boosts the brain levels of serotonin, which is the good-mood or feel-good neurotransmitter (Stoll 2001). Intakes of omega-3 fatty acids with higher levels of DHA alone have however shown no effect on depression as demonstrated by the randomized clinical trial by Marangell et al. (2003) where there was no significant difference between DHA mono-therapy (2 g/day) and placebo in the treatment of adult outpatients with non-psychotic major depression. Further research by Makrides et al. (2010) with 800 mg/day of DHA and 100 mg/day of EPA among depressed pregnant women did not also find any significant difference in depression response

Whether DHA or EPA is the most important therapeutic ingredient in omega-3 fish oil has been a controversial issue in omega-3 fish oil and depression research (Rees et al. 2008). Emerging research evidence however now indicates that it is the high EPA content and not

DHA in omega-3 that is responsible for reducing depressive symptoms. Lesperance and colleagues (2010) reported that omega-3 fish oil with a higher concentration of EPA than DHA is effective in management of depressive symptoms among depressed adults.

2.6 Omega-3 fatty acids in pregnancy and depression

Adequate intake of long chain omega-3 fatty acids is essential during pregnancy to support normal growth and maturation of many foetal organ systems, particularly the brain and eyes (Hornstra 2003; Innis and Friesen 2008). For pregnant women therefore, their level of omega-3 fatty acids rapidly decline, especially after the second trimester of pregnancy, as some of it is transferred to the foetus for the rapid formation of the foetal brain cells (Hornstra 2003).

Although some studies have shown contradictory results of the effect of omega-3 fatty acids on depression after delivery (Jones and Papamandjaris 2001; Llorente et al. 2003) levels of omega-3 fatty acids have been reported by other researchers to be significantly lower in women with depression after delivery than in non-depressed women (Su et al. 2008). A significant increase in plasma levels of omega-3 fatty acids has also been reported in some of the clinical trials among depressed pregnant women (Rees et al. 2008; Su et al. 2008) . This could be an indication of the reduced levels of the nutrient in pregnancy. The resultant depletion in omega-3 might precipitate the occurrence of depression in pregnant women unless the nutrient deficit is met through dietary intake of omega-3 rich foods or supplementation. Clinically, 2 to 5 grams of omega-3 fish oil supplements per day is recommended for adults (Stoll 2001).

Studies on the efficacy of omega-3 fatty acids on depression during pregnancy have revealed mixed results. The question of whether administration EPA and DHA content of

the omega-3 is effective in the prevention or treatment of prenatal depression still remains unanswered (Jans et al. 2010). The inconsistent findings are not only from different study designs, but also from similar designs of randomized double-blind, placebo-controlled trials (Freeman 2008; Makrides et al. 2010; Rees et al. 2008; Su et al. 2008). However, some of the randomized clinical studies (Freeman 2008; Rees et al. 2008; Su et al. 2008), concluded that omega-3 polyunsaturated fatty acids may have therapeutic benefits on depression during pregnancy. The differences in the results among these randomized studies is attributed to methodological differences, particularly the total omega-3 supplement dosage and EPA and DHA content of the supplement, as well as differences in measurement tools for efficacy levels.

When DHA fatty acids alone (Makrides et al. 2010) or higher content of DHA than EPA (Rees et al. 2008) were used, no significant reduction in depression following omega-3 fish oil supplementation in depressed pregnant women was found. In another study, there was a significant reduction in depressive symptom scores from baseline, although there was no significant benefit of omega-3 fatty acids over placebo (Freeman 2008). Even though a higher EPA content than DHA was used in studies that reported significant reduction in depressive symptoms, the total omega-3 content of the supplement in Freeman and colleague's study was less than the recommended dose of 2 to 5 grams per day (Stoll 2001). There was however a significant 50% reduction in depression among pregnant depressed women was observed following omega-3 EPA rich supplementation of 3.4g/day (EPA=2.2g/day; DHA=1.2g/day) (Su et al. 2008). The high EPA content in the omega-3 supplement therefore seems to be responsible for improvements in depressive symptoms as seen in the trend from the research finding summaries in Table 1.

Table 1- Dosage of Omega-3 fatty acids supplementation in Pregnancy

Reference	Omega-3 supplementation	Major findings
(Rees et al. 2008). (n=26)	6g fish oil/day (27.3% DHA; 6.9% EPA) for 6 weeks	Although there was no significant difference in depression between omega-3 and placebo group there was a significant change in depression scores over the intervention period. There was a significant rise in plasma levels of omega-3 fatty acids (83.7 (SD=26.5) μL^{-1} at baseline and 139.7 (SD=56.1) μL^{-1} at the end of the study while there was no difference in placebo group.
(Su et al. 2008) (n=36)	EPA=2.2g/day; DHA=1.2g/day; For 8 weeks	50% reduction in depression at end of intervention ($p<0.05$).
(Freeman et al. 2008) (n=49)	Four capsules daily (8 weeks) 1.9g/day (EPA=1.1g/day; DHA=0.8g/day)	There was no significant benefit of omega-3 fatty acids over placebo. There was however significant decreases in EPDS and HAM-D scores ($P<0.0001$) from baseline.
(Marangell et al. 2003) (n=35)	DHA 2g/day for 6 weeks	There was no statistical difference in response rates between DHA group and placebo group.
(Marcus et al. 2004) (n=3,472)	No omega-3. Only screened for depression	Past depression history, health status, alcohol use smoking, being unmarried, unemployment, and lower educational level were significantly associated with prenatal depression.

2.7 Omega-3 fatty acids and HIV/AIDS

Interventions on omega-3 fatty acids in HIV infection have focused on the effect on triglycerides and cluster of differentiation 4 (CD4) cell count. For example, omega-3 fatty acid supplementation has been shown to reduce triglyceride levels in patients receiving antiretrovirals (Oliveira and Rondo 2011; Wohl et al. 2005). Omega-3 fatty acid supplementation has also been shown to increase CD4 cell count. Where as, Gerber et al. (2008) and Ranieri (2007) did not find any effect of fish oil omega-3 on CD4 cell count, other studies (de Luis et al. 2005a; Isaac et al. 2008) found that dietary intake of omega-3 fatty acids increases CD4 cell count.

2.8 Safety of omega-3 supplements in pregnancy and HIV/AIDS

Safety of omega-3 fatty acids in pregnancy and HIV/AIDS has been established and documented (Gerber et al. 2008; Su et al. 2008). The fish oil has also been found to be safe, well tolerated for these HIV infected patients receiving highly active antiretroviral therapy (Gerber et al. 2008; Wohl et al. 2005). Gerber and colleagues also found that use of omega-3 fatty acids among HIV infected patients is immunologically safe at doses up to 4.8g/day of omega-3 (EPA=3.0g/day; DHA=1.86g/day). Fish oil has therefore been recommended as second-line therapy for HIV patients with hyper-triglycerimia (Bennett et al. 2008).

Research also shows that fish oil supplementation during pregnancy is safe for the foetus and may have beneficial effects on infant development (Dunstan et al. 2008). In pregnancy, doses of up to 3.4g/day (EPA 2.2g/day and DHA 1.2g/day) omega-3 has been found to be safe and effective on major depressive disorders with no major side effects apart from fishy taste (Su et al. 2008) However, the use of this amount of omega-3 dose has not been documented among HIV-infected pregnant women. Other side effects among non-HIV infected pregnant women have been reported as mild stomach upset, nose bleeding and loose stools. Higher doses of omega-3 which do not have a balanced proportion of omega-6 fatty acids may however cause a partial and time-limited reduction in platelet's ability to form clots, (Stoll 2001) In HIV infection, up to 4.8g/day of omega-3 (EPA=3.0g/day; DHA=1.86g/day) has been found to be immunologically safe (Gerber et al. 2008).

2.9 Methodological issues in fish oil omega-3 and depression research

2.9.1 Tools for screening and measuring depression in pregnancy

Studies on depression in pregnancy and HIV infection have used a variety of assessment tools. Among the screening tools that have been used for assessment of depression in pregnancy are Hamilton Rating Scale for Depression (HAM-D) (Freeman et al. 2008;

Marangell et al. 2003; Rees et al. 2008; Su et al. 2008); Centre for Epidemiological Studies-Depression scale (CES-D) (Marcus et al. 2004), Beck Depression Inventory (BDI) and Beck Depression Inventory-Second Edition (BDI-II) (Su et al. 2008) and Edinburgh Postnatal Depression Scale (EPDS) (Freeman et al. 2008; Goodman and Tyer-Viola 2010; Manikkam and Burns 2012; Rees et al. 2008; Su et al. 2008). These tools however use different cut-off points to define depression levels (Table 11).

Table 2: Depressive symptom assessment tools used in pregnancy studies

Reference	Depression assessment tools
(Makrides et al. 2010) (n=2,320)	EPDS; Cut-off point of more than 12 scores
(Rees et al. 2008) (n=26)	EPDS – Cut-off point of more than 13 scores; HAM-D – Cut-off point of more than 14 scores; Montgomery Asberg Depression Rating Scale – at least 25 scores;
(Su et al. 2008) (n=36)	HAM-D – Cut-off point of at least 18 scores; EPDS – Cut-off point at least 12 scores and BDI-II
(Freeman et al. 2008) (n=49)	HAM-D; EPDS.
(Marcus et al. 2004) (n=3,472)	CES-D – Cut-off point of at least 16 scores;
(Marangell et al. 2003) (n=35)	Montgomery-Asberg Depression Rating Scale – Cut-off point of at least 12 scores. HAM-D; Cut-off point at least 17 scores.
(Smith Fawzi et al. 2007)	Hopkins Symptom Checklist-25 (HSCL-25)
(Goodman and Tyer-Viola 2010)	EPDS - Cut-off point of more than or equal to 10 scores
(Manikkam and Burns 2012)	EPDS

The overlapping of the symptoms and the variation in assessment tools therefore requires careful consideration for a tool that will give comparable results of the prevalence of depression with other similar studies. In this study, the BDI-II was considered the most appropriate tool for assessment of depressive symptoms for several reasons. First, it was not the intention of the study to diagnose for specific depressive disorders. Second, the BDI-II tool has a comprehensive list of depressive symptoms that are exhibited in both pregnancy

and HIV infection conditions. Third, the BDI-II tool has been demonstrated to be reliable and valid in screening for depression (Bennett et al. 2004). Fourth, the tool has been used and validated in HIV-positive patients in other parts of the world (Lipps et al. 2010a) and in African study settings, including HIV context (Kagee et al. 2014; Kim et al. 2014; Ndetei et al. 2010). The individual questions of the BDI-II (Beck et al. 1996) is a set of 21-item tool which assess mood, pessimism, sense of failure, self-dissatisfaction, guilt, punishment, self-dislike, self-accusation, suicidal ideas, crying, irritability, social withdrawal, body image, work difficulties, insomnia, fatigue, appetite, weight loss, bodily pre-occupation, indecisiveness and loss of libido. The 21 items on BDI-II are scored and interpreted as follows: Scores of 0-13: minimal depression; 14-19: mild depression; 20-28: moderate depression and 29-63: severe depression.

2.9.2 Dosage of fish oil omega-3 fatty acid supplementation

Studies on omega-3 fatty acids and depression in pregnancy have used varying dosages of omega-3 fish oil supplements. The EPA and DHA content of the omega-3 fatty acid supplements used are also different in various studies. Initially, it was thought that DHA alone was responsible for mood. Studies that have used higher ratios of DHA in the management of depression demonstrated intake of DHA alone did not lead to a significant reduction in depression (Makrides et al. 2010; Marangell et al. 2003). Su and colleagues (Su et al. 2008) from Taiwan, in a randomized, double-blind, placebo-controlled trial reported a significant reduction in depression with omega-3 supplementation using a higher EPA of 2.2 g than DHA of 1.2 g providing a daily dosage of 3.4g omega-3 supplement per day. Although this dosage of 3.4g/day is within the daily range of 2g to 5g which is recommended for clinical conditions (Stoll 2001), it is higher than what is commonly

produced by most omega-3 manufacturing companies and can only be obtained through special request.

Studies on effect of omega-3 fatty acids on HIV/AIDS conditions have also used varying dosages. Wohl et al. (2005) used a higher dose of EPA than DHA in their omega-3 dose content for the study and found that it was safe and well tolerated by HIV patients receiving antiretroviral (ARV) therapy. Gerber et al. (2008) demonstrated that doses as high as 3g/day of EPA and 1.8g/day of DHA are clinically safe for HIV/AIDS patients on highly active antiretroviral therapy (ARV). Both studies have used a higher EPA than DHA omega-3 supplement dose content.

2.9.3 Determination of omega-3 fatty acid cellular levels

Omega-3 fatty acids have been investigated extensively to determine their effect on various health conditions using different biomarkers to determine the fatty acid status (Klingler et al. 2011). Among the biomarkers that have been used to determine omega-3 fatty acid levels are blood, adipose tissue and cheek cells (Fekete et al. 2009; Grindel et al. 2013; Klingler et al. 2011). Collection of samples of blood or adipose tissues is often the most commonly used method in most studies (Fekete et al. 2009; Grindel et al. 2013). However, obtaining these samples is difficult as it requires painful invasive procedures that are not well accepted (Abraham et al. 2013). Use of phospholipid fatty acid composition of human cheek cells (buccal mucosa) as a fatty acid biomarker was proposed by McMurchi et al. (1984). Lapillonne et al. (2002), in their fatty acid profile of the piglets found that the cheek cell phospholipid content of most of the PUFA reflects that of liver, skeletal muscle, and adipose tissue phospholipids as well as the contents of plasma and/or erythrocyte phospholipids. Other human studies have also confirmed that cheek cell phospholipids

compare well with plasma and erythrocyte phospholipids (Connor et al. 2000; Grindel et al. 2013; Harris et al. 2004; Hoffman et al. 1999).

Cheek cell levels of omega-3 fatt acids reflect the physiological status of the human body with respect to these fatty acids. They are easy to access and therefore a non-invasive technique that can be used where collection of blood or adipose tissue samples may pose methodological difficulties (Connor et al. 2000; Grindel et al. 2013; Klingler et al. 2011). Samples can be collected by mouthwash (McMurchi et al. 1984) or scrapping the buccal membrane (Connor et al. 2000; Harris et al. 2004) without a trained clinical personnel; hence the method is suitable for large intervention studies (Klingler et al. 2011). Cheek cell sampling with mouth rinse yields adequate cells for fatty acid analysis (Hoffman et al. 1999; Klingler et al. 2011). The fatty acid concentration in cheek cell samples may however be lower than in blood or plasma samples when small quantities of the buccal mucosa tissues are collected. The small quantities of cheek cells can however be increased through several mouthwash swabs although the mouth wash methods seems to be more hygienic and non-invasive way of collecting these cheek cells than the swabs method.

Once the biological specimens have been collected, analysis of fatty acids is normally a 3-step process: extraction of lipids, conversion (trans-esterification) of the extracted lipids to a volatile derivative, often to fatty acid methyl esters (FAMEs) and analysis of the FAMEs by gas chromatography (GC) for the fatty acid profile (Ratnayake and Galli 2009). The method of Bligh and Dyer (Bligh and Dyer 1959) is the one widely used for extraction of lipids in solution. Separation of lipid fractions is normally through Thin Layer chromatography (TLC). Separation of individual fatty acids however requires use of Gas Chromatography (GC), Gas Chromatography-Mass Spectrometry (GC-MS) or high performance liquid chromatography (HPLC) (Arab and Akbar 2002; Handayani and Budimarwanti 2010).

Identification and quantitation of specific fatty acids requires addition of a known internal standard of similar composition as the samples while interpretation of the levels of fatty acids demands for an understanding of factors that can influence either the measurement or actual levels of fatty acids in the tissues collected and analyzed (Arab and Akbar 2002).

2.9.4 Assessment of compliance in fish oil omega-3 supplementation

Adherence to omega-3 fish oil supplement intake in a clinical trial may be a challenge for some study subjects, and can lead to biased study results. Among the reasons that may lead to lack of adherence could be forgetting to take the medication. Rees et al. (2008) however found that forgetting to take the omega-3 fish oil capsules occurred only occasionally across the trial, and equally across both treatment groups.

Minor adverse effects of unpleasant breath/taste, heartburn, burping and nausea resulting from taking the fish oil supplements (Freeman et al. 2008; Rees et al. 2008) may contribute to poor compliance in omega-3 fish oil interventions. The contribution of these adverse effects to the fish oil supplements have not been reported by study subjects in most clinical trials. Assessment of compliance in interventions with omega-3 fish oil is equally a challenge to researchers, just the same way adherence may be to the subjects. Inquiry on missed doses and pill counts at each visit (Freeman et al. 2008) is one of the methods that has been used. Plasma levels of omega-3 fatty acids have also been used to confirm the study subjects adherence to the study dosage (Makrides et al. 2010; Rees et al. 2008).

2.9.5 Intervention duration for omega-3 interventions for depression

It is documented that some individuals may feel the positive effects of omega-3 supplements 2-4 days after initial dosage, while others may take several weeks to months

(Stoll 2001). The duration of randomized controlled trials on effects of omega-3 fatty acids on depression among pregnant women is documented as between six to eight weeks (Freeman 2008; Makrides et al. 2010; Marangell et al. 2003; Rees et al. 2008; Su et al. 2008). Su and colleagues (2008) reported a significant statistical differences in change in depressive symptoms after six weeks and eight weeks of intervention with fish oil omega-3 EPA-rich supplements while other studies did not report a significant difference. It is however worth noting that different studies have used different ratios of EPA and DHA. The interpretation of the research findings on the actual duration of taking omega-3 supplements for depression relief however still remains inconclusive as variations exist due to differences in total omega-3 dosage used, differences in the EPA and DHA ratios of the omega-3 fish oil and tools for measuring depression.

2.9.6 Soybean oil as a placebo in fish oil omega-3 research

The choice of the most appropriate placebo for the control group in essential fatty acid research is a challenge (Bradbury et al. 2004). Most studies on the role of long chain omega-3 EPA and DHA fatty acids have used plant-based oils such as olive oil, soybean, canola, palm oil, corn oil or sunflower (Grosso et al. 2014) for control groups. These plant-based omega-3 sources do not contain EPA and DHA but are a source of monounsaturated fatty acids and polyunsaturated shorter long chain omega-3 ALA fatty acids. The ALA is the main source of dietary omega-3 EPA and DHA which is consumed as plant-based in soybean, green leafy vegetables, and certain seed oil (flaxeed, chia, canola), Although soybean oil has been shown to increase omega-3 levels in cases of deficiency (Holman et al. 1982), the metabolic conversion of ALA omega-3 fatty acid to EPA and DHA among healthy adults is limited (Burdge and Wootton 2002; Pawlosky et al. 2001; Stoll 2001) and only about 1% in infants (Turchini et al. 2011). The conversion process may be further reduced by about 50% due to high dietary intake of omega-6 fatty acid.

2.10 Gaps in knowledge on omega-3 fatty acids and depression research

The studies reviewed here examined the role of omega-3 EPA and DHA fatty acids on depression, pregnancy, HIV infection and depression in pregnancy. There is however a gap in research and documentation of the role of omega-3 fatty acids on depression in pregnancy in the context of HIV infection. While depression is common in pregnancy and HIV infection also induces depression, a combination of both pregnancy and HIV infection in the complex context of high nutrient demand, yet poor intake and metabolism may worsen the depression effects in HIV-infected pregnant women. Variations exist on the actual duration of taking fish oil omega-3 supplements as well as well as depressive symptom assessment tools. This study was an attempt to answer the research question of whether fish oil omega-3 EPA-rich supplements reduce depressive symptoms among HIV-seropositive pregnant women.

CHAPTER 3: STUDY DESIGN AND METHODOLOGY

3.1 Introduction

The debate on efficacy of omega-3 fatty acids on depression is inconclusive due to variations in study designs although some randomized clinical trials (Freeman 2008; Rees et al. 2008; Su et al. 2008) indicate therapeutic benefits during pregnancy. The observed inconsistency in several previous study findings (Freeman 2008; Makrides et al. 2010; Rees et al. 2008; Su et al. 2008) is attributed to differences in the ratio of omega-3 EPA and DHA, depression assessment tools, intervention duration and physiological status of study participants. For example, whereas Freeman and colleagues (2008) used a lower omega-3 fatty acid ratio of 1.1 g/day EPA and 0.8 g/day DHA and found no significant benefit of omega-3 over placebo, Su and colleagues (2008) reported a significant reduction in depression with a higher omega-3 content of 2.2 g/day EPA and 1.2 g/day DHA. Thus, this can be a presumption that a higher dose of omega-3 EPA may be more beneficial in reduction of depressive symptoms.

The purpose of this study was to contribute to the scientific debate on use of fish oil omega-3 EPA-rich fatty acids in nutritional support and management of depression and on related health complications in HIV-infected pregnant women. The objective of this chapter of the thesis is therefore, to describe and rationalize the study design. The methods used to achieve specific objectives, including data collection methods are however described in separate relevant chapters in this thesis.

3.2 Study design

This was a randomized controlled trial (RCT) with two parallel groups. The RCT was double-blind where neither the research administrators, including the principle investigator, nor participants were aware of the difference between fish oil omega-3 and soybean oil soft

gels during the trial period. Both study products were similar in physical appearance. The intervention group received fish oil omega-3 supplements while the control group received soybean oil soft gels. Only the statistician who assigned participants to the study groups as per the randomization list and the person who conducted the allocation concealment knew the difference between the two products. These two people did not participate in any other research activity involving direct contact study participants nor the data generated from the research.

3.3 Trial products

The treatment group received three soft gels of fish oil omega-3 fatty acid for each day, each containing more EPA (0.715 grams) than DHA (0.340 grams). The total daily intake of omega-3 fatty acids from the three fish oil soft gels was therefore 3.17 grams (EPA=2.15 grams/day; DHA=1.02 grams/day) for each participant in the treatment group. The control group received three soft gels of soybean oil per day, each containing saturated fatty acids (0.178 grams), monounsaturated fatty acids (0.299 grams) and polyunsaturated fatty acids (0.985 grams) with traces of EPA (0.115 grams). The total EPA intake in the control group was 0.345 grams/day. Both the fish oil omega-3 fatty acids and soybean oil soft gels were provided by Innovix Pharma Inc, California, manufacturers of OmegaVia fish oil products. The trial products were similar in shape, colour and taste.

The trial products were analysed by an independent laboratory in Nairobi at the International Centre of Insect Physiology and Ecology (ICIPE) to confirm their actual content of omega-3 fatty acids. Omega-3 fatty acid content of the trial products of fish oil and soybean soft gels as per the manufacturer's specifiction and confirmatory test are as shown in Table 2 and *Appendix 10.* The confirmatory test indicated that fish oil omega-3 soft gels had less EPA (-34.62 mg) and more DHA (+89.95 mg) than indicated on the

manufacturerer's analysis certificate. In the soybean oil soft gels, the test showed that there was more EPA (+115.09 mg) than the levels shown on the analysis certificate.

Table 3- Confirmatory tests of trial products' omega-3 content

Product		Manufacturer specification per soft gel (mg)	Confirmatory test results per soft gel (mg)	Actual omega-3 FAs in the 3 soft gels in Trial (mg)
Fish oil omega-3 (intervention)	Total omega-3	1060	1066.59	3199.77
	EPA	750	715.38	2146.14
	DHA	250	339.95	1019.85
Soybean oil (Control)	Polyunsaturated fatty acids	700.	985.38	2956.14
	EPA	-	115.09	345.27

3.4 Study setting

The study was conducted in Nairobi at the city county's, Riruta Health Centre, Mathare North Health Centre, Kariobangi North Health Centre and Kayole-II Sub-district Hospital. These were health facilities under the management of the City Council's Health Department and were located within low to middle-income residential estates of Nairobi City (Figure 4). The four study sites of Kayole II Sub-district Hospital, Mathare Health Centre, Riruta Health Centre and Kariobangi North Health Centre were purposively sampled because they provided antenatal care (ANC) and a comprehensive PMTCT services to HIV infected pregnant women. These facilities were also selected because they had the highest number of enrolments in PMTCT in the City Council of Nairobi's health department during the preparatory phase of this study in August to October 2011. Their proximity to the University of Nairobi, School of Medicine, also conferred logistical advantage.

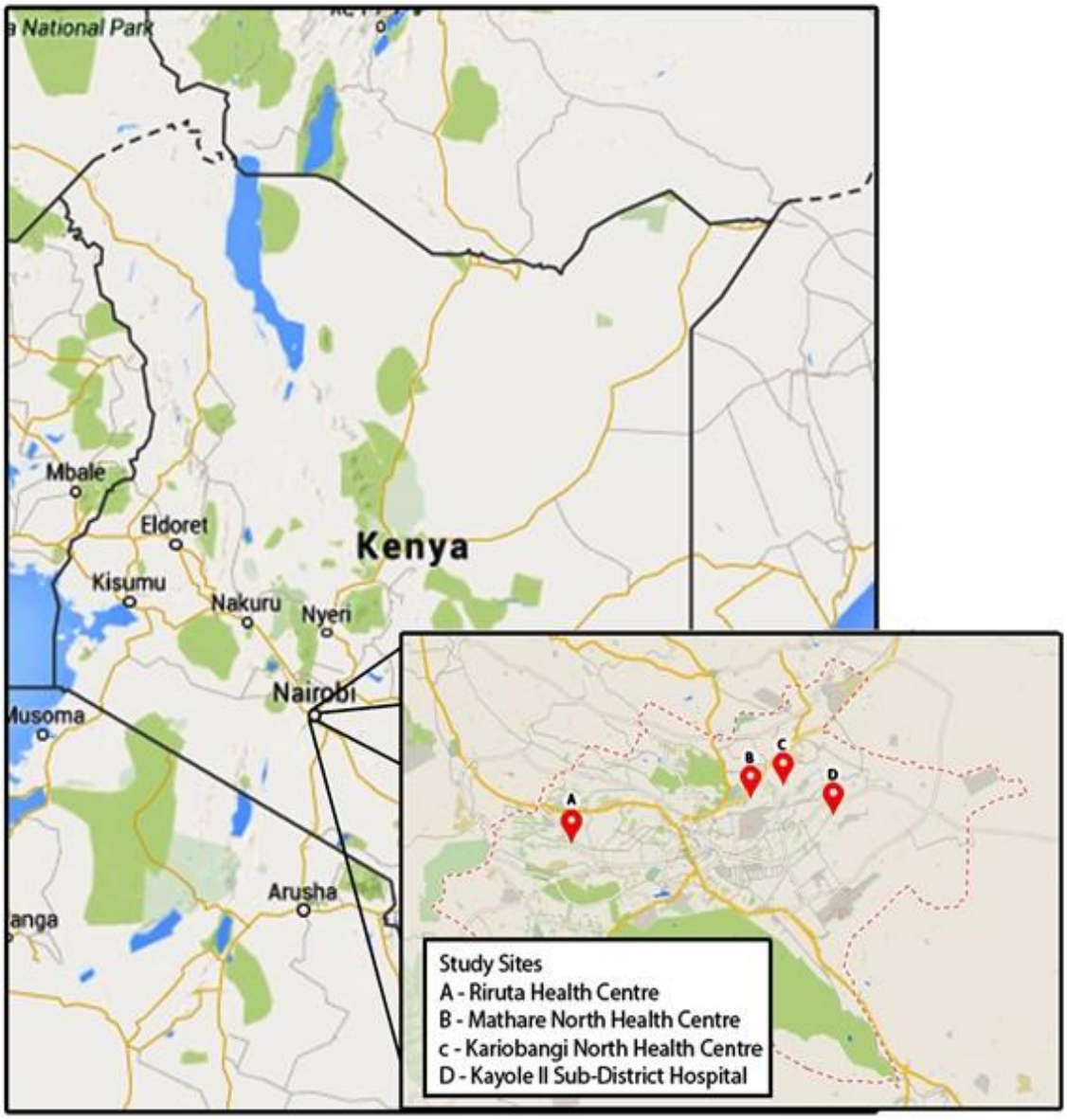


Figure 4 - Map of Kenya showing study sites in Nairobi

3.5 Study population

The study population was HIV-positive pregnant women enrolled in the Prevention-of Mother-to-Child-Transmission (PMTCT) of HIV AIDS programs and attending antenatal clinics (ANC) at Nairobi City Council's facilities. The choice of these already persuing PMTCT services was an advantage in that they were already committed to a programme and hence were more likely to discern the possible benefits and remain compliant to the usage of prescribed medication. The ANC clinic data captured at the City Council Health Department every three months indicated that during August to October 2011, the HIV-positive pregnant women attendance was 263 from the four selected facilities.

3.6 Sample size

The sample size was determined based on an assumption of omega-3 intervention difference of lowering depressive symptom scores by an estimated 20% as measured by Beck Depression Inventory, Second Edition (BDI-II) Scores. Su and colleagues reported a 50% reduction in depressive symptom scores after eight weeks of intervention with fish oil omega-3 (Su et al. 2008). A change of 20% would be a change of at least 4 units. A sample size of 91 women per study group gave an 85% power to detect as statistically significant at 5% level (α=0.05), a true difference of four scores in the mean depressive symptom scores between women given fish oil omega-3 EPA-rich supplements and women given soybean oil, assuming a within group standard deviation of nine in depressive symptom scores. Assuming that the mean depressive symptom scores after eight weeks in the control group was 20 scores; a decrease of four units corresponded to a 20% reduction in the depressive symptom scores. This sample size was calculated in STATA version 11 statistical software (*Box 1*).

Box 1: Study sample size calculated in STATA version 11

Using the estimated sample size calculation for two-sample comparison of means, we
Test Ho: m1 = m2, where m1 is the mean in population 1 and m2 is the mean in population 2
Assumptions:
sampsi 20 16, sd1(9) sd2(9) n1(91) n2(91)

Estimated power for two-sample comparison of means Test Ho: m1 = m2, where m1 is the mean in population 1 and m2 is the mean in population 2

Assumptions:
alpha = 0.0500 (two-sided)
m1 = 20
m2 = 16
sd1 = 9
sd2 = 9
sample size n1 = 91
n2 = 91
n2/n1 = 1.00

Estimated power: 0.8504.

The sample size was adjusted for a 10% non-response due to drop-outs and losses to follow-ups, the following formula was used to calculate the final adjusted sample size: n* = n/(1-q), where n*=adjusted sample size, n=sample size before adjusting, and q=the proportion expected for non-response (Friedman et al. 1998), estimated at 10%. When adjusted for the 10% non-response due to drop-outs, a total of 200 participants with at least mild depressive symptoms (Beck et al. 1996) were enrolled in the study, with 100 participants randomized in each of the study groups. However, during the trial, 16% of the study participants dropped out instead of the planned 10%. An additional 16 participants were therefore recruited with approval from Kenyatta National Hospital/University of Nairobi Ethical Review Committee (KNH/UoN – ERC), the institutional review board. Hence, a total of 216 participants were enrolled in the study, 109 on fish oil omega-3 intervention and 107 on soybean soft gel control arm.

3.7 Randomization procedure

Randomization allocation sequence was computer generated by an independent statistician, not involved in the study, to ensure allocation concealment of the intervention. Block randomization of four fixed blocks. A 1:1 allocation ratio was used to achieve balance in allocating the intervention to participants in the four study sites and to reduce within group variability. For every block of four participants, two were allocated to each intervention group. The four blocks used were consistent with the four study sites of Kariobangi, Riruta, Mathare and Kayole health centres. Six possible balanced combinations of assignment of either fish oil or soybean oil group in four blocks were computer generated as: BBAA BABA, BAAB, ABBA, ABAB and AABB. Participants were allocated to each group by randomly selecting one of the six combinations and assigning them according to the specified sequence as described by Efird (2011). In each study site, the allocation of intervention followed a general pattern illustrated in Box 2, depending on the combination block randomly selected and the number of participants sampled in each site.

Box 2: Allocation of intervention to participants

Combination of allocation	ABBA BBAA BABA BAAB …
Participant ID	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16…

The randomization sequence generated for this study (*Appendix 3*) was confidentially kept from everyone who was blinded, including the principal investigator, until the end of the trial activities. The sequence was only known to the statistician who generated it and assigned participants to the interventions. A backup of the sequence was also confidentially and securely kept in the Director's office at the School of Public Health incase anything happened to the statistician at the time of unblinding.

3.8 Allocation concealment

Allocation concealment refers to the technique used to implement the randomization sequence (Schulz and Grimes 2002) by protecting the randomization list and allocation codes such that the allocated treatment is not known until the study is un-blinded. This was achieved in this study by involving someone who did not participate in recruitment, enrolment, data collection and monitoring or data management to allocate the intervention to participants using plastic bottles. The plastic bottles were identical, opaque, securely sealed and sequentially numbered according to the study randomization sequence generated. During enrolment therefore, each participant received a sequentially numbered, securely sealed opaque bottle containing either fish oil omega-3 fatty acid supplement or soybean oil soft gels to take for two weeks before returning for re-supply.

Unblinding of the trial products was performed by the statistician in the presence of the researcher, and witnessed by at least one academic supervisor and the Director of School of Public Health after completion of data collection, data entry and preliminary analysis (Appendix 12). This procedure of unblinding the trial completely eliminated any source of bias during administration of the study products.

3.9 Sampling and recruitment of participants

Participants' sampling frame consisted of HIV-seropositive pregnant women with known CD4 cell count of not more than 500 cells/µl, on antiretroviral therapy (ART) and in their second trimester of pregnancy at 14 to 27 weeks when omega-3 fatty acid levels are expected to decline during pregnancy (Hornstra 2003) and enrolled in PMTCT program at these facilities. The CD4 cell count of not more than 500 cells/µl was chosen as criterial for selection because during the protocol writing phase of this study, 500 cells/µl was the WHO thresh-hold for antiretroviral therapy initiation (WHO 2010).

Recruitment of participants started in July 2012 and was a continuous process which ended in May 2013. It was not practical to get all the pregnant women who met the study inclusion criteria at the same time. All eligible HIV-positive pregnant women were recruited as they visited the PMTCT in respective clinics using the study standard operating procedure (SOP 2 in *Appendix 7*) which defines the inclusion criteria.

Mentor mothers of *mother2mothers (m2m)* non-governmental organization who provide peer education and psychosocial support in PMTCT assisted with the recruitment process. Two approaches were used during recruitment where one approach was applicable for pregnant women who had enrolled previously in the PMTCT, and the other approach was applicable for pregnant women who visited PMTCT for the first time during recruitment period. The first approach of recruitment was therefore used to recruit women who were already in the PMTCT program. The mentor mothers of *m2m* compiled a list from their PMTCT records of HIV-positive pregnant women with CD4 cell count of not more than 500 cells/µl and were in their second trimester of pregnancy at 14 to 27 weeks. The listed women were contacted by phone and requested to visit the clinic. The women who came to the clinic were informed of the study and its purpose as well as benefits. Those who accepted to participate in the study were screened for inclusion criteria. The second recruitment approach was used to recruit all HIV-infected pregnant women who were not registered in the PMTCT program but were probably visiting the facility for the first time. They were invited to participate in the study and were screened for enrolment in the study. Those who met the eligibility criteria and gave their consent to participate were enrolled by research assistants for the 8-weeks study period. Monitoring and data collection were conducted every two weeks: at the beginning, half way during the trial and at the end of the trial (SOP 3 in *Appendix 6*).

3.10 Training of data collection team

The data collection was done by nutrition and clinical psychology graduates. They were trained by the researcher, with assistance from a psychiatrist and biochemist using a pre-developed training curriculum (Appendix 4), on data collection tools and principles of good clinical practices (GCP). They undertook the GCP online course at East African Consortium for Clinical Research (EACCR) website "http://www.eaccr.org/nodes/training/" and printed out a certificate of completion of the course. This online course equipped the research assistants with the basic principles of good clinical practices in the conduct of clnical trials. Specifically, the online GCP course helped the research team understand what GCP is, the basic principles of GCP, what it means to be GCP "qualified", responsibilities of the investigator and practical approaches to GCP.

3.11 Pre-test of the study

Data collection tools were pre-tested on a sub-sample of HIV-infected pregnant women at Langata, Mathare North and Kariobangi North health centres. Participants in the pre-test sub-sample were not part of the study sample. The main purpose of the pre-test was to determine reliability of the data collection tools and to test the safety of the trial products. Although omega-3 fish oil supplements have already been shown to be safe in pregnancy (Su et al., 2008) and in HIV infection (Gerber et al. 2008), it was necessary to conduct this safety test among HIV-infected pregnant women. The pre-test involved randomizing a sub-sample of six HIV-seropositive pregnant women who had depressive symptoms with a BDI-II score of more than 14 to receive either omega-3 or soybean soft gels for two weeks. The standard individual questionnaire, the BDI-II depressive symptom scoring tool and food frequency questionnaire were administered during the pre-test. The pre-test participants were monitored for 8 weeks for adherence and any serious adverse events (SAE) or suspected unexpected serious adverse reactions. There was no adverse side effect reported

or observed during the 2-weeks pre-test period and throughout the 8-weeks period among these pre-test participants. One of the pre-test participants on fish oil omega-3 who had varicose veins on her legs during randomization reported the disappearance of the veins after 4 weeks in the study. She was not on any medication for the varicose veins.

3.12 Ethical considerations

Ethical approval to conduct the study was obtained from the Kenyatta National Hospital/University of Nairobi Ethical Review Committee (KNH/UoN – ERC, P266//6/2011), the Ministry of Education, Research and Technology (NCST/RRI/12/1/MED011/167) and from Pharmacy and Poisons Board of Kenya (PHD/MOH/R.1/101/2011/ac). Copies of ethical approval are in Appendix 11. This study was also registered at the international web-based resource registry, ClinicalTrials.gov, as ID-NCT01614249. Both written and oral informed consent were obtained from the study participants after providing them with information on the objectives, risk and benefits of study. The written consent explanation form was in two language-versions: English language and Kiswahili language versions (*Appendix 1* and *Appendix 2*). Confidentiality was maintained through use of participant code numbers instead of their names.

All participants from both study groups received all the necessary treatments for HIV-seropositive pregnant women given at the PMTCT throughout the trial period. Participants also received transport reimbursements of Kenya Shillings, 150.00 during the study period.

Although no serious side effects of the intervention products have been reported before, both known and unknown possible adverse events from the study were closely monitored. There was no adverse side effect reported or observed during the 2-weeks pre-test period and throughout the 8-weeks trial period among the pre-test participants and all trial

participants. However, other non-serious adverse events were reported, and the proportion of participants reporting was below the 5% threshold level (*Appendix 8*).

The trial sample products of fish oil omega-3 and soybean oil were only used for purposes of the study. The principal investigator was responsible for disposal of the remaining samples as per the approved bio-safety rules. It was not the intention of this study to diagnose specific depressive disorders. However, BDI-II scores of more than 30 were considered as severe depression and participants were closely monitored for referral to psychiatrists for diagnosis and management.

3.13 Inclusion criteria

All HIV-seropositive pregnant women with known CD4 cell count of not more than 500 cells/µl, were on antiretroviral therapy (ART) and in their second trimester of pregnancy at 14 to 27 weeks were invited to participate in the study. Only those who consented to participate by signing the consent form and had at least 14 scores on Beck Depression Inventory Second Edition (BDI-II) scoring questionnaire were enrolled.

3.14 Exclusion criteria

i. Underweight with Mid-upper arm circumference (MUAC) less than 22 cm and overweight with MUAC more than 33 cm;
ii. Anyone with a medical history of use of a blood thinning medication/anti-clotting medication for health conditions like liver problem, varicose veins, peptic ulcers; or use of Vitamin K supplement was excluded as omega-3 supplements may increase the effects of these medications;
iii. Use of antidepressant medications two weeks prior to the study;
iv. Anyone who was on diabetic medication to lower blood sugar was excluded because

Omega-3 may increase the blood sugar level.

3.15 Selection of participants for enrolment

Only those women who met the inclusion criteria, with a normal MUAC of 22 cm to 33 cm, not on medication for blood thinning conditions, diabetes or antidepressants two weeks prior to the study and had signed the consent form were screened for depressive symptoms. Participants who had depressive symptoms and scored 14 or more on the BDI-II depressive symptom scoring tool were enrolled in the study. They were randomly assigned to either fish oil omega-3 or soybean oil intervention group as per the Consolidated Standards of Reporting Trials (CONSORT 2010) statement (Schulz et al. 2010).

3.16 Quality control measures

3.16.1 Blinding of all individuals involved in trial activities

This study was double-blinded such that both the study participants and those administering the interventions, including the principal investigator were unaware of the intervention/treatment allocation. Specifically, the following individuals involved in the trial activities were blinded from the intervention: principal investigator, study participants, health facility personnel, and research assistants and data clerk handling the data.

3.16.2 Standard operating procedures

A summary of standard operating procedures were developed as a step-by-step guidelines to ensure uniformity in intervention implementation and data collection as per the research protocol (Appendix 7). The principal investigator and research assistants followed these guidelines to carry out all the research activities in the four study sites.

3.16.3 Calibration of equipment

The gas chromatograph machine for analysis of cheek cells for omega-3 fatty acids cellular levels was calibrated by the Kenya Bureu of Standards. A calibration curve was further generated (Chapter 4, section 4.3) for quantification of the EPA and DHA fatty acids.

3.16.4 Data and safety monitoring board

A data and Safety Monitoring Board (DSMB) was constituted to monitor the safety and treatment efficacy of the omega-3 supplements during the clinical trial period. The DSMB was an independent group of experts comprising of a pharmacist, gynaecologist, biostatistician and a psychiatrist (SOP 10 in *Appendix 7*). The committee held two meetings, at the beginning of the trial and at the end of the trial. However, during the trial, communication on progress and updates of the trial between the Principal Investigator and committee members was through exchange of emails and cell-phone.

3.16.5 Monitoring of compliance in taking trial products

Each participant provided cell-phone contact details during recruitment. Those who did not have personal numbers or cell-phones provided numbers of their husbands, relatives or neighbours. Each participant was encouraged to use the research cell-phone contact on the treatment container to contact the research team whenever necessary. Regular cell-phone contacts were therefore established with each participant immediately after enrolment in the study. Participants were contacted at least two times a week to monitor intake of the soft gels and to remind them of the next visit to the study site. Further confirmation as to whether all the soft-gels were swallowed was done during the bi-weekly face-to-face visits for re-supply of trial products and data collection. During these visits, each participant was asked whether she had any soft gel left in her container at home, and the number. Participants were expected to have remained with only one or two soft gels in their last container before starting the next re-supply container. Any participant, who reported more

soft gels left in her container than the expected number, was required to give reasons for non-completion. Participants who had soft gels for five days or more were dropped out of the study for non-compliance. Analysis of compliance in the study, which did not significantly vary between the two groups, is presented in Appendix 8 of this thesis.

3.17 Data collection methods

3.17.1 Demographic, socio-economic, health and HIV-related characteristics

Collection of baseline data was a continuous process depending on when a participant was recruited, screened and enrolled in the study. Standardized individual questionnaires were used to collect data on participants' demographic, socio-economic, health and HIV-related characteristics (*Appendix 6*). Participants' age, gestational age and CD4 cell counts data were obtained from their individual health records and recorded in the questionnaires. The data on health blood pressure and anthropometry were obtained by taking participants' actual measurements instead of recording from their health booklets where some data were missing. Each participant's blood pressure was measured on the left arm using a digital blood pressure monitor (*Citizen CH-453:* C*itizen Systems Japan Co. Ltd.*). Participants had their blood pressure taken while in a sitting position, with the left arm on the table.

The anthropometry measurements taken were weight and mid-upper arm circumference (MUAC). The MUAC, which is a measure of the diameter of the upper arm, and is considered as an indicator of body wasting, was used as a selection criterion for screening for participants' nutritional status during recruitment. Only participants with normal nutritional status as defined by MUAC measurements of not less than 22 cm and not more than 33 cm met the inclusion criteria and were recruited. The MUAC measurement was taken in centimetres using a fibreglass MUAC tape-measure for use in adults. Each measurement was taken at mid-point between the bony tip of the shoulder (acromion

process) and the point of the elbow olecranon process. The measurements were taken at mid-study and end of study data collection points to monitor nutritional status of participants throughout the study period.

Since MUAC is independent of gestational age and varies little during pregnancy (Bruce 2001), pregnancy weight gain indicator was used to monitor nutritional status of study participants during the trial. Weight measurements were taken using electronic weighing scales *(seca 881 U, seca Deutschland)* to the nearest 0.1 kilograms. Participants stood on the weighing scale with their bare feet and light clothing. All jackets and cardigans were removed. In case a participant had items in her pockets, she was asked to remove everything before standing on the weighing scale. Each participant who completed the study had her weight taken three times: at enrolment, at week-four after enrolment and at week-eight during the main data collection points.

3.17.2 Collection of Dietary intake data

Dietary intake data were collected using a combination of diet history checklist, recall of foods eaten and semi-qualitative food frequency questionnaire. A summary of dietary data collection and nutrient intake computation methods are shown on Figure 5. The dietary intake data were collected in two phases. First, a rapid household survey was conducted in 13 conveniently sampled households, and second, dietary intake data were collected among study participants at baseline data collection, mid- study and end of the study.

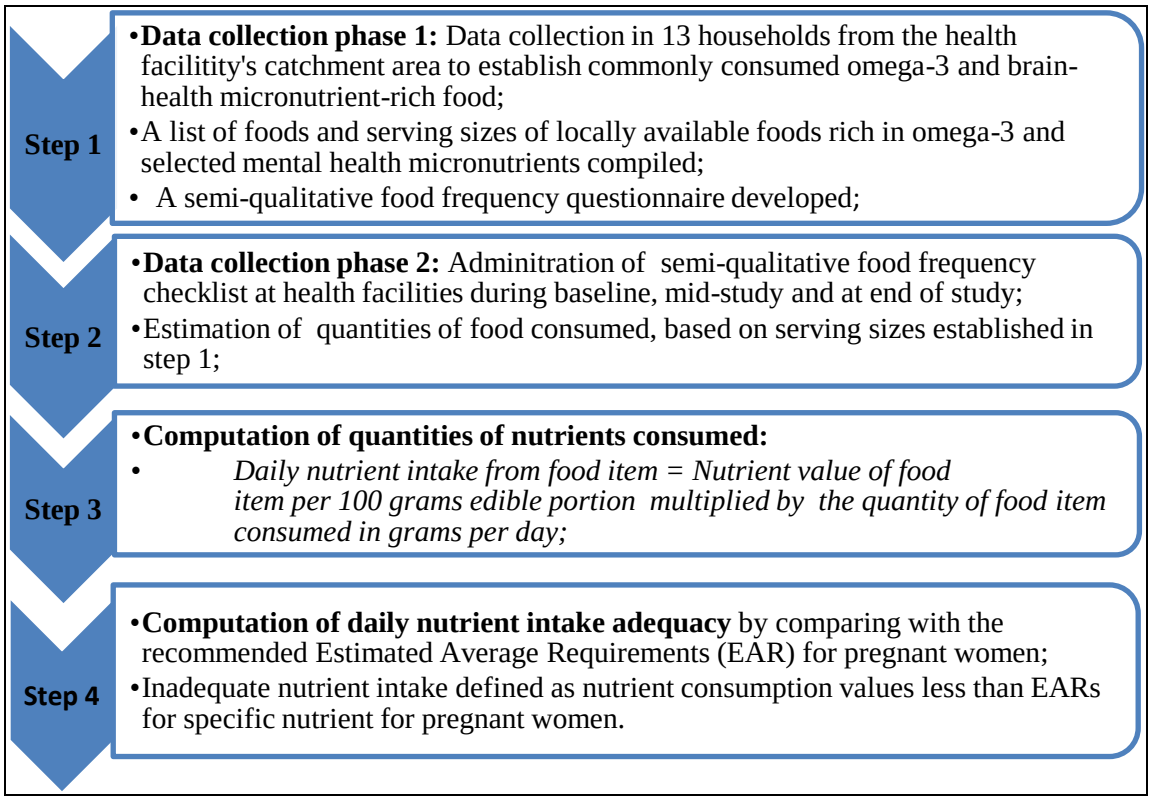


Figure 5 - Summary of dietary data collection and nutrient intake computation

The first phase of dietary intake data collection aimed at establishing locally available foods rich in omega-3 and mental health micronutrients of B-complex vitamins, vitamin C, vitamin E, iron, calcium, selenium, folate and zinc. All the 13 households which were included in this phase of the study had women of child-bearing age and were within the study health facilities' catchment area. These households were identified by a community healthcare worker attached to the health facility. The mothers were interviewed using a checklist containing food rich in omega-fatty acids and brain-health micronutrients. They were asked the types of foods they normally consume and quantities in local serving utensils (spoons, cups and plates) per meal. Mothers were asked to show utensils they normally used for serving food. The serving size of foods that were consumed whole (eggs, fruits, slice of bread) was determined by weighing each unit of the food that was purchased by the researcher in the locality. A similar method was used to determine the serving size of food consumed as cuttings of a unit (chicken and fish). Vegetables, meat and legumes were

served using serving spoons or table spoons; hence the amount of food consumed was estimated from these utensils' scoops. Cooking fat and oil were estimated using table spoons while milk was estimated using volumes purchased. Whole grain maize meal (*ugali*) and millet porridge were estimated from the amount of flour used per serving. All the food items were weighed on a 5000 g/1g - digital kitchen scale *(Ramtons Kitchen Scale, Model No: RM/299).* A list of the locally available foods rich in omega-3 and mental health micronutrients and their serving sizes that were established for this study are found in *Appendix 5*.

The second phase of dietary intake data collection was at the health facility with participants who had been enrolled in the clinical trial. Participants were interviewed using a semi-qualitative food frequency questionnaire that required them to recall the foods eaten and amount served. Food quantities consumed were estimated from the pre-established serving sizes and utensils. The quantities of nutrients consumed from each food item were computed from existing food composition data bases (Maundu et al. 1999; Nutrisurvey.de 2007; Sehmi 1993; The National Academies 2005; The National Agricultural Library 2011). The daily nutrient consumption values of each food item were computed in *IBM SPSS* Statistics soft-ware version 20 using the following formula:

> *Daily nutrient intake from food item = Nutrient value of food item per 100 grams edible portion X the quantity of food item consumed in grams per day*.

The individual total daily dietary nutrient intake was then computed by summing up the nutrients calculated from all the foods consumed in the last 24 hours. Nutrient adequacy was determined by comparing the study population's median nutrient intake values with the Estimated Average Requirements (EAR) for pregnant women (FAO/WHO 2005). The

EARs are the amount of nutrient required to meet the nutrient requirement of half the healthy individuals in a life-stage and sex group (Murphy and Vorster 2007). They were computed by dividing the recommended dietary allowances (RDAs) for pregnant women (FAO/WHO 2005; The National Academies 1998; The National Academies 2001) by the corresponding conversion factor for each nutrient (Gibson 2012). Nutrient bioavailability was considered and computed at 15% for iron (WHO/FAO 2004) and 30% for zinc (Gibson 2012; WHO/FAO 2004) Inadequate micronutrient intake was defined as nutrient consumption values below the EARs for pregnant women for a given nutrient.

Due to lack of internationally agreed upon dietary reference intakes (DRI) for omega-3 EPA and DHA fatty acids, it was not possible to determine adequacy of their dietary intake in this thesis. Although some countries (Canada, Sweden, United Kingdom, Australia and Japan) and international organizations have recommendations for EPA and DHA intake of 200 to 500 mg/day for healthy adults, formal DRI values for these nutrients are yet to be established (Flock et al. 2013; Food and Agriculture Organization of the United Nations 2010; Kris-Etherton et al. 2009; Opperman 2013).

3.17.3 Cheek cell samples for omega-3 fatty acid celluar levels

Collection of cheek cell samples

The data was collected from HIV-seropositive pregnant women who received either fish oil omega-3 EPA-rich supplements (EPA= 0.715 grams; DHA=0.340 grams) as intervention arm or soybean oil (saturated fatty acids=0.178 grams), monounsaturated fatty acids=0.299 grams) and polyunsaturated fatty acids (0.985 grams) with traces of EPA (0.115 grams) as control group. The fish oil omega-3 for intervention group and soybean oil soft gels for control group were from Innovix Pharma Inc, California.

Data was collected at three points. The first data collection point was at baseline before randomizing participants into intervention groups. The second point was four weeks after intervention with fish oil omega-3 and soybean oil and the third point was at the end of the study, eight weeks after intervention. During each data collection point, participants were asked to thoroughly rinse their mouth with a swirl three times using distilled water and expectorate the second and third wash into a disposable cup. The first mouth rinse was assumed to contain food particles; hence all participants were required to discard it. This procedure is described in the research standard operating procedure (SOP 6 *Appendix 6*). From each participant, a sample of 60-70 millilitre (ml) of mouth wash containing cheek cells was collected. This was transferred into labelled centrifuge tubes, placed in a cool box containing ice packs and transported to the laboratory for storage at minus 20 ^{o}C, extraction and analysis of omega-3 fatty acids.

Chemical solvents for analysis of omega-3 fatty acids

Chloroform and methanol (HPLC grade), were used to extract lipids from cheek cell samples. Methanol and dry toluene were used to prepare fatty acid methyl esters (FAMEs) and saturated sodium chloride solution added to break emulsions and allow separation of methyl esters from water soluble impurities. Hexane (HPLC grade) solvent was used for extracting FAMEs. Excess water that could interfere with the esterification process was further removed by adding sodium hydrogen carbornate.

Analytical procedure for omega-3 fatty acids in cheek cells

Analysis of omega-3 eicosapentaenoic (EPA, C20:5n-3) and docosahexaenoic (DHA, C22:6n-3) fatty acids from cheek cells (buccal mucosa epithelial tissues) in the mouthwash samples involved a series of three steps: extraction of lipids from cheek cell samples,

preparation of fatty acid methyl esters (FAMEs) and gas chromatography (GC) identification and quantification of the corresponding EPA, C20:5n-3 and DHA, C22:6n-3 fatty acid methyl esters. The analytical procedure is summarized in Figure 6 and Figure 7.

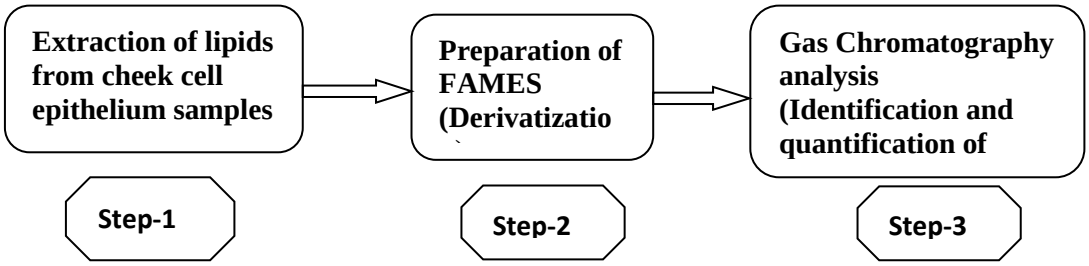


Figure 6- Summary of analytical procedure

i. Extraction of lipids from cheek cell samples

The extraction of lipids was done by solvent extraction method with chloroform-methanol (2:1 by volume) as described by Folch et al. (1957). The mouthwash was first centrifuged to obtain about one gram pellets of epithelium tissues. This was then mixed with 5 ml of chloroform-methanol (2:1) and shaken for one hour in orbital shaker to extract lipids. The mixture was filtered through whatmans filter paper to remove the debris, leaving only lipids in the filtrate. The filtrate was washed in 0.5% sodium chloride (NaCl) solution (volume = 0.2 x volume of filtrate) to remove any contaminants from the lipids. It was shaken well and left to stand for one hour to give a two-phase system of sodium chloride solution at the top and chloroform-methanol and lipids at the bottom. The chloroform phase was recovered according to Bligh and Dyer (1959), by leaving it in an oven at 65 ^{0}C overnight to evaporate to 50 microlitre of chloroform-lipid solution.

Confirmatory tests for the presence of lipids was performed on all the mouthwash samples using a thin-layer chromatography (TLC) before derivatization. This was done by depositing spots of samples on TLC plates. The TLC tank was equilibrated with developing

solvents of Hexane, Diethyl ether and Acetone in a ratio of 50:50:1 for one hour. The samples were then applied as 2 cm streaks to TLC plates (20 cm ×20 cm ×0.15 mm) precoated with Merck silica gel 60 (Schlechtriem et al. 2008)and visualised using iodine in a beaker.

ii. Lipid derivatization

Preparation of FAMEs, also known as derivatization process was conducted by first dissolving the extracted lipid in 1.0 ml of toluene. About 2.0 ml of dry methanol was added and mixed well. The mixture was incubated at 55 ^{0}C overnight, and 4.0 ml of saturated sodium chloride solution added and mixed well by vortex. About 800 microlitre of hexane, HPLC grade, was added to the mixture to extract the FAMEs, and 2% of 3.0 mls of sodium hydrogen carbornate added and vortexed well to absorb any excess water in the samples. This mixture was centrifuged and the upper phase containing hexane and lipids taken and stored in a glass labeled *vial* bottle at minus 20 ^{0}C for gas chromatography analysis

Extraction, derivatization and quantification of fatty acids

Steps in extraction of lipids and derivatization procedures are summarised in *Figure 7*

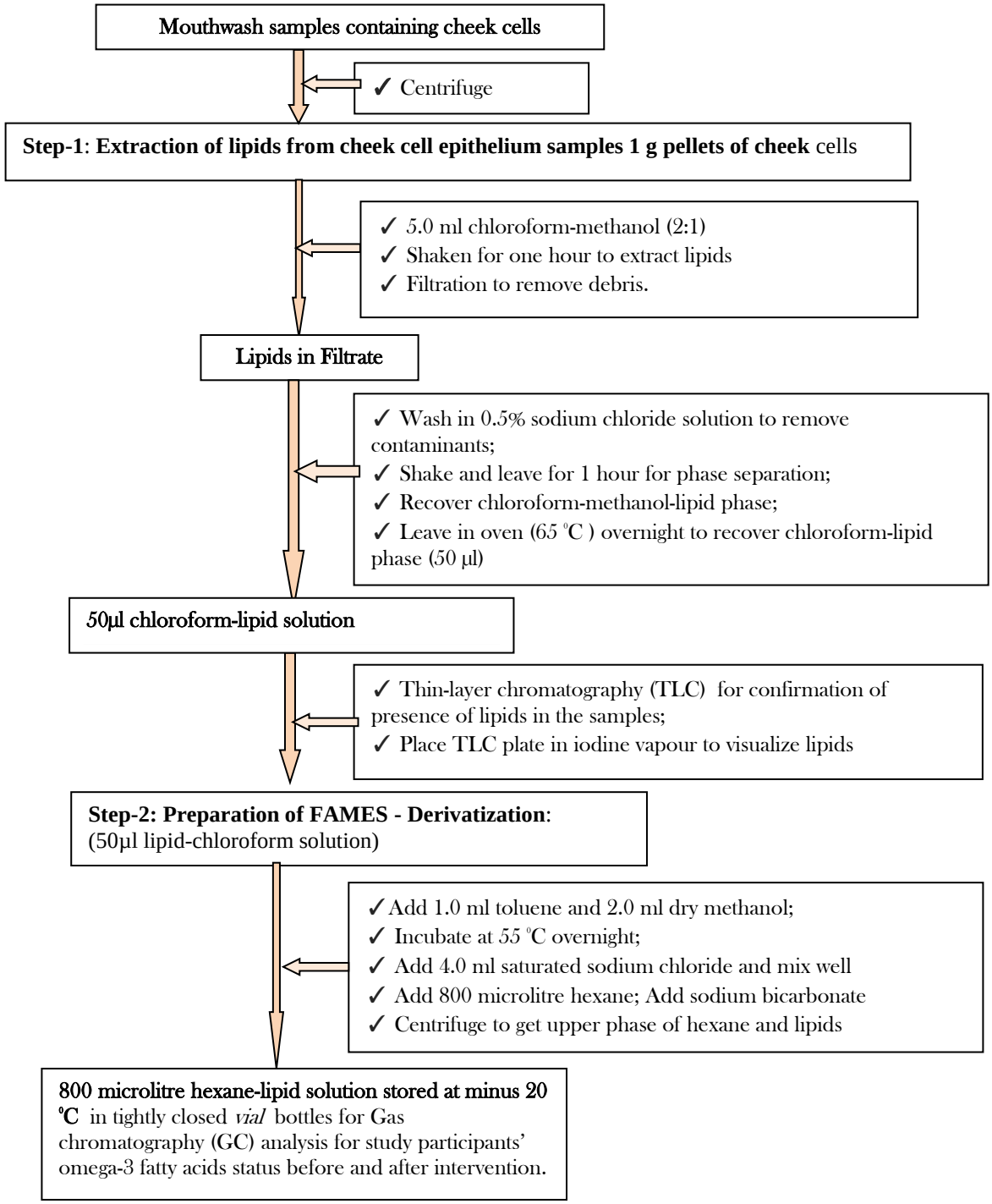


Figure 7 - Steps in extraction of lipids and derivatization procedures

iii. Quantification of EPA (C20:5n-3) and DHA (C22:6n-3) methyl esters

The fatty acid methyl esters were analyzed using *Shimadzu GC-2014* gas chromatograph with flame ionization detector (FID) at the University of Nairobi, Department of Public Health, Pharmacology and Toxicology. Sub-samples of the analyzed samples were also re-analyzed at ICIPE for validation of findings. The analytical column was HP-88 60m by 0.25mm by 0.20µm (*Agilent Technologies-Part No.112-8867).* The instruments and analytical conditions for the GC analysis are summarised in *Appendix 9.*

Quantification of the fatty acids required, first, generation of a standard calibration curve to determine the concentration of EPA and DHA fatty acids from known concentrations of the compounds in an external standard solution. Second, determination of limit of detection (LoD), which is the lowest level of concentration of the analytes (Shrivastava and Gupta 2011), EPA and DHA, in the cheek cell samples that could be detected under the study analytical conditions was necessary. Third, the limit of quantification (LoQ) as the lowest concentration of the analytes in a sample that could be determined with acceptable precision and accuracy under the stated analytical conditions (Shrivastava and Gupta 2011) was determined. Both LoQ and LoD were based on the 0.05% concentration of the Marine oil FAME Mix standard *(RESTEK - catalogue number 35066)* containing 10 g/ml EPA and 12 g/ml DHA methyl esters. A GC-solution Software Version 2.3 (*Shimadzu Corporation catalogue number 223-06290*) was used in quantification of EPA and DHA (*Appendix 9*).

Marine oil FAME Mix standard GC-solution Software Version 2.3

3.17.4 Assessment of depressive symptoms and intervention effect

Data on depressive symptoms were collected using BDI-II 21-item scoring tool. The BDI-II 21-item tool was orally administered to participants after screening for inclusion in the trial. The study inclusion and exclusion criteria are outlined in Chapter 1 and the screening tool is in SOP 5 (*Appendix 6*). Each symptom on the BDI-II tool was scored on a 4-point Likert scale of 0 to 3 based on the severity of each item. In this study, the inclusion criteria was a cut-off score of 14 and above, for mild depression. Therefore, only participants who had scored a minimum of 14 on the BDI-II tool were considered to have symptoms of depression and were enrolled in the study.

Validity and reliability of BDI-II scoring tool

The KII (*Appendix 6*) were first conducted among health workers and a sub-sample of HIV-infected pregnant women to pre-test BDI-II tool and determine content validity of the tool in the context of the current study setting and population. Data from KIIs was collected by note-taking. The KIIs were analyzed under two themes: one, depressive symptoms among HIV-infected pregnant women and two, methods used by health workers in assessment of depressive symptoms. In addition to the pretesting and validation of the data collection tools, qualitative data from KII was also used to support the quantitative findings.

Internal reliability of the BDI-II tool was determined by Cronbach's alpha reliability coefficient (Santos 1999). This was computed in STATA version-11 software (Stata 2009) on pre-test data and on all participants' baseline data to determine the internal consistency of the 21 depressive symptoms on the BDI-II scale in measuring depression among the study population. Normally, Cronbach's alpha values above 0.90 are considered excellent, 0.80 and above are considered good, 0.70 and above are acceptable, up to 0.60 are

questionable, up to 0.50 are poor and less than 0.50 are unacceptable (George and Paul 2010). The BDI-II scoring tool was translated into Kiswahili language, and orally administered to each participant.

3.18 Data analysis

Data were entered in SPSS version 17 software and analyzed in STATA version 11 software (Stata 2009). Continuous data were summarized as median and inter-quartile ranges instead of means and standard deviations. This is because the data do not follow a normal distribution. Categorical data were summarized as proportions using both numbers and percentages. The distribution and flow of participants in the study is summarized according to the CONSORT 2010 requirements for clinical trials (Moher et al. 2010). Proportions of participants reporting each depressive symptom were summarized as counts (n) and percentages (%) for each intervention group

Shapiro-Wilk Test and normal quantile plot were used to test if continuous variables were normally distributed and met the proposed analysis requirements. Descriptive statistics of mean values, median and percentages were used to summarize the data. Reliability of the BDI-II scoring tool was assessed using Cronbach's alpha test of reliability. The distribution of categorical variables across the two study arms was compared using the Chi square test for depression symptoms and levels. The two-sample Wilcoxson rank-sum (Mann-Whitney) test was used to compare dietary intake, cellular level omega-3 EPA and DHA and depression scores which were not normally distributed across the two study arms.

The total BDI-II scores for the 21 items were added and interpreted as follows: minimal range (0 to 13), mild depression (14 to 19), moderate depression (20 to 28), and severe depression (29 to 63). Proportions of participants within each depression level were summarized as counts (n) and percentages (%). Participants' BDI-II total scores throughout the intervention period were not normally distributed, based on Shapiro-Wilk statistical test of normality. They were therefore summarized as median values with IQR. Two-sample Wilcoxon rank-sum (Mann-Whitney) non-parametric equality-of medians test was used to compare the total scores between the two groups since the data was not normally distribted.

The change in BDI-II depressive symptom scores was computed as suggested by Jamieson (2004) in his article on analysis of covariance (ANCOVA) with difference score as follows: *Post-intervention BDI-II scores (at week 4 or week 8) minus baseline BDI-II scores (at week 0).* The change in BDI-II scores at weeks four (mid-study) and eight (end of study) were tested for normality and summarized as mean values with standard deviations (SD), reporting 95 percent confidence interval levels (95% CI). The magnitude and difference in change in BDI-II depressive symptom scores compared between the two study groups by Student t-test. Before adjusting for baseline covariates, simple linear regression model was fitted to determine the variability in change in BDI-II scores that was explained by the intervention. The level of significance for all the inferential tests was at 5%. Regression to the mean effect by any suspected extreme values was controlled by randomization at the study design stage and by analysis of covariance (ANCOVA) model during data analysis. The ANCOVA adjusts each participant's follow-up measurement according to their baseline measurement (Barnett et al. 2005). The analysis followed per-protocol analysis approach, where participants who did not complete the trial were not included for the primary outcome analysis at the end of the study. Those who dropped out of the study before the end of four weeks after enrolment were not included in the mid-study analysis.

Similarly, all participants who dropped out of the study before the end of the trial at eight weeks were not included in the final data analysis.

The intervention effect was compared between the two groups by fitting the ANCOVA regression model. The model included change in BDI-II scores after intervention, all the baseline characteristics and intervention arms. All baseline characteristics were included in the model as covariates to adjust for any possible variations at baseline before randomization into fish oil intervention group or soybean control group, and to control for regression to the mean. Bivariate and multiple linear regression analysis were used to determine which variables were key determinants of BDI-II depressive symptom scores. Heteroskedasticity due to differences in variance in the errors across observations was controlled by robust standard errors analysis in the regression model. The presence of interaction between the covariates (predictor variables) that were found to be significantly associated with the change in BDI-II scores in the ANCOVA model was tested. There was no interaction between the predictor variables.

CHAPTER 4: RESULTS

4.1 Socio-demographic and health-related characteristics of study participants

4.1.1 Distribution and flow of participants in the study

The number of participants recruited; enrolled and completed the study from each study site is summarized in Table 4. The study recruited 282 pregnant women who met the inclusion criterion of HIV-seropositive with CD4 count of not more than 500 cells/mm^3 and gestation of 14 to 27 weeks of pregnancy. A total of 216 participants were enrolled and randomly assigned into two study arms: 109 received fish oil omega-3 and 107 received soybean oil soft-gels for eight weeks.

Table 4 - Distribution of participants by study sites and group

Health Facility*	**Recruited by May 2013****	**Randomized**		**Completed Study**	
		Fish oil	**Soybean oil**	**Fish oil**	**Soybean oil**
Kariobangi H/Centre	51	18	18	13	16
Riruta Health Centre	72	31	30	26	26
Mathare-North	80	28	26	24	24
Kayole	79	32	33	23	30
Total	**282**	**109**	**107**	**86 (78.9%)**	**96 (89.7%)**

**Health facilities were the study sites;*

*** May 2013 was the last date of recruiting participants in the study.*

Participants' flow in the study is summarized in (Figure 5). After screening for enrolment, 66 of the 282 were excluded due to exclusion criteria of mid-upper arm circumference (MUAC) measurement of more than 33 cm (n=1) and depressive symptoms scores being less than 14 (n=65). Administration of fish oil omega-3 and soybean oil soft-gels were after every two weeks with follow-up and monitoring visits.

A total of 182 participants completed the 8-week study period. A total of 34 of them dropped out; 23 from omega-3 arm and 11 from the soybean arm respectively (Figure 5).

Overall completion rate was 84.3% for all the enrolled participants. Completion rates were 78.9% and 89.7% for omega-3 and soybean oil arms respectively. Among the 34 participants who did not complete the eight-week study period, 21 of them had travelled to the rural homes, nine failed to return for bi-weekly re-supply for unknown reasons and four of them gave birth before the end of the trial.

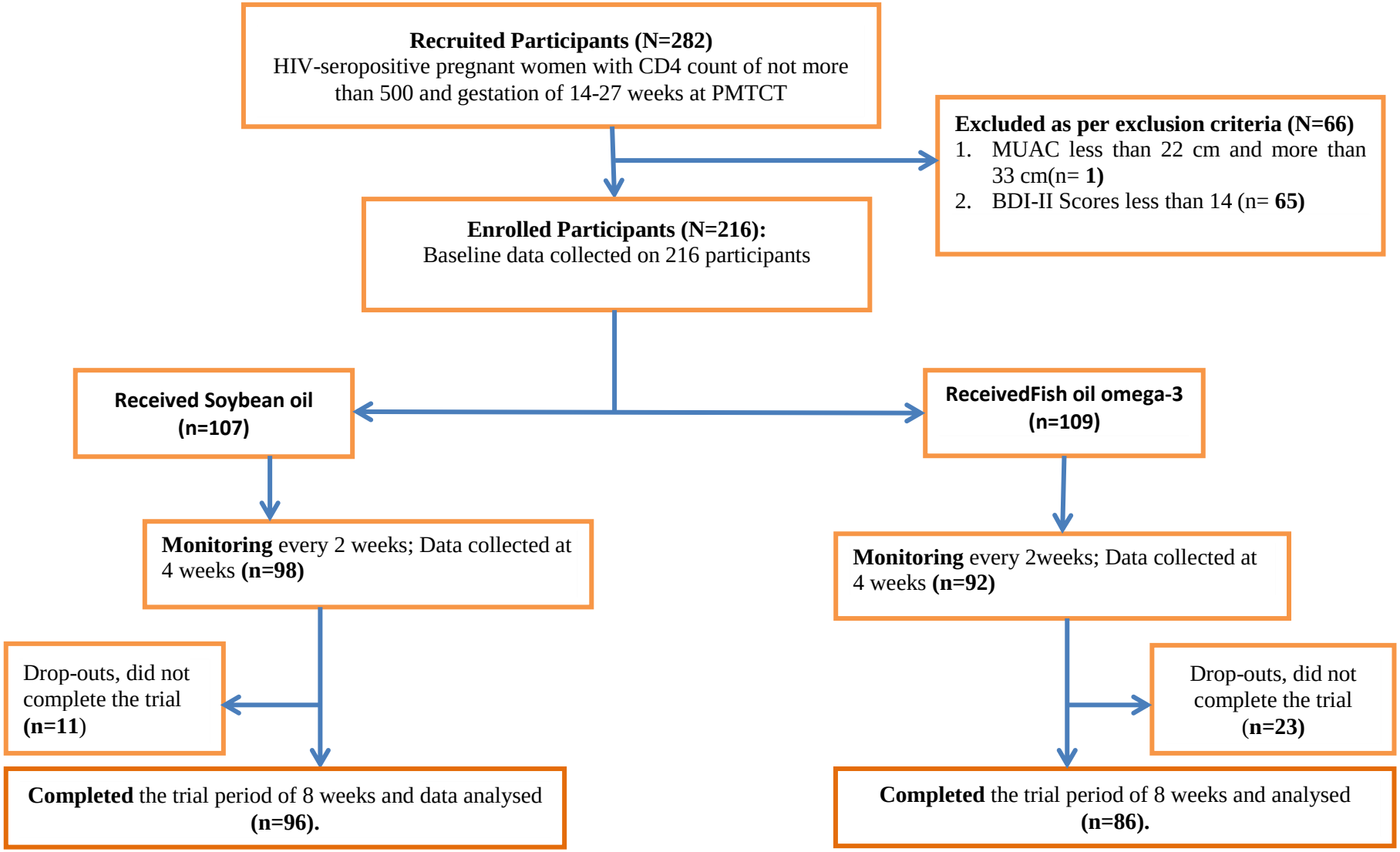


Figure 8 – Participatants' distribution and flow during the trial

4.1.2 Demographic and socio-economic characteristics of participants

Participants' baseline characteristics of demographic, socio-economic, health and HIV status are shown on Table 5. The age distribution was similar in both groups with a median and interquartile range (median (IQR)) of 26 (22-30). The median (IQR) gestational age was 22 (18-24) and 22 (19-24) weeks for fish oil and soybean oil groups respectively. The total household income per month, in Kenya Shillings was a median of 6,000 (3000 – 8000) for fish oil group and 5,025 (3000-8000) for the soybean group. This was the income from the participant and her spouse, together with any other person living with them. Based on this income level, the proportion of households that were below the poverty line, earning less than KSh. 2,913 were also similar in the two intervention groups with 17.5% in fish oil omega-3 and 19.4% in soybean oil group.

At least three quarters of participants in each group were married and living with their spouse, and for more than 75% of them, this was not their first pregnancy (fish oil omega-3=78.7%; soybean=78.3%,). It was also noted that participants had similar education status where those with at least high school education were 49.5 % in fish oil group and 48.6% in soybean oil group. Experiences of stressful life events two weeks prior to the study were also similar in both intervention arms (33.0% in fish oil and 30.0 % in soybean oil group). The stressors included knowledge of HIV status before pregnancy and disclosure (Fish oil = 39.0%; soybean oil = 51.5%), domestic and marital related problems (Fish oil =25.0%; soybean = 45.0%) and financial related issues (Fish oil = 36.1%; soybean oil = 3.0%).

Table 5 - Participants' baseline characteristics by intervention arm

Baseline characteristic	Fish oil intervention group (N=109)	Soybean oil control group (N=107)
Continuous Variables (Median (IQR))		
Age (single years)	26 (22-30)	26 (22-30)
Gestation age (weeks)	22 (18-24)	22 (19-24)
CD4 count (cells/mm^3)	361 (287- 440)	360 (288 – 414)
Weight (Kg)	60.5 (54.2-66.4)	62.0 (57.2-69.0)
MUAC (cm)	26.0 (23.9-27.6)	26.0 (24.3-28.0)
Household income per month (KSh)	6000 (3000-8000)	5025 (3000 - 8000)
Categorical Variables (n (%))		
Age group (in years)		
15- 25	53 (48.6)	48 (44.8)
26-45	56 (51.4)	59 (55.1)
Marital status		
Single (not married, divorced, widowed)	27 (24.8)	22 (20.6)
Married	82 (75.2)	85 (79.4)
Parity status		
First pregnancy	24 (22.0)	24 (22.4)
Not a first pregnancy	85 (78.0)	83 (77.6)
Education status		
No high school education	55 (50.4)	55 (51.4)
At least high school education	54 (49.5)	52 (48.6)
Employment status		
Not in gainful employment	61 (56.0)	64 (59.8)
In gainful employment	48 (44.0)	43 (40.2)
Household income status per month		
Below poverty-line (Less than Ksh.2913)	16 (17.5)	20 (19.4)
At least on poverty line (At least Ksh.2913)	75 (82.4)	74 (78.7)
Experienced stressful life events		
No stressful event 2 weeks before study	73 (66.7)	75 (70.1)
Had stressful event 2 weeks before study	36 (33.0)	32 (30.0)
CD4 cell count levels		
Less than 350 cells/mm^3	44 (40.7)	47 (43.9)
350 to 500 cells/mm^3	64 (59.3)	60 (56.1)
Knew HIV status before pregnancy		
Newly tested (Less than 6 months)	56 (51.4)	56 (52.3)
Known positive (KP) 6 months or more	53 (48.6)	51(47.6)
HIV status Disclosure to anyone		
Status not disclosed to anyone	18 (16.5)	25 (23.4)
Status disclosed to someone	91 (83.5)	82 (76.6)
PMTCT support group meetings attendance		
Not attended support group meeting	62 (56.8)	54 (50.4)
Attended support group meeting	47(43.1)	53 (49.5)

**Household income: Fish oil experimenta group - n=91; Soybean oil control group - n=94*
***CD4 cell count levels: Fish oil - n=108 (participant with missing CD4 was lost to follow-up).*

4.1.3 Health and HIV-related baseline characteristics

The CD4 cell count (cells/mm^3) was similar for participants in both intervention arms. In fish oil omega-3 group, it was (median (IQR)) 361 (287- 440) and in soybean oil groupit was 360 (288 – 414) (p=0.39). About half of participants from each intervention group (Fish oil = 51.4% had knowledge of their HIV status at least six months before the pregnancy while the other half had been newly tested for HIV. More than 75% of them from each group had disclosed their status to a friend, a relative or husband.

All HIV-infected pregnant women were routinely invited by *m2m* peer educators to attend support group meetings as part of the PMTCT program in the study sites. However, only 43.1% and 49.5% of participants from fish oil and soybean oil reported to have attended these meetings. Although attendance in these *m2m* meetings was slightly higher in the soybean oil group (49.5%) than in fish oil group (43.1%) at baseline, this difference was by chance alone since bias in selection of participants was controlled by randomization and allocation concealment. The study observed that during these *m2m* support group meetings, the HIV-infected pregnant women received support and encouragement from the *m2m* peer educators and women who were either pregnant or had given birth with HIV infection. The meetings involved shairing experinces of HIV-infection, pregnancy and child care, followed by a health talk by *m2m* peer educators. Sharing a meal at the end of the health talk gave participants in these meetings an opportunity to interact with each other.

Normal nutritional status based on MUAC was a criterion for inclusion. Participants had similar MUAC measurements of a median of 26.0 (IQR=23.9-27.6) cm and 26.0 (IQR=24.3-28.0) cm in omega-3 and soybean groups respectively.

4.2 Dietary patterns among HIV-seropositive pregnant women

4.2.1 Food consumption patterns

Consumption of various brain-health micronutrient-rich foods was reported by participants at baseline Table 6. The consumption pattern was similar at baseline in both the fish oil and soybean oil intervention groups. Regular consumptions, for at least once per week, were reported for kale (fish oil=63.3%; soybean=61.7%), spinach (fish oil=56.1%; soybean=56.1%), milk (fish oil=54.1%; soybean=51.4%) and vegetable cooking fat (fish oil=oil=50.5%; soybean=55.1%). Other popular foods reported were ripe bananas (fish oil=50.5%; soybean=46.7%), avocado (fish oil=46.8%; soybean=43.0%), whole-grain maize-meal (fish oil=40.4%; soybean=40.2%) and Irish potatoes (fish oil=50.5%; soybean=39.2%).

Regular consumption of meat, chicken and fish was reported by less than 30.0% of participants from both intervention groups. Beef was the most commonly consumed meat (fish oil=22.9%; soybean=28.9%) while goat, mutton and pork were consumed by less than 10.0% participants from each intervention group. *Rastrineobola argentea* (*Omena)* was the most common fish consumed at least once a week by participants from both groups (fish oil=29.4%; soybean=26.2%). The other fish reported were tilapia (fish oil=14.7%; soybean=17.7%) and Nile perch (fish oil=13.8%; soybean=15.9%). None of the study participants mentioned to have ever eaten sea fish.

Table 6 - Brain-health nutrient-rich foods consumed by participants at baseline

Food category	Specific food item	Proportion of participants reporting regular consumption of food by study group (n(%)	
		Fish oil intervention Group (N=109)	Soybean oil control Group (N=107)
Vegetables	Cabbage	39(35.8)	34(31.8)
	Carrots	28(25.7)	21(19.6)
	Kales (S*ukuma Wiki*)	69(63.3)	66(61.7)
	Spinach	61(56.1)	60(56.1)
	Cow pea leaves	38(34.8)	47(43.9)
	Amaranthus (*Terere*)	38(34.9)	41(38.3)
Fruits	Avocado	51(46.8)	46(43.0)
	Mango	41(37.6)	34(31.8)
	Orange	34(31.2)	40(37.4)
	Ripe banana	55(50.5)	50(46.7)
	Pawpaw	12(11.0)	14(13.1)
Whole grain cereals and products	Maize meal	44(40.4)	43(40.2)
	Millet porridge	13(11.9)	6(5.6)
	Wheat (Brown bread)	17(15.6)	18(16.8)
	Pasta/noodles	13(11.9)	8 (7.5)
Fish	Tilapia	16(14.7)	19(17.7)
	Nile Pearch (*Mbuta*)	15(13.8)	17(15.9)
	Rastrineobola argentea (o*mena)*	32(29.4)	28(26.2)
Meat products	Beef	25(22.9)	31(28.9)
	Liver	17(15.6)	15(14.0)
	Goat	6 (5.5)	10(9.3)
	Chicken	11(10.1)	11(10.3)
	Mutton	1(0.9)	6(5.6)
	Pork	3(2.7)	5(4.7)
Milk and milk products	Fresh milk	59(54.1)	55(51.4)
	Yoghurt	22(20.2)	18(16.8)
Eggs	Boiled egg	16(14.7)	12(11.2)
	Fried egg	33(30.3)	28(26.2)
Legumes, nuts, seeds	Cooked Beans	43(39.4)	36(33.6)
	Black beans (*Njahi*)	9(8.3)	5(4.7)
	Green gram stew	26(23.8)	21(19.6)
	Soybean flour	3(2.7)	2(1.9)
	Roasted groundnuts	18(16.5)	17(15.9)
Potatoes	Irish potatoes	55(50.5)	42(39.2)
	Sweet potatoes	25(22.9)	14(13.1)
Edible oils	Vegetable cooking fat	55(50.5)	59(55.1)
	Cooking oil (liquid)	43(39.5)	38(35.5)
	Margarine	20(18.3)	19(17.7)

4.2.2 Multivitamin and other nutritional supplement intake

None of the study participants from both intervention arms reported access and use of purchased nutritional supplements. Use of nutritional supplements received from the health facility as part of routine pregnancy care medication was however reportedly low (Fish oil=19.3% (n=21), soybean=15.9% (n=17)). Except for two participants from fish oil group, those who reported use of nutritional supplements mainly referred to the iron and folic acid (Fish oil = 17.4%; Soybean=15.9%) they received at the health facilities. The two participants who reported use of other supplements referred to the multivitamin tablets (*Univit multivitamin*, a *PMC product, Kenya Reg. No. H97/408)* received during ANC visits. The labels on the product containers had vitamin A (2500 IU), vitamin D_3 (250 IU), vitamin B_1 (1.0 mg), vitamin B_2 (0.5 mg), vitamin C (15.0 mg) and nicotinamide (7.5 mg) per tablet.

4.2.3 Adequacy of dietary brain-health nutrient intake levels

The dietary brain-health nutrient intake values from the reported foods are as listed in Table 7. At baseline, the median nutrient intake was below the EAR for pregnant women for all nutrients consumed by participants in each of the intervention groups. The intake values were least for omega-3 EPA and DHA fatty acids whose median (IQR) EAR values were zero in the Fish oil group and 0 (0–0.01) grams in the Soybean oil group. Throughout the study period, Vitamin C median (IQR) intake values were higher than its EAR value of 71 mg/day for pregnant women at week-4 (Fish oil: 96.5 (39.1–226.5) mg/day; Soybean oil: 84.8 (39.4–188.4) mg/day) and week-8 (Fish oil: 91.2 (31.2-196.6) mg/day; Soybean oil: 93.9 (38.2- 18.6). At week 4, Vitamin B1 intake was also more than the EAR (1.2 mg/day) for pregnant women in the fish oil group in week-4 (1.3(IQR: 0.6-2.6) and week 8 (1.6 (IQR: 0.7-2.9) mg/day and

soybean oil group in week 8 (1.5 (IQR: 0.8-2.9)) mg/day. The median intake for zinc (EAR=2.8 mg/day) also increased at week-4 in fish oil group (1.6 (IQR: 0.5-2.9)) mg/day; and week-8 in both groups (Fish oil: 3.2(IQR: 1.7-4.6) mg/day; Soybean oil: 2.9 (IQR: 1.8 – 5.1). The median intake values for the rest of the nutrients remained below the EAR for pregnant women throughout the study.

Table 7 – Median dietary nutrient intake levels by study arm and period

		Nutrient intake levels by study arm and period					
Nutrient	**[a]EAR**	**Fish oil arm (Median (IQR))**			**Soybean oil arm (Median (IQR))**		
		Baseline (N=109)	**Week-4(N=92)**	**Week-8 (N= 86)**	**Baseline (N=109)**	**Week-4(N=98)**	**Week-8 (N= 96)**
Vitamin C (mg);	71	63.5 (15.0 - 121.6)	96.5(39.1– 226.5)	91.2 (31.2-196.6)	61.5 (15.1-191.2)	84.8(39.4-188.4)	93.9(38.2-218.6)
Vitamin B1 (mg)	1.2	0.8 (0.2 - 1.8)	1.3(0.6- 2.6)	1.6 (0.7-2.9)	0.7 (0.2-1.8)	0.9(0.5-2.4)	1.5(0.8-2.9)
Vitamin B6 (mg)	1.6	0.7 (0.3 – 1.4)	1.1 (0.7 – 2.1)	1.0 (0.7-2.2)	0.7 (0.4-1.3)	1.0(0.6-1.5)	1.1(0.6-2.7)
Vitamin B12 (mcg)	2.6	0.5 (0 – 1.4)	0.9(0 – 2.1)	0.9 (0.4-2.3)	0.4 (0-1.4)	0.8 (0.1-2.4)	1.0(0.4-2.7)
Folate (mcg)	520	106.4 (46.2-302.7)	255.1(88.9-461.7)	296.2(99.0-511.9)	178 (23.7-360.0)	192.1(68.9-503.2)	293.7(95.1-508.6)
[b]Iron (mg)	3.3	1.8 (0.6 – 3.6)	3.1(1.5 – 4.1)	2.8(1.7-4.2)	2.3(1.1 – 3.2)	3.2(1.6 – 3.3)	3.2(2.2 – 4.7)
Vitamin E (IU)	12.5	4.6 (2.5-8.6)	7.1 (4.7-11.8)	6.3(4.4-10.1)	4.7 (2.0-9.6)	6.6(3.7-11.5)	6.9(4.0-14.4)
Calcium (mg)	833	411.0 (238.1-916.1)	600.9(337.2-1653.8)	598(415.6-1506)	448.8 (206.5-930.6)	652.7(378.2-2347.1)	665.8(403.4-2210.8)
Selenium (mcg)	50	10.1 (4.0-50.1)	34.3(6.7-75.2)	31.8(7.8-85.6)	9.5 (3.3 -56.7)	21.8(7.5-63.6)	41.3(9.3-96.6)
[c]Zinc (mg)	2.8	1.6(0.5-2.9)	2.7(0.9- 4.6)	3.2(1.7-4.6)	1.9 (0.4 – 3.4)	2.6 (1.1 – 5.2)	2.9(1.8 – 5.1)
[d]Total Omega-3 (g)	1.4	0.54 (0.31-0.95)	1.1(0.7-1.6)	1.0(0.6-1.4)	0.62 (0.32-1.14)	0.9(0.5-1.5)	1.1(0.6-1.5)
Omega-3 EPA (g)	N/A	0 (0,0)	0 (0 – 0.5)	0(0 – 0.1)	0 (0,0.01)	0 (0-0.5)	0(0-0.3)
Omega-3 DHA (g)	N/A	0 (0,0)	0(0-0.5)	0 (0-0.2)	0 (0, 0.01)	0(0-0.5)	0(0-0.4)

[a]EAR: Estimated Average Requirements - computed by dividing the recommended dietary allowances (RDAs) for pregnant women (FAO/WHO 2005; The National Academies 1998; The National Academies 2001) by the corresponding conversion factor for each nutrient (Gibson 2012). ;
[b]Iron [c]Zinc: Median dietary intake values computed at bioavailabilitylevels of 15% for iron and 30% for zinc (Gibson 2012; WHO/FAO 2004)
cTotal Omega-3: Dietary reference intake valuesused in place of EAR (The National Academies 2005)
N/A: Not Applicable; recommended nutrient values are yet to be established (Flock et al. 2013).

The proportion of study participants whose nutrient intakes were below the estimated requirements for pregnant women is as shown on Figure 9. At baseline, more than 60.0% of participants from each intervention arm had their dietary nutrient intake levels below the EARs for pregnant women for most nutrients. Only Vitamin C (Fish oil = 56.9%, Soybean = 55.1%) had less than 60.0% of study participants whose median intake were below the EAR level. At the end of the study at week-8, more than 50.0% of participants had their nutrient intake values below the EAR for pregnant women for all nutrients except for Vitamin C (Fish oil: 44.2%, soybean oil: 40.6%), Vitamin B1 (Fish oil: 46.5%, soybean oil: 42.7%) and zinc (Fish oil: 44.2%, soybean oil: 46.9%).

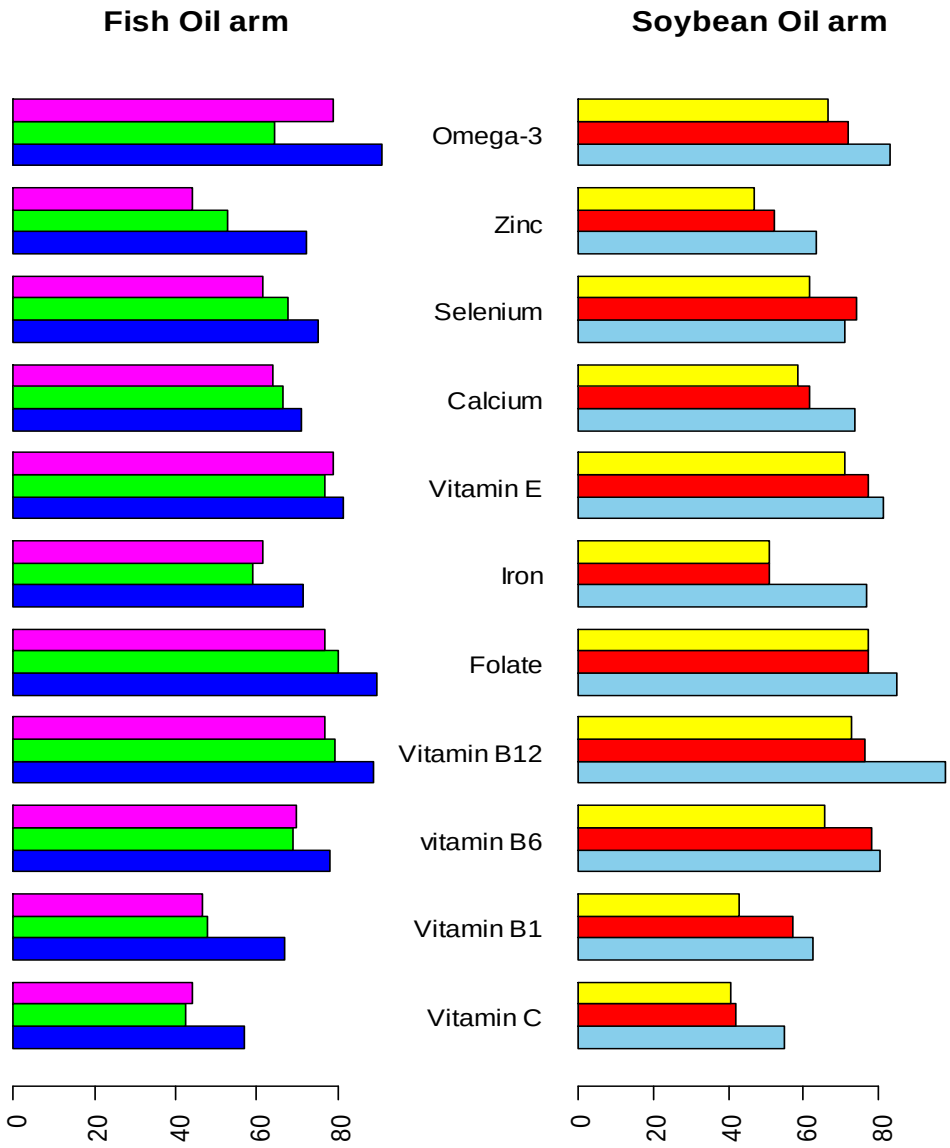


Figure 9: Proportion of participants with nutrient intake levels below the EAR values for pregnant women

Fish oil	Legend	Soybean oil
	Week- 0 (Baseline)	
	Week-4 (Mid-study)	
	Week-8 (End of study)	

4.3 Omega-3 EPA and DHA fatty acid cellular levels in HIV-seropositive pregnant women after fish oil supplementation

Confirmatory TLC test for presence of lipids in cheek cell samples indicated that lipids were present in all the samples. Spots of lipids were present on the TLC plates for all the samples that were tested. The calibration curves were generated from seven levels of concentrations prepared from 0.05%, 0.5%, 5%, 10%, 20%, 30%,40% and 50% of Marine oil FAME Mix standard *(RESTEK -catalogue number 35066)* solution containing 10 g/ml of EPA (C20:5n-3) and 12 g/ml DHA (C22:6n-3) methyl esters. These were used in the quantification of EPA (C20:5n-3) and DHA (C22:6n-3) in this study are shown in Figure 10.

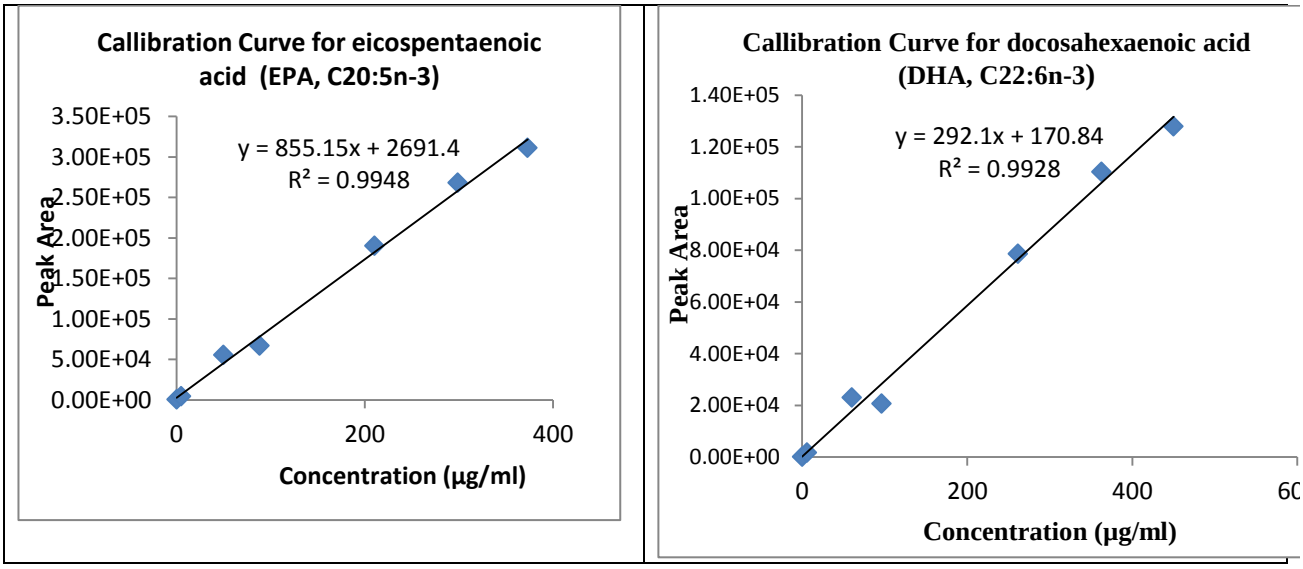


Figure 10 - Calibration curves for EPA and DHA in Marine oil FAME Mix standard

The limits of detection (LoD) for EPA (C20:5n-3) was 0.0086 µg/ml and for DHA (C22:6n-3) it was 0.0043 µg/ml. The limit of quantififcation (LoQ) was 0.026 µg/ml for EPA (C20:5n-3) and 0.013 µg/ml for DHA (C22:6n-3). Table 9 shows the number of cheek cell samples that were analysed in GC for omega-3 EPA and DHA fatty acid concentration levels by intervention arm and data collection period. The minimum and maximum concentration levels of EPA (C20:5n-3) detected in the study samples

analyzed were 0.007 µg/ml and 7.82 µg/ml respectively. The DHA (C22:6n-3) was detected in 100 (17.6%) of the samples (Table 8), with a minimum and maximum concentration levels of 0.011 µg/ml and 7.88 µg/ml respectively. Confirmatory GC re-analysis of samples at an independent laboratory at ICIPE also confirmed similar findings. There was no significant difference in the proportion of samples which had the fatty acid concentrations by intervention group and data collection period.

Table 8: Samples analysed in GC by study group and period

Omega-3 EPA and DHA concentrations (µg/ml)	Fish oil Group	Soybean oil Group	X^2	p-value
Week-0 (Baseline):				
EPA				
Conc. = 0	61	70	0.02	0.88
Conc. > 0	22	21		
DHA				
Conc. = 0	89	84	0.86	0.35
Conc. > 0	15	20		
Week-4 (Mid-study)				
EPA				
Conc. = 0	58	70	0.17	0.68
Conc. > 0	22	23		
DHA				
Conc. = 0	58	77	2.36	0.12
Conc. > 0	17	12		
Week-8 (End of study)				
EPA				
Conc. = 0	65	77	0.57	0.45
Conc. > 0	17	15		
DHA				
Conc. = 0	71	81	0.85	0.36
Conc. > 0	11	8		

The Shapiro Wilk statistical test for normal distribution of data indicated that the omega-3 fatty acids concentration levels (µg/ml) were not normally distributed in the analysed samples ($p<0.05$), hence the data was summarized as medians with interquartile range (IQR). Table 9 shows the concentration levels of omega-3 EPA and DHA that were detected in cheek cell sample by study period. At baseline, the median EPA (C20:5n-3) and DHA (C22:6n-3) levels for the fish oil group were 0.000 µg/ml

(IQR = 0.00 – 0.17, minimum = 0.00 and maximum = 3.47) and 0.000 µg/ml (IQR = 0.00 – 0.00, minimum = 0.00 and maximum = 7.88) respectively. For the soybean group, their median EPA (C20:5n-3) and DHA (C22:6n-3) levels were 0.000 µg/ml (IQR = 0.00 – 0.04, minimum = 0.00 and maximum = 3.19) and 0.00 µg/ml (IQR = 0.00 – 0.00, minimum = 0.00 and maximum = 4.18). There was no significant difference in the fatty acid concentration levels between the two intervention groups at baseline for omega-3 EPA (z=0.32; p=0.74) and DHA (z=-0.78;p=0.43).

At week 4 of the intervention, the median EPA (C20:5n-3) and DHA (C22:6n-3) levels for the fish oil group were 0.000 µg/ml (IQR = 0.00 – 0.21, minimum = 0.00 and maximum = 5.63) and 0.000 µg/ml (IQR = 0.00 – 0.00, minimum = 0.00 and maximum = 4.74) respectively.In the soybean group, the median EPA (C20:5n-3) and DHA (C22:6n-3) levels were 0.000 µg/ml (IQR = 0.00 – 0.00, minimum = 0.00 and maximum = 2.43) and 0.00 µg/ml (IQR = 0.00 – 0.00, minimum = 0.00 and maximum = 6.12).

By week 8, which was also the end of the study, the median EPA (C20:5n-3) and DHA (C22:6n-3) levels for the fish oil group were 0.000 µg/ml (IQR = 0.00 – 0.00, minimum = 0.00 and maximum = 5.75) and 0.000 µg/ml (IQR = 0.00 – 0.00, minimum= 0.00 and maximum = 5.94) respectively. In the soybean group, the median EPA (C20:5n-3) and DHA (C22:6n-3) levels were 0.000 µg/ml (IQR = 0.00 – 0.00, minimum=0.00 and maximum=7.82) and 0.00 µg/ml (IQR=0.00 – 0.00, minimum=0.00 and maximum=5.90).

Table 9 – Concentration levels of omega-3 EPA and DHA in cheek cell samples

Omega-3 fatty acid By period	Fish oil intervention group			Soybean oil control group			Statistical test*	
	n	Median (IQR) (µg/ml)	Minimum, maximum	n	Median (IQR) (µg/ml)	Minimum, maximum	z-value	p-value
Week-0 (Baseline)								
EPA	83	0.00 (0.00– 0.17)	0, 3.47	94	0.00(0.00 – 0.04)	0, 3.19		
DHA	104	0.00 (0.00 – 0.00)	0, 7.88	104	0.00 (0.00 – 0.00)	0, 4.18		
Week-4 (Mid-study)								
EPA	80	0.00 (0.00 – 0.21)	0, 5.63	93	0.00(0.00 – 0.00)	0, 2.43	0.61	0.54
DHA	75	0.00 (0.00 – 0.00)	0, 4.74	89	0.00 (0.00 – 0.00)	0, 6.12	1.70	0.09
Week-8 (End of study)								
EPA	82	0.00 (0.00 – 0.00)	0, 5.75	92	0.00(0.00 – 0.00)	0, 7.82	0.77	0.44
DHA	82	0.00 (0.00 – 0.00)	0, 5.94	89	0.00 (0.00 – 0.00)	0, 5.90	0.91	0.36

**Statistical test based on Two-sample Wilcoxon rank-sum (Mann-Whitney)*

4.4 Depressive symptoms in HIV-seropositive pregnant women

4.4.1 Validity and reliability of BDI-II scoring tool

Qualitative data from KII provided insight on understanding the meaning of depressive symptoms among health workers (Two nurses and one clinical officer), three PMTCT *m2m* personnel and three pregnant HIV-infected women at the health facilities. From the discussions, it was observed that depression is common among HIV-infected pregnant women. Among the depressive symptoms reported by the health workers during these KII were crying, sadness, lack of sleep, loss of appetite, lack of interest in everything, loss of pleasure, feelings of past failure, blaming partners and general mood changes. The HIV-positive pregnant women also concurred that those pregnant women who have HIV infection can be depressed. Two of the women shared their own experiences.

"I bought the rat-rat poison to take after learning about my status…."

"I feel useless because I know I can die even before giving birth to this child, but my husband encourages me."

The third woman gave an example of her friend who was always pessimistic about the HIV status of her unborn child. She said.."*Since she knows I am also HIV positive because I told her, she always asks me: do you think it is true that if I take the medicine and follow what I am told at the clinic my child will not be infected?"*

All the health workers and PMTCT *mentor-mothers* interviewed however observed that although women with depressive symptoms were only identified during counseling sessions, there are no guidelines or tools for assessment of these symptoms.

"There is no particular way of assessing for depression in HIV positive pregnant women. In most cases, those suffering from depression are identified

*through counselling and in support groups where people open up and are advised accordingly" (*PMTCT *mentor-mother).*

According to these health workers, women with depressive symptoms are only identified during counselling, before and after HIV testing, when they open up and are willing to talk about their life**.**

"In most cases clients are assessed for depression during counseling where the client opens up about their view of life with HIV infection, then the counselor advices the client accordingly" (Nurse-in-charge)

Internal validity of the BDI-II scoring tool was assessed using Cronbach's alpha reliability statistical test. The Cronbach's alpha reliability coefficient for the 21 symptoms on the BDI-II tool was 0.91 (95 % Confidence Interval: 0.76 – 0.98; N=6) for pre-test data and 0.73 (95% Confidence Interval: 0.70 – 0.78; N=216) for all participants' data.

4.4.2 Depressive symptoms

All the 21 depressive symptoms on the BDI-II tool were reported by participants from both study arms at baseline (Table 10). More than 50.0% of participants in both arms reported to have experienced 20 of these depressive symptoms. The only symptom which was reported by less than half of participants from both groups was indecisiveness (Fish oil = 45.0%; Soybean oil = 47.6%) where most of them indicated that they had no difficulty making decisions. Any imbalance in baseline characteristics of study participants in the two study groups was controlled in the study design by randomization, block combination of allocation codes and allocation concealment of the randomization list and allocation codes.

Table 10: Baseline prevalence of BDI-II depressive symptoms

Depressive symptom	Fish oil (N=109) n(%)	Soybean oil (N=107) n(%)
Fatigue	105 (96.3)	104 (97.2)
Loss of energy	103 (95.4)	104 (97.2)
Sadness	103 (94.5)	100 (93.4)
Crying	93 (85.3)	97 (90.6)
Changes in sleeping pattern	95 (87.2)	90 (84.1)
Loss of interest in sex	91 (83.5)	94 (87.8)
Changes in appetite	98 (89.9)	87 (81.3)
Irritability	93 (85.3)	88 (82.2)
Past failure	88 (80.7)	91 (85.0)
Pessimism	85 (78.0)	92 (86.0)
Agitation	86 (78.9)	79 (73.8)
Anhedonia (Loss of Pleasure)	69 (63.3)	71 (66.3)
Guilty feelings	76 (69.7)	75 (70.1)
Punishment feelings	58 (53.2)	55 (51.4)
Self-dislike	71 (65.1)	72 (67.3)
Self-criticalness	71 (65.1)	75 (70.1)
Worthlessness	70 (64.2)	83 (77.5)
Suicidal thoughts	65 (60.0)	69 (64.5)
Loss of interest	67 (61.2)	59 (55.1)
Concentration difficulty	66 (60.5)	67 (62.6)
Indecisiveness	49 (45.0)	51 (47.6)

Table 11 shows proportion of participants who reported the depressive symptoms on the BDI-II tool at the end of the 8-week study period. The depressive symptoms experienced by most participants were changes in appetite (Fish oil=73.3%; Soybean oil=65.6%), changes in sleeping pattern (Fish oil=66.3%; Soybean oil=52.1%), loss of interest in sex (Fish oil=60.5%; Soybean oil=60.4%), fatigue and loss of energy (Fish oil=51.2%; Soybean oil=62.5%). The rest of the symptoms were reported by less than 35.0% of participants from each of the study arms.

Table 11 – End of study prevalence of BDI-II depressive symptoms

Depressive symptom	Omega-3 (N=86) n(%)	Soybean (N=96) n(%)	p-value*
Fatigue	51(59.3)	59(61.5)	0.87
Loss of energy	44(51.2)	60(62.5)	0.14
Sadness	13(15.1)	25(26.0)	0.09
Crying	17(19.8)	28(29.2)	0.18
Changes in sleeping pattern	57(66.3)	50(52.1)	0.07
Loss of interest in sex	52(60.5)	58(60.4)	1.00
Changes in appetite	63(73.3)	63(65.6)	0.33
Irritability	26(30.2)	32(33.3)	0.75
Past failure	21(24.4)	21(21.8)	0.72
Pessimism	10(11.6)	12(12.5)	1.00
Agitation	27(31.4)	26(27.1)	0.62
Anhedonia (Loss of Pleasure)	15(17.4)	13(13.5)	0.54
Guilty feelings	21(24.4)	18(18.7)	0.37
Punishment feelings	13(15.1)	15(15.8)	1.00
Self-dislike	14(16.3)	17(17.7)	0.84
Self -criticalness	25(29.1)	29(30.2)	0.87
Worthlessness	17(19.8)	21(21.9)	0.85
Suicidal thoughts	5(5.8)	8(8.3)	0.57
Loss of interest	13(15.1)	11(11.5)	0.51
Concentration difficulty	12(13.9)	8(8.3)	0.24
Indecisiveness	22(25.6)	17(17.7)	0.21

All the p-values are based on Chi square test (df =1) statistic.

4.4.3 Depressive symptom scores

Shapiro Wilk Test for normality indicated that the BDI-II depressive symptom scores were not normally distributed in the fish oil intervention group and soybean oil control group through out the study period. Data was therefore summarised as median BDI-II scores and not mean scores. At baseline, the minimum BDI-II symptom score was 14 while the maximum was 40 and the median scores were 20 (IQR=16 – 25) and 21(IQR=17 – 25) in fish oil intervention and soybean oil control groups respectively. These baseline BDI-II scores were similar in the two study groups (Table 12).

At mid-study, four weeks after randomization, participants in the intervention fish oil group had a median BDI-II depressive symptom score of 9 (IQR = 5 - 14) and 10

(IQR = 6 - 13) in Soybean oil control group. Again, the BDI-II scores at mid-study were not significantly different between the two study arms (z=-0.26; p=0.79).

Data collected at the end of the intervention period, eight weeks after randomization revealed that the median BDI-II score in the intervention fish oil group was 6 (IQR=5-11) while in the soybean oil control group it was 7 (IQR=4-10). There was however no significant difference in the scores between those participants in the intervention fish oil and soybean oil control group (Table 12).

Table 12 - BDI-II depressive symptom scores by intervention period and study arm

	BDI-II scores (Median (IQR)		**Wilcoxon rank-sum test**	
	Fish oil	Soybean oil	z- test	p-value
Baseline at week-0	20(16 - 25)	21(17- 25)		
Mid-study at week-4	9(5 - 14)	10(6 - 13)	-0.26	0.79
End of study at week-8	6(5 - 11)	7(4 - 10)	0.49	0.62

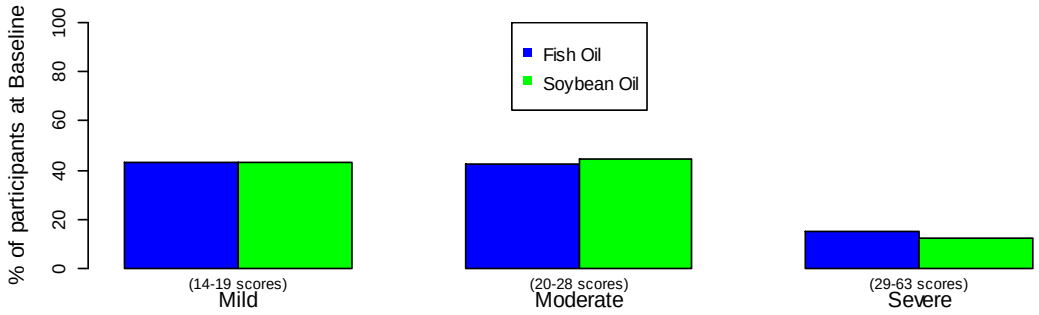
IQR (interquartile range, 25% - 75%);

4.4.4 Depressive symptom levels

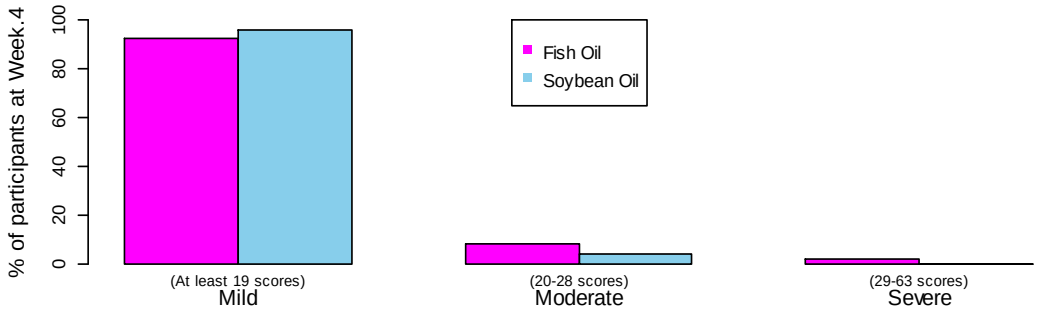
At baseline, most participants in both, fish oil and soybean had mild BDI-II symptom score levels of 14 to 19 and moderate BDI-II scores of 20 to 28 (*Figure 10*). Participants who had severe depressive symptoms with BDI-II scores above 29 were 14.7% and 12.1 % in the fish oil and soybean arms respectively. Four weeks after taking the intervention, more than 90% of study participants (Fish oil = 92.4%, Soybean=95.5% had minimal to mild depressive symptoms with BDI-II scores of at least 19 (Figure 10)). Participants who had moderate depressive symptom levels were 5.4% in Fish oil arm and 4.1% in Soybean arm. The ones with severe symptom levels

were 2.2% in Fish oil arm and none in the soybean oil arm. The difference in the proportions of participants with the three depressive symptom levels, four weeks after the intervention was not significantly different between the two intervention arms (X^2=2.4, *d.f*=2, p-value=0.30).

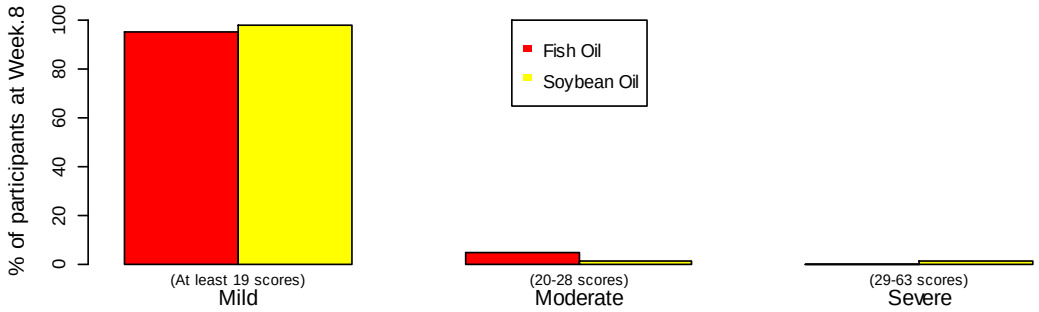
After 8 weeks of intervention, more than 95% of participants in both intervention groups had minimal to mild BDI-II symptom score levels of at least 19 scores (Fish oil = 95.3%, Soybean oil=97.9%) as seen in *Figure 10*. Participants with moderate symptom levels were 4.6% in the fish oil group and 1.0% in the soybean oil group. Only 1.0% of them in the soybean group had score levels of severe symptoms. The difference in the proportions of participants with different BDI-II depressive symptom levels after eight weeks of intervention was not significantly different between the two intervention arms (X^2=3.1, *d.f*=2, p-value=0.21).



Baseline BDI-II depressive symptom score levels by study arm



Week.4 BDI-II depressive symptom score levels by study arm



Week.8 BDI-II depressive symptom score levels by study arm

Figure 11 - Proportion of participants by BDI-II score levels, group and period

4.5 Effect of fish oil omega-3 EPA-rich supplements on change in BDI-II depressive symptom scores among HIV-seropositive pregnant women

4.5.1 Normality of the change in BDI-II scores data distribution

The Shapiro-Wilk statistical test of normality of data indicated that the change in BDI-II depressive symptom scores were normally distributed four weeks after intervention (fish oil: w=0.97, p=0.07; soybean oil: w=0.98, p=0.13) and eight weeks after intervention (fish oil: w=0.98, p=0.35; soybean oil: w=0.98, p=0.24). Further statistical test for normality with normal quantile plot (q-plot) also confirmed that the change in baseline scores was normally distributed within each group (Figure 10) at four weeks and after eight weeks.

Any variability present in the distribution of the scores in the samples was not statistically significant, based on Levene's test of variance at four weeks (mid-study) for sample means (F (1,188) =0.79, p=0.37) and medians (F (1,180) =0.80, p=0.37). The variability in the distribution of data was also not statistically significant at week eight (end of study) for sample means, (F (1,180) =1.96 p=0.16), medians (F (1,180) =1.45, p=0.23) and standard deviation test of variance (p=0.24). Any difference in variability in errors across observations was controlled for by applying *robust* analysis in STATA statistical analysis software. These statistical tests proved that the primary outcome data of the study, change in BDI-II depressive symptom scores, met the regression analysis assumptions of normality.

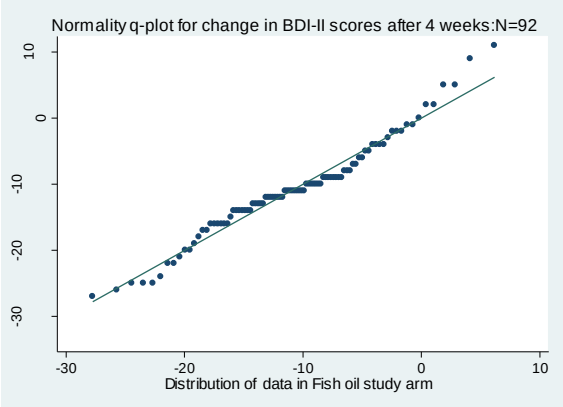
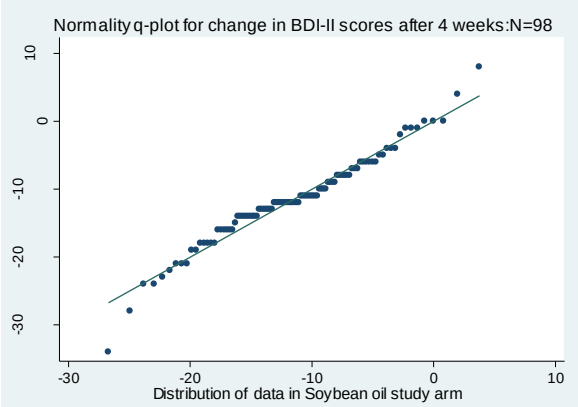
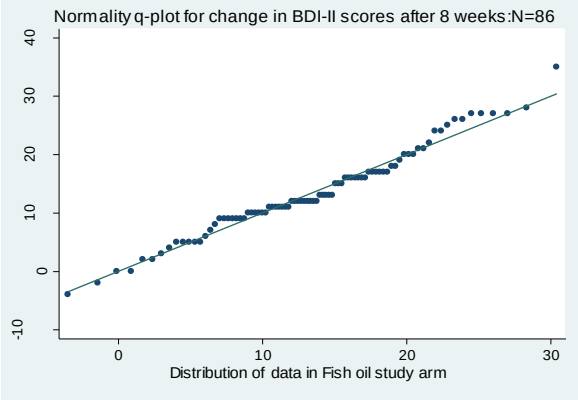
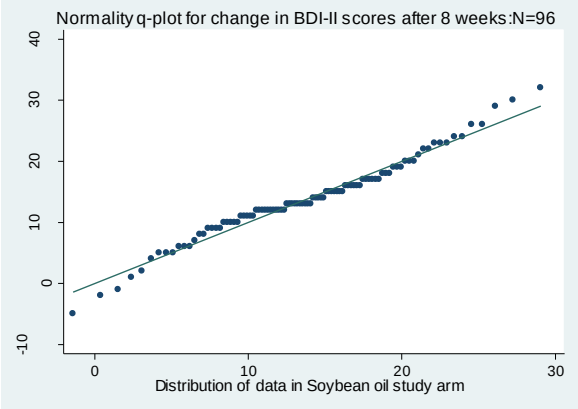
<table>
<tr><th colspan="2">Normality test for change in BDI-II scores by study arm and period</th></tr>
<tr><th>Fish oil study arm</th><th>Soybean Oil study arm</th></tr>
<tr><td>Shapiro-Wilk test
1. After 4 weeks: w=0.97, p=0.07,
2. After 8 weeks: w=0.98, p=0.35;</td><td>Shapiro-Wilk test
1. After 4 weeks: w=0.98, p=0.13
2. After 8 weeks: 0.98, p=0.24</td></tr>
<tr><td> 
</td><td> 
</td></tr>
<tr><td> 
</td><td> 
</td></tr>
<tr><td colspan="2">Test of variance in the distribution of change in BDI-II scores between Fish oil and soybean oil study arms:
After 4 weeks: Sample means (F(1,188)=0.79, p=0.37,) medians (F(1,180)=0.80, p=0.37)
After 8 weeks: Sample means (F(1,180)=1.96, p=0.16), medians (F(1,180)=1.45, p=0.23).</td></tr>
</table>

Figure 12 - Normality tests for change in BDI-II scores by study arm

4.5.2 Change in BDI-II scores before adjusting for baseline covariates

Table 13 shows the mean change and mean difference in change in BDI-II scores before adjusting for baseline covariates at four and eight weeks post intervention. After four weeks, fish oil intervention group had a mean (SD) change in BDI-II depressive symptom scores of -10.8 (±7.3) with a 95% confidence interval (CI) of -12.3 to -9.3scores. The mean (SD) change in scores after the four weeks for the soybean oil control group was -11.5(± 6.5) with a 95% CI of -12.3 to -10.2 scores. At the end of the 8-week study period, participants in the fish oil intervention group had a mean (SD) change in BDI-II depressive symptom scores of -13.3 (±7.4) with a 95% CI of -14.9 to -12.0 scores. The mean (SD) change in scores among soybean oil control group was -13.9 (± 6.5) with a 95% CI of -15.3 to -12.6 scores. The calculated 95% CI for the two groups overlapped substantially at week four and week eight when the median scores were compared (Figure 13), suggesting that there was no significant difference in intervention effect on depressive symptoms between the two study groups.

The mean difference in the change in BDI-II scores between the two intervention groups was 0.7 (se=1.0) with a 95% CI: 1.3 to 2.7 after four weeks and 0.6 (se=1.0) with a 95% CI: 1.5 to 2.6 after eight weeks of intervention. These differences in change in BDI-II scores were however not significantly different (week 4: p=0.49; week 8: p=0.58). By fitting simple linear regression model, the intervention could only explain 0.25% of the variability in change in depressive symptom scores at week four (R^2=0.0025) and 0.07% (R^2=0.0017) at week eight. The intervention alone was not statistically significant in explaining the variability in change in BDI-II scores at week four 4 (F (1,188) =0.47, p=0.49) and week eight (F (1,180) =0.31, p=0.58).

Table 13 - Change in BDI-II scores after intervention

Intervention period	Intervention group	Change in BDI-II scores (Mean (SD, 95% CI)	Difference between groups in change in BDI-II scores (Mean (SE, 95% CI)	Statistical test		
				t	df	p
After 4 weeks	Fish oil (N=92)	-10.8(-12.3 - -9.3)	0.7 (1.0, -1.3 – 2.7)	0.69	188	0.49
	Soybean oil (N=98)	-11.5(-12.3 - -10.2)				
After 8 weeks	Fish oil (N=86)	-13.3(-14.9 - -12.0)	0.6 (1.0, -1.5 -2.6)	0.55	180	0.58
	Soybean oil (N=96)	-13.9(-15.3 - -12.6)				

SD = Standard Deviation

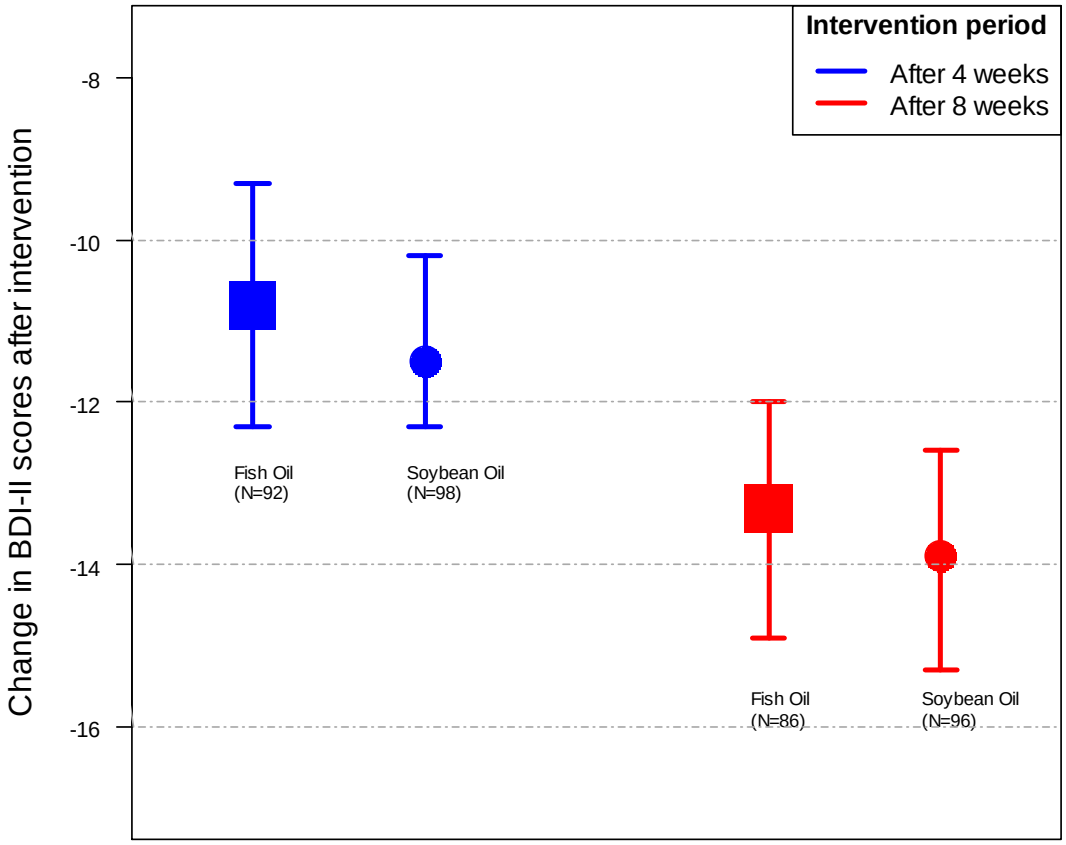


Figure 13: 95% confidence interval bars for change in BDI-II scores by study arm and period

4.5.3 ANCOVA Analysis of change in BDI-II scores between groups

Table 14 shows the ANCOVA analysis where all baseline characteristics were held constant by including them in the model. The changes in BDI-II depressive symptom scores were at week four 0.14 (95% CI: -1.51 – 1.78) and at week eight 0.85 (95% CI: -0.73 – 2.44) scores times higher in fish oil intervention group than in soybean oil control group. This intervention effect was however not statistically significant at both week 4 (p=0.87) and week 8 (p=0.29). The baseline characteristics in the ANCOVA model explained 47.9% (R^2=0.479) and 56.6% (R^2=0.566) of the variance in changes in BDI-II depressive symptoms scores at week 4 and week 8.

At week 4, the change in BDI-II depressive symptom scores was significantly associated with baseline BDI-II scores, -0.76 (95% CI: -0.94 - -0.61, p=0.000), dietary omega-3 intake -3.03(95% CI: -5.54 – -0.52, p=0.02), dietary vitamin B1 intake -3.49(95% CI: -5.49 - -1.49, p= 0.001), attendance in PMTCT m2m support group meetings attendance 2.07(95% CI: 0.39 – 3.76, p=0.02), vitamin B6 2.72(95% CI: 0.13 – 5.31, p=0.04) and zinc (-2.53 (95% CI: -4.39 – -0.67, p=0.01). The presence of interaction between baseline BDI-II depressive symptom scores and these variables that were significantly associated with change in BDI-II scores was tested. There was no interaction between the baseline BDI-II scores and any of the variables, suggesting that each variable influenced the change in BDI-II scores independently.

At the end of the study at week 8, change in BDI-II depressive symptom scores was significantly associated with baseline BDI-II symptom scores -0.87 (95% CI: -1.02 - -0.72; p=0.000) and parity status -2.23(95% CI: -4.38 – -0.09, p=0.04) assuming that all other variables were held constant in the ANCOVA model. The presence of interaction

between baseline BDI-II depressive symptom scores and the number of times a participant had been pregnant (parity) was tested. There was no interaction between the baseline BDI-II scores and any of the two variables and each variable influenced the change in the BDI-II scores independently.

Table 14 - ANCOVA analysis of mean change in BDI-II depressive symptom scores by baseline characteristics and intervention duration

Baseline characteristics	**Duration of intervention**			
	Week 4		**Week 8**	
	Regression Coefficient (95% CI)	P-value	**Regression Coefficient (95% CI)**	P-value
Intervention Group	0.14 (-1.51 – 1.78)	0.87	0.85 (-0.73 – 2.44)	0.29
Baseline BDI-II scores	-0.76 (-0.94 – -0.61)	0.00*	-0.87 (-1.02 – -0.72)	0.00*
Age	0.03 (-0.15 – 0.21)	0.75	0.11 (-0.06 – 0.28)	0.21
Gestational age	0.01 (-0.21 – 0.24)	0.92	0.03 (-0.18 – 0.25)	0.75
CD4 cell count	0.01 (-0.001 – 0.02)	0.08	-0.002 (-0.01 – 0.01)	0.60
Employment	-0.73 (-2.65 – 1.18)	0.45	-.57 (-2.19 – 1.05)	0.49
Knew HIV status when pregnant	-1.40 (-3.22 – 0.41)	0.13	-0.79 (-2.64 – 1.06)	0.40
HIV status disclosure	-0.81 (-2.81 – 1.20)	0.43	-0.37 (-2.10 – 1.36)	0.67
Marital status	0.87 (-1.32 – 3.06)	0.43	0.72 (-1.14 – 2.57)	0.45
Education Level	-0.27 (-2.14 – 1.60)	0.77	-.17 (-1.78 – 1.44)	0.84
Parity	-0.75 (-2.79 – 1.29)	0.47	-2.23 (-4.38 – -0.09)	0.04*
Stressful life event	-0.35 (-2.11 – 1.41)	0.69	0.99 (-.5701 – 2.5405)	0.21
PMTCT *m2m* meeting attendance	2.07 (0.39 – 3.76)	0.02*	1.06 (-.49 – 2.62)	0.18
MUAC	0.02 (-0.35 – 0.39)	0.93	-0.05 (-0.36 – 0.27)	0.76
Dietary total Omega-3 FA	-3.03 (-5.54 – -0.52)	0.02*	-0.32 (-2.75 – 2.12)	0.80
Dietary Vitamin C	-.79 (-2.60 – 1.03)	0.40	0.94 (-0.96 – 2.84)	0.33
Dietary Vitamin B1	-3.49 (-5.49 – -1.49)	0.001*	-1.43 (-3.61 – 0.76)	0.20
Dietary Vitamin B6	2.72 (0.13 – 5.31)	0.04*	2.26 (-0.19 – 4.71)	0.07
Dietary Vitamin B12	1.22 (-1.26 – 3.71)	0.33	-0.35 (-2.94 – 2.23)	0.79
Dietary Folate	1.95 (-0.95 – 4.86)	0.19	1.94 (-.92 – 4.81)	0.18
Dietary Vitamin E	0.07 (-2.58 – 2.72)	0.96	-1.26 (-3.30 – 0.78)	0.22
Dietary Zinc	-2.53 (-4.39 – -0.67)	0.01*	-0.59 (-2.92 – 1.75)	0.62
Dietary Selenium	1.46 (-0.97 – 3.88)	0.24	1.87 (-.25 – 4.00)	0.08
Dietary Calcium	1.63 (-0.31 – 3.58)	0.10	-1.49 (-3.41 – 0.44)	0.13
Dietary Iron	0.08 (-2.44 – 2.61)	0.95	0.76 (-1.87 – 3.39)	0.57

CHAPTER 5: DISCUSSION

5.1 Review and discussion of key findings

5.1.1 Introduction

The main objective of the study was to assess the effect of fish oil omega-3 EPA-rich supplements on BDI-II depressive symptom scores among HIV-seropositive pregnant women. This study demonstrated that participants were randomised to either fish oil intervention group or soybean oil control group while they were all at the same level with regard to their demographic, socio-economic, dietary, depressive symptoms and other health-related attributes. Bias in the selection and allocation of participants to intervention groups was controlled by randomization, block combination of allocation codes and allocation concealment of the randomization list and allocation codes. Block randomization method used in this study increased the probability that each arm contained an equal number of individuals by sequencing participant assignments by block (Efird 2011). The use of securely sealed, sequentially numbered, opaque plastic identical bottles for allocation concealment in this study further reduced selection bias in allocating the intervention products.

The significance of the observed baseline findings in this study was that the participants' demographic, socio-economic, dietary, depressive symptoms and other health-related attributes were the covariates in explaining change in BDI-II depressive symptom scores. In randomized trials where groups of participants should differ only with respect to the intervention, if randomisation is performed correctly, participants in treatment and control groups are expected to be similar with regard to their baseline characteristics. This is important for generalization of the study to the overall patient population with the same health conditions (Wang et al. 2006). No statistical tests were used in this study to compare whether participants were similar before they were

randomized into fish oil or soybean oil group. Use of statistical tests and p-values in comparing balance of baseline characteristics between intervention groups in clinical trials is considered inappropriate since it is already known that if randomization was properly performed, any observed difference could have occurred by chance alone (Burgess et al. 2003; Moher et al. 2010).

Key findings on the research objectives of this thesis are summarised in Figure 14.

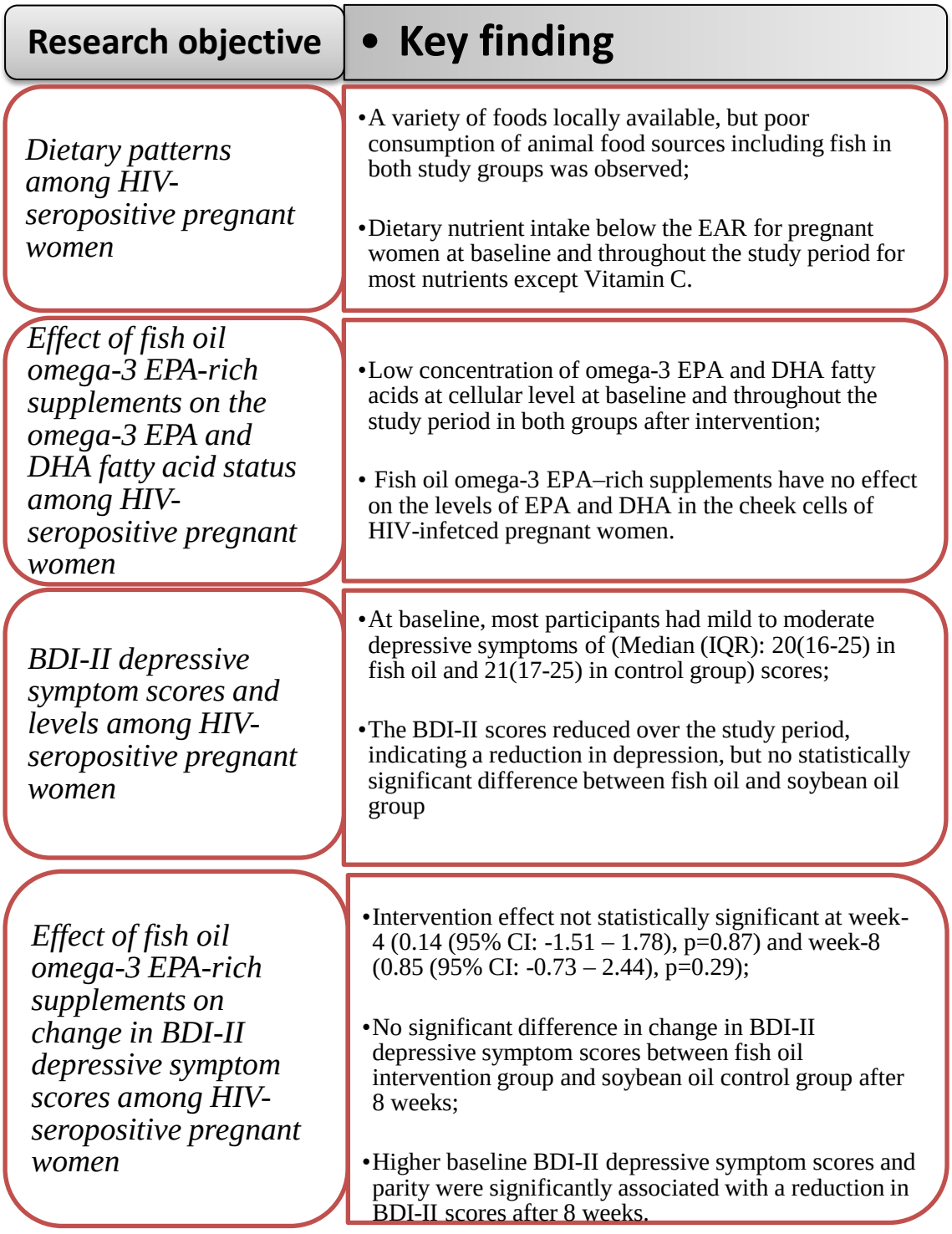


Figure 14: Key findings on the research objectives

5.1.2 Dietary patterns among HIV-seropositive pregnant women

The food consumption patterns results revealed an intake of a variety of locally available foods, and the pattern was similar in both study groups at baseline. This ensured that study participants were randomized in the trial at the same dietary nutrient adequacy levels. Dietary patterns were characterist by regular consumption of vegetables, fruits, whole-maize meal (*ugali)*, beans, potatoes, milk, vegetable cooking fat and occasional consumption of animal food sources and fish. Regular consumption of meat products was low while consumption of sea fish, the main source of omega-3 EPA and DHA was not reported even though sea fish was available at Nairobi County's major markets of Gikomba and City Market. These observations on the expensive meat products and fish were expected among the study participants who were drawn from low to medium income catchment areas of the study sites. The nutrients intake was below the recommended requirements for pregnant women at baseline and throughout the study period.

For most nutrients, intake levels were below the estimated average reuirements for pregnant women (FAO/WHO 2002; FAO/WHO 2005; Food and Agriculture Organization of the United Nations 2010; The National Academies 2001; The National Academies 2005) for all nutrients except for Vitamin C throughout the study period. The vitamin C nutrient adequacy levels were achieved due to the observed high consumption of green leafy vegetables and fruits. The availability of nutritionaly adequate food did not translate into nutrient adequacy for the pregnant HIV-infected women. Similar findings of low nutrient intakes among pregnant women have been established in Kenya (Steyn et al. 2012a) Portugal (Pinto et al. 2009), India (Samuel et al. 2013; Singh et al. 2009), Australia (Blumfield et al. 2011) and United Kingdom (Mouratidou et al. 2006). Although recommended intake values for EPA and DHA

omega-3 fatty acids were not available at the time of conducting and reporting this study, their intakes in the study were negligible. Low dietary intake of EPA and DHA omega-3 fatty acids has been reported among urban women in Nairobi (Gitahi 2012) and low-income pregnant women in the Michigan (Nochera et al. 2011). Fish oil supplement is a convenient source of omega-3 EPA and DHA. However none of the study participants from both intervention arms reported access or use of these supplements probably due to lack of awareness of their existence and health benefits and the cost. The only nutritional supplements reported were iron and folic acid provided at the health facility as part of the antenatal care package.

The observed lack of statistical interaction between the covariate nutrients in this study suggested that each nutrient influenced the depressive symptom independently. Interaction between micronutrients when dietary intake of a single nutrient is inadequate has been known to affect absorption and bioavailability of other nutrients (Sandstrom 2001). The intake levels of antioxidants vitamins (C and E) and minerals (zinc and selenium) which were investigated in this study, have been previously found to be important in enhancing bioavailability of omega-3 fatty acids (Bourre 2004; Logan 2004; Singh et al. 2009).

5.1.3 Effect of fish oil omega-3 EPA-rich supplements on EPA and DHA fatty acid status among HIV-seropositive pregnant women

Evidence shows that fish oil omega-3 supplementation among pregnant women significantly increases the EPA and DHA fatty acid levels in the body tissues (Mozurkewich et al. 2013; Rees et al. 2009; Su et al. 2008). This study did not demonstrate any significant increase in EPA and DHA fatty acid levels in the cheek

cell samples. These findings suggest that the fish oil omega-3 EPA–rich supplements have no effect on the levels of EPA and DHA in the cheek cells of pregnant HIV-infetced pregnant women. Supplementation with EPA and DHA in humans has however been shown to increase the levels of these fatty acids in cheek cells (Harris et al. 2004) and the cheek cell phospholipids have been previously shown to compare well with plasma and erythrocyte phospholipids (Connor et al. 2000; Grindel et al. 2013; Harris et al. 2004; Hoffman et al. 1999). In this study, fatty acids were observed in all the cheek cell samples collected, suggesting that the samples collected contained the lipids. However, low concentration levels of EPA and DHA fatty acids were detected in the samples from both the fish oil and soybean oil groups throughout the study period. These findings suggested that the cellular level of EPA and DHA was low among the study participants from both study arms. Yet, after randomization, the EPA and DHA fatty acid levels were expected to be significantly higher in the fish oil intervention group compared to the soybean oil control group.

The fish oil soft gels had pre-formed EPA (0.715 grams per capsule) and DHA (0.340 grams per capsule) omega-3 fatty acids while the soybean oil soft gels taken by the control group had only traces of EPA (0.115 grams per capsule) and no DHA faty acids, hence a higher concentraion level of EPA and DHA in the fish oil intervention group was expected from the cheek cell samples analysed. Soybean oil mainly contains ALA, which is a precursor of EPA and DHA in the body, hence a lower concentration levels of EPA and DHA was expected from the soybean oil control group samples. Furthermore, due to the documented low metabolic conversion rate of ALA omega-3 fatty acid to EPA and DHA in healthy adults, (Burdge and Wootton 2002; Pawlosky et al. 2001; Stoll 2001) the EPA and DHA fatty acid levels in the cheek cell samples were only expected to be similar at baseline before in the intervention and control

group samples. There was however observed consistent but insignificant trend in increase in maximum values for EPA in the fish oil group at week-4 (mid-study) and week-8 (end of study), suggesting a minimal change in the fatty acid levels following intervention. The pattern was not observed in the EPA values for soybean oil where there was a decrease at week four followed by an increase at week 8.

In comparison with previous studies among healthy pregnant women, this study was conducted among HIV-infected pregnant women. In HIV infection, omega-3 EPA and DHA fatty acid absorption may be impaired like other nutrients (Kapembwa et al. 1990; Piwoz and Preble 2000) due to altered and reduced activity of pancreatic lipase enzyme as has been previously observed in HIV patients on HAART (Manfredi et al. 2004). The hydrolysis of omega-3 fatty acids by the pancreatic lipase enzyme may be further reduced when the fatty acids are in ethyl esters form compared to the triglyceride or phospholipid forms (Opperman 2013). Since the fish oil omega-3 fatty acids soft gels used in the study were in form of ethyl esters, their hydrolysis was therefore probably low. The observed low consumption of animal food sources and use of fats and oils in cooking may have also contributed to poor absorption of the fish oil omega-3 ethyl esters. The findings on low dietary nutrient intake levels and omega-3 cheek cell levels highlight the importance of routine dietary assessment, promoting and supporting adequate nutrient intake among HIV-infected pregnant women.

5.1.4 Depressive symptoms and BDI-II scores and levels among HIV-seropositive pregnant women before and after intervention

The BDI-II scoring scale was consistent in measuring depression among HIV-infected pregnant women in this study as confirmed by the statistical test for inter-item reliability for this tool which was within the acceptable range (Cronbach's alpha=0.7). Kim et al. (2014) in Malawi reported an alpha of 0.80 among HIV-infected adolescents while (Lipps et al. 2010a) from Jamaica reported 0.89 among HIV infected adults. The reliability of the BDI-II tool in this study was further confirmed by key informant interviews conducted among the health workers, PMTCT *mentor-mothers* and HIV-positive pregnant women who also revealed presence of almost similar symptoms among this population. The use of BDI-II scoring tool to measure depressive symptoms in this study population was therefore valid.

The study also established that all the 21 depressive symptoms on the BDI-II assessment tool were equally reported by study participants from both fish oil and soybean oil study groups throughout the study period. These findings supported existing evidence that depression is common among HIV-infected pregnant women (Kwalombota 2002; Manikkam and Burns 2012; Ross et al. 2009; Smith Fawzi et al. 2007). Fatigue was the most common symptom of depression reported by over 95% of participants from both fish oil intervention group and soybean oil control group at baseline. Indepth interviews with the health workers, PMTCT *mentor-mothers* and HIV-infected pregnant women did not however indicate that fatigue is considered as a symptom of depression. This was probably because fatigue is one of the physical symptoms exhibited in both pregnancy and ill-health conditions (Lipps et al. 2010b; Psaros et al. 2009). Since the health workers and PMTCT *mentor-mothers* did base their assessment of depressive symptoms on any structured tool a structured guideline,

it was unlikely that they considered fatigue as a depressive symptom. These findings suggested that awareness and recognition of depressive symptoms among HIV-infected pregnant women by the health workers and PMTCT *mentor-mothers* were inadequate and mainly occurred during counselling sessions before or soon after HIV testing. This implies that those HIV-infected women who develop depressive symptoms later in pregnancy after the routine counselling that precedes or follows the HIV testing may not be identified for timely management of their depression condition. The observed poor awareness and recognition of depressive symptoms in antenatal care by health workers support the evidence from other studies (Goodman and Tyer-Viola 2010; Rochat et al. 2006) that have highlighted the need for routine screening for antenatal depression. Probably, if the health workers routinely used a structured tool to screen for depressive symptoms among this population, they would be able to recognize and mention more symptoms than they did in this study. This study demonstrated that depression is prevalent among HIV-infected pregnant women, yet screening is not routinely done at the antenatal care facilities. Lack of routine screening for depressive symptoms in antenatal care had been decried before by Santoro and Peabody (2010), Goodman and Tyer-Viola (2010) and Rochat and colleagues (2006).

5.1.5: Effect of fish oil omega-3 EPA-rich supplements on change in BDI-II depressive symptom scores among HIV-seropositive pregnant women

This study tested the hypothesis that there is no difference in the magnitude of change in BDI-II scores between HIV–seropositive pregnant women with depressive symptoms taking fish oil omega-3 EPA-rich supplements and the control group taking soybean oil soft gels. The study had hypothesized to detect as statistically significant at 5% level (α=0.05) a true difference of at least 4 BDI-II scores, equivalent to a 20%

change, between the intervention and control group. Although each group experienced a 20% change (4 units) in BDI-II scores from baseline to week 8, The differences in reduction in BDI-II scores were 0.7 and 0.6 times more in the fish oil intervention group than in the soybean oil control group at week four and week eight respectively. These differences were not statistically significant. The fish oil intervention alone could only explain 0.25% and 0.07% of the variability at week four and week eight respectively. The change in BDI-II depressive symptom scores was only 0.14 (95% CI: -1.51 – 1.78) times higher at week four and 0.85 (95% CI: -0.73 – 2.44) times higher at week eight in fish oil EP-rich omega-3 intervention group than in soybean oil control group. The statistical change in the BDI-II scores could not be explained by either fish oil or soybean oil alone. These findings suggested that fish oil omega-3 was not effective in reducing depressive symptoms among HIV-infected pregnant women.

Several possible explanations could have contributed to the observed lack of a statistically significant difference in change in BDI-II scores between the fish oil intervention group and the soybean oil control group. First, in this study, participants had mild, moderate and severe depressive symptoms at baseline while previous studies had only participants with major depressive symptoms. Second, the previous studies on omega-3 supplementation were conducted among pregnant women without HIV infection. Even though the prevalence of depression in HIV-infected pregnant women has been documented before (Kwalombota 2002; Manikkam and Burns 2012; Ross et al. 2009; Smith Fawzi et al. 2007), change in depressive symptoms scores following omega-3 EPA-rich fatty acid supplementation had not been previously demonstrated in this population group. Yet, in HIV-infected pregnant women, the omega-3 EPA and DHA fatty acid absorption may be impaired (Kapembwa et al. 1990; Piwoz and Preble

2000) due to reduced activity of the pancreatic lipase enzyme (Manfredi et al. 2004), thus contributing to low response to the fish oil omega-3 fatty acid supplementation.

The third explanation for lack of a statistically significant difference in change in BDI-II scores between the groups could have been the "placebo effect". Due to the "placebo effect" some participants in the soybean oil control group believed that they were taking fish oil soft gels and reported a reduction in their depressive symptom scores might have contributed to the observed lack of a significant statistical difference between the intervention and control group. Both the fish oil soft gels for the intervention group and soybean oil for the control group were physically similar in shape and color. This "placebo effect" might have influenced the mean change in BDI-II depressive symptom scores of the groups, causing "regression to the mean" value, where the mean change in depressive symptom scores showed a trend towards the treatment effect (Bradbury et al. 2004). "Regression to the mean" was controlled in this study through the ANCOVA analysis model which adjusted for any extreme baseline values and variabilities during analysis.

The fourth possible explanations for lack of a statistically significant difference in change in BDI-II scores between the fish oil intervention group and the soybean oil control group in this study was the intensive follow-ups of participants throughout the study period which could have contributed to "Hawthorn Effect". McCarney and colleagues demonstrated that intensive follow-up of participants resulted in a better outcome in clinical trial than minimal follow-up, and they defined "Hawthorn Effect" as increase in treatment response due to psychological stimulus of being singled out and being made to feel important (McCarney et al. 2007). Regular follow-ups of all participants in both study groups after every two weeks to re-supply the treatment, and

regular cell-phone contacts for purposes of monitoring adherence in taking the treatment might have made the HIV-infected pregnant feel worthwhile and more positive about their pregnancy outcome. The findings on depressive symptoms revealed that the symptom of worthlessness was reported by more than 60% of participants from intervention and control group at baseline, but less than 25% of participants from each group at the end of the study. This is an indication that more than 75% of participants had their BD-II score for the symptom of worthlessness reduced at the end of the study. Although routine PMTCT *m2m* support group peer education meeting attendance did not significantly statistically influence the change in BDI-II scores, it might have also contributed to the positive response and feeling of "important" exhibited by participants during follow-ups in both study groups. It was not possible to control for the Hawthorn Effect. Regular follow-up of participants in this study was necessary to re-supply the intervention and monitor compliance in the trial.

Although there was no significant difference in baseline BDI-II scores between the intervention and control groups before randomization, participants who had higher BDI-II scores at baseline experienced less change in their depressive scores than those who had lower scores. The findings suggest that severity of depressive symptoms was a predictor of the magnitude of change in BDI-II depressive symptoms scores rather than the fish oil omega-3 intervention. These findings on severity of baseline depressive symptom scores influencing change in the scores after intervention support the observation made by Kilt and colleagues that response to depression treatment decreased with increasing baseline symptom severity (2009). These research findings highlight the need for routine screening for depressive symptoms to guide timely identification and management of depressive symptoms in HIV-infected pregnant women.

5.2 Strengths and weaknesses of the study

This is the first study to explore the role of fish oil omega-3 fatty acids on depression among HIV-infected pregnant women. It examined change in individual pregnant women's BDI-II depressive symptom scores before and after the intervention. Most potential confounding demographic, socio-economic and health-related factors were controlled for in the study design by the randomization. The ANCOVA analysis model used in analysing the study outcome further controlled for any imbalance due to baseline variations. Other baseline covariates which were most likely to change over the intervention period such as BDI-II depressive symptom scores, CD4 cell count levels, frequency of interaction with PMTCT peer educators, occurrence of stressful life events and omega-3 fatty acid levels in the body were controlled for in the ANCOVA analysis model.

The other strength of this study was the use of BDI-II tool which contains depressive symptoms exhibited in both HIV and pregnancy conditions. The reliability test for the BDI-II scale in assessing depressive symptoms in HIV infected pregnant women confirmed that the tool was reliable. The reliability of the tool was further confirmed in key informant interviews with HIV-infected pregnant women and health workers. Their local understanding of the depressive symptoms matched with the symptoms listed on the BDI-II tool. Furthermore, the use of BDI-II tool in screening for depressive symptoms had been previously validated in other studies among adults, pregnant women and HIV-infected individuals and in the African context (Kagee et al. 2014; Kim et al. 2014; Ndetei et al. 2010).

Although varicose veins were not part of the research objectives in this thesis, is worth mentioning that three of four participants who had swollen varicose veins in the legs and were in the fish oil group had their swollen veins disappear after 8 weeks. However, the fourth participant with varicose veins who was in the soybean oil control group did not notice any change in her veins at the end of the study. These observations suggested that fish oil omega-3 may be effective in management of varicose veins.

These study findings may not be generalized to other populations who are neither pregnant nor HIV-seropositive. This is for two reasons. First, participants were depressed partly because of their HIV condition, pregnancy or a combination of both HIV and pregnancy. However, the BDI-II depressive symptom screening tool used in this study comprises of a comprehensive list of symptoms that are exhibited in both pregnancy and HIV infection. Second, pregnancy and HIV infection on their own are also associated with nutrient depletion and increased nutrient demand. This implies that the recommended EARs for pregnancy alone are not adequate for both pregnancy in HIV infection conditions. Due to lack of omega-3 EPA and DHA fatty acids from the Kenya national food composition databases, international sources where the nutrient content of locally available foods may not be the same were used to compute the dietary intake of these nutrients.

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

This study demonstrated that there is no difference in the magnitude of change in BDI-II scores between HIV–seropositive pregnant women with depressive symptoms taking fish oil omega-3 EPA-rich supplements with a daily dosage of 3.17 grams (EPA=2.15 grams; DHA=1.02 grams) and a control group taking soybean oil of a daily dosage of saturated fatty acids (0.53 grams), monounsaturated fatty acids (0.89 grams) and polyunsaturated fatty acids (0.985 grams) with traces of EPA (0.34 grams). Based on this, the study concludes that the fish oil omega-3 is not effective in reduction of depressive symptoms among HIV-infected pregnant women with mild, moderate and severe depression symptoms. Severity of depressive symptoms at baseline and maternal parity status can however significantly cause a reduction in change in depressive symptoms severity in an 8-week intervention period.

Low concentrations of omega-3 EPA and DHA fatty acids in the cheek cell samples analysed suggest that the fish oil omega-3 supplementation has no effect on change in cellular levels of EPA and DHA fatty acids among HIV-infetced pregnant women. The fish oil omega-3 supplements were however welll tolerated, with no adverse side effects among the HIV-infected pregnant women.

Although a variety of food is locally available in Nairobi City, most HIV-infected pregnant women are not likely to meet their nutrient needs through regular dietary intake due to poor consumption of animal food sources and fish which translate into inadequate nutrient levels below the estimated average requirements for pregnant women. The low nutrient intake levels are likely to impact negatively on the health status of the women since the nutrients' demand is higher than normal due to the increased need by the developing foetus and the woman's HIV-infection status.

Depressive symptoms are common among HIV-infected pregnant women. The evidence of the BDI-II depressive symptoms scores reducing over the study period in this study implied a reduction in depressive symptoms among the HIV-infected pregnant women. Although the difference in reduction in BDI-II scores between the two study groups was not statistically significant, depressed individuals participating in an intervention aimed at relieving them of the condition are sometimes likely to get better even when the intervention is not effective due to Hawthorn effect of receiving attention and feeling important.

Findings from this study have several policy and research implications. First, it was clear that whereas screening for depressive symptoms is not a routine activity in PMTCT or at the antenatal clinics, there are no specific guidelines in assessment for depressive symptoms at ANC. Yet, health workers receive women with depressive symptoms during pre- and post HIV testing counselling sessions. This is the time women open up in the discussions on how they feel. There is therefore need for routine screening for depression since it was only during counseling before and after HIV status testing when health workers suspected some women to be depressed. Furthermore, health workers did not have a standard screening tool for assessment of depression. Whereas resources may be a constraint in the health facilities, these findings suggest the need for a rapid tool or guideline for routine screening of depressive symptoms among HIV-infected pregnant women for timely management of severe cases of depression.

Second, although nutrition education on healthy dietary practices to women of reproductive age is in the Kenya National Nutrition Action Plan this study established

that although assessment of dietary intake in nutritional counseling are in the Kenya National Nutrition Policy document, dietary intake among HIV–infected pregnant women remained below the EARs for pregnant women for all nutrients except for vitamin C and B1. Effective promotion and support of adequate nutrient intake including omega-3 fatty acids calls for a more focused nutrition counseling based on individual assessments for this population during their routine visits to the PMTCT or home-based follow-up visits.

Three areas of future research are suggested in this study. First, the observed lack of omega-3 EPA and DHA fatty acids from the Kenya national food composition databases in this study calls for a national research on omega-3 fatty acids in locally available foods and a review of the national food composition table and other databases to include these nutrients. Second, research on fish oil omega-3 supplementation and change in depressive symptoms in HIV-infected pregnant women should focus on either moderately depressed or severely depressed women separately, with a control group that does not contain omega-3 fatty acids. The third area of future research suggested in this study is on the effect of fish oil omega-3 on varicose veins among HIV-infected pregnant women. Although this was not one of the study objectives, the observation made among the three participants whose varicose veins disappeared after taking fish oil omega-3 suggested that the fish oil omega-3 may be effective in management of the varicose veins. Further study on a larger sample is however needed to confirm the fish oil omega-3 benefits in treatment and management of varicose veins.

This research supports the existing body of knowledge that HIV-infected pregnant women experience depression yet minimal measures are in place for routine timely

screening and management of the symptoms. The research also contributes to the ongoing debate on role of fish oil omega-3 in reduction of depressive symptoms. The evidence that depression was common among HIV-infected pregnant women calls for routine screening for depressive symptoms at the PMTCT to allow for timely management of severe depressive symptoms among this vulnerable population.

REFERENCES

Abraham, R. A., Bahl, V. K., Parshad, R., Seenu, V., Roy, A., Golandaz, S., Dorairaj, P., and Ramakrishnan, L. (2013). "Content of Trans Fatty Acids in Human Cheek Epithelium: Comparison with Serum and Adipose Tissue." *BioMed Research International*, 2013, 7.

Arab, L., and Akbar, J. (2002). "Biomarkers and the measurement of fatty acids." *Public Health Nutr*, 5(6a), 865-71.

Barnett, A. G., van der Pols, J. C., and Dobson, A. J. (2005). "Regression to the mean: what it is and how to deal with it." *International Journal of Epidemiology*, 34(1), 215-220.

Beck, A., Steer, R., and Brown, G. (1996). *BDI-II: Beck Depression Inventory Manual* Harcourt Publishers.

Bennett, H., Einarson, A., Taddio, A., Koren, G., and Einarson, T. (2004). "Prevalence of depression during pregnancy: Systematic review." *Obstetric and Gynecology* 103(4), 698-709.

Bennett, M., John, K., and Bondy, G. (2008). "Current and future treatments of HIV-associated dylipidemia. Review. ." *Future Lipidol.*, 3(2), 175-188.

Bligh, E., and Dyer, W. (1959). "A rapid method for total lipid extraction and purification." *Canadian Journal of Biochemistry and Physiology* 37, 911-917

Blumfield, M. L., Hure, A. J., Macdonald-Wicks, L. K., Patterson, A. J., Smith, R., and Collins, C. E. (2011). "Disparities exist between National food group recommendations and the dietary intakes of women." *BMC Womens Health*, 11, 37.

Bodnar, L. M., and Wisner, K. L. (2005). "Nutrition and depression: implications for improving mental health among childbearing-aged women." *Biol Psychiatry*, 58(9), 679-85.

Bourre, J. M. (2004). "[The role of nutritional factors on the structure and function of the brain: an update on dietary requirements]." *Rev Neurol (Paris)*, 160(8-9), 767-92.

Bradbury, J., Myers, S. P., and Oliver, C. (2004). "An adaptogenic role for omega-3 fatty acids in stress; a randomised placebo controlled double blind intervention study (pilot) [ISRCTN22569553]." *Nutr J*, 3, 20.

Bruce, C. (2001). "Anthropometric indicators measurement guide." *Food and nutrition technical assistance project, Academy for Educational Development, Washington DC*, 1-98.

Burdge, G., and Wootton, S. (2002). "Conversion of alpha-linolenic acid to eicosapentaenoic, docosapentaenoic and docosahexaenoic acids in young women." *Br J Nutr*, 88(4), 411-20.

Burgess, D. C., Gebski, V. J., and Keech, A. C. (2003). "Baseline data in clinical trials." *The Medical journal of Australia*, 179(2), 105.

Burr, G., and Burr, M. (1929). "A new deficiency disease produced by the rigid exclusion of fat from the diet. ." *The Journal of Biological Chemistry* 82, 345-367.

Charrondiere, U. R., Stadlmayr, B., Wijesinha-Bettoni, R., Rittenschober, D., Nowak, V., and Burlingame, B. (2013). "INFOODS Contributions to Fulfilling Needs and Meeting Challenges Concerning Food Composition Databases." *Procedia Food Science*, 2(0), 35-45.

Chetty, N., Reavis, S., Immelman, A., Atkinson, P., and JG, v. A. (1989). " Fatty acid composition of some South African fresh-water fish." *South African Medical Jopurnal*, 76(7), 368-370.

Connor, S., Zhu, N., Anderson, G., Hamill, D., Jaffe, E., Carlson, J., and EW, C. (2000). "Cheek cell phospholipids in human infants: a marker of docosahexaenoic and arachidonic acids in the diet, plasma, and red blood cells. ." *American Journal of Clinical Nutrition*, 71(1), 21-27.

Cook, J., Cohen, M., Burke, J., and Grey, D. (2002). "Effects of depressive symptoms and mental health quality of life on use of highly active antiretroviral therapy among HIV-seropositive women.Cited in Cruess, D.G., Douglas,S.D., Petitto, J.M., et.al., (2005) " *Journal of Acquired Immune Deficiency Syndrome* 30, 401-409.

Coppen , A., and Bolander-Gouaille, C. (2005). "Treatment of depression: Time to consider folic acid and vitamin B12 " *Journal of Psychopharmacology* 19(1), 59-65.

Cruess, D., Douglas, S., Petitto, J., Have, T., Gettes, D., Dubé, B., Cary, M., and Evans, D. (2005). "Association of resolution of major depression with increased natural killer cell activity among HIV-seropositive women." *American Journal of Psychiatry* 162(11), 2125-2130.

de Luis, D., Bachiller, P., Izaola, O., and Eiros Bouza, J. (2005a). "Relation between intake of omega 3 fatty acids and CD4 count in patients with HIV infection: a transversal study [Article in Spanish]." *Anales de medicina interna*, 22(7), 323-5.

de Luis, D. A., Bachiller, P., Izaola, O., and Eiros Bouza, J. M. (2005b). "[Relation between intake of omega 3 fatty acids and CD4 count in patients with HIV infection: a transversal study]." *An Med Interna*, 22(7), 323-5.

Denomme, J., Stark, K. D., and Holub, B. J. (2005). "Directly quantitated dietary (n-3) fatty acid intakes of pregnant Canadian women are lower than current dietary recommendations." *J Nutr*, 135(2), 206-11.

Dilley, J., Perl, M., Ochitill, H., and Volberding, P. (1985). "Findings in psychiatric consultations with patients with acquired immunodeficiency syndrome." *American Journal of Psychiatry* 142(1), 82-86.

Dubé, B., Benton, T., Cruess, D. G., and Evans, D. L. (2005). "Neuropsychiatric manifestations of HIV infection and AIDS." *Journal of Psychiatry and Neuroscience*, 30(4), 237-246.

Dunstan, J., Simmer, K., Dixon, G., and Prescott, S. (2008). "Cognitive assessment of children at age 2 1/2 years after maternal fish oil supplementation in pregnancy:

A randomised controlled trial." *Archives of Disease in Childhood - Fetal and Neonatal Edition*, 93 (1), F45-50.

Edwards, R., Peet, M., Shay, J., and Horrobin, D. (1998). "Omega-3 polyunsaturated fatty acid levels in the diet and in red blood cell membranes of depressed patients
" *Journal of Affective Disorders*, 48(2–3), 149-155.

Efird, J. (2011). "Blocked randomization with randomly selected block sizes." *Int J Environ Res Public Health*, 8(1), 15-20.

Emken, E., Adlof, R., and Gulley, R. (1994). "Dietary linoleic acid influences desaturation and acylation of deuterium-labeled linoleic and linolenic acids in young adult males." *Biochim Biophys Acta*, 1213(3), 277-88.

Erasmus, U. (2007). *Fats that heal, fats that kill*, Summertown, Tenn.: Alive Books.

FAO/WHO. (2002). *Human vitamin and mineral requirements. Report of a joint FAO/WHO expert consultation*. Rome.

FAO/WHO. (2005). "Vitamin and mineral requirements in human nutrition."

Fekete, K., Marosvolgyi, T., Jakobik, V., and Decsi, T. (2009). "Methods of assessment of n-3 long-chain polyunsaturated fatty acid status in humans: a systematic review." *Am J Clin Nutr*, 89(6), 2070s-2084s.

Flock, M. R., Harris, W. S., and Kris-Etherton, P. M. (2013). "Long-chain omega-3 fatty acids: time to establish a dietary reference intake." *Nutrition Reviews*, 71(10), 692-707.

Folch, J., Lees, M., and Sloane, S. (1957). " A simple method for the isolation and purification of total lipides from animal tissues." *J Biol Chem*, 226, 497-509.

Food, and Agriculture Organization of the United, N. (2009). *Right to food methodological toolbox*, Rome: Food and Agriculture Organization of the United Nations.

Food and Agriculture Organization of the United Nations. (2010). "Fats and fatty acids in human nutrition: Report of an expert consultation "*Food and Nutrition Paper 91*. City: FAO, Rome.

Freeman, M. (2008). "Omega-3 fatty acids and perinatal depression: A review of the literature and recommendations for future research." *Prostaglandins, Leukotrienes and Essential Fatty Acids*, 75(4-5), 291-297.

Freeman, M., Davis, M., Sinha, P., Wisner, K., Hibbeln, J., and Gelenberg, A. (2008). "Omega-3 fatty acids and supportive psychotherapy for perinatal depression: a randomized placebo-controlled study." *The Journal of Affective Disorders* 110, 142-148.

Friedman, L., Furberg, C., and DeMets, D. (1998). "Fundamentals of Clinical Trials: Springer.".

George, D., and Paul, M. (2010). *SPSS for Windows step by step : a simple guide and reference, 17.0 update*, Boston: Allyn & Bacon.

Gerber, J., Douglas, W., Kitch, D., Fichtenbaum, C., Zackin, R., Charles, S., Hogg, E., Edward, P., Acosta, E., Connick, E., Wohl, D., Kojic, M., Benson, C., and Aberg, J. (2008). "Fish Oil and Fenofibrate for the Treatment of Hypertriglyceridemia in HIV-Infected Subjects on Antiretroviral Therapy Results of ACTG A5186." *Journal of Acquired Immune Deficiency Syndrome*, 47, 459-466.

Gibson, R. S. (2012). "A Historical Review of Progress in the Assessment of Dietary Zinc Intake as an Indicator of Population Zinc Status." *Advances in Nutrition*, 3(6), 772-782.

Giddens, J. B., Krug, S. K., Tsang, R. C., Guo, S., Miodovnik, M., and Prada, J. A. (2000). "Pregnant adolescent and adult women have similarly low intakes of selected nutrients." *J Am Diet Assoc*, 100(11), 1334-40.

Gitahi, T. (2012). *Diffrerences in cardiovascular disease, biochemical risk markers, physical activity and nutrition between an urban and pastoral sample in Kenya*.

Goodman, J., and Tyer-Viola, L. (2010). "Detection, Treatment, and Referral of Perinatal Depression and Anxiety by Obstetrical Providers." *Journal of Women's Health*, 19(3), 477-490.

Greenfield, H., and Southgate, D. (2003). *Food composition data : production, management and use*, Rome: FAO.

Grindel, A., Staps, F., and Kuhnt, K. (2013). "Cheek cell fatty acids reflect n-3 PUFA in blood fractions during linseed oil supplementation: a controlled human intervention study." *Lipids Health Dis*, 12, 173.

Grosso, G., Galvano, F., Marventano, S., Malaguarnera, M., Bucolo, C., Drago, F., and Caraci, F. (2014). "Omega-3 fatty acids and depression: scientific evidence and biological mechanisms." *Oxid Med Cell Longev*, 2014, 313570.

Handayani, S., and Budimarwanti, C. (2010). "DEVELOPMENT OF SIMPLE ANALYTICAL METHOD OF OMEGA-3 FATTY ACID BY PROPANOIC ACID DETERMINATION USING ALKALIMETRIC TITRATION." *Indonesian Journal of Chemistry*, 6(3), 322-324.

Harris, W., Sands, S., Windsor, S., Ali, H., Stevens, T., Magalski, A., Porter, C., and Borkon, M. (2004). "Omega-3 Fatty Acids in Cardiac Biopsies from Heart Transplantation Patients. Correlation with Erythrocytes and Response to Supplementation." *Circulation American Heart Association, Inc.*, 110, 1645-1649.

Hibbeln, R., Blasbalg, T., Rigs, J., and Land, W. (2006). "Healthy intakes of n-3 and n-6 fatty acids: estimations considering worldwide diversity." *American Journal of Clinical Nutrition*, 83 (Suppl), 1483S-93S.

Hoffman, D. R., Birch, E. E., Birch, D. G., and Uauy, R. (1999). "Fatty acid profile of buccal cheek cell phospholipids as an index for dietary intake of docosahexaenoic acid in preterm infants." *Lipids*, 34(4), 337-42.

Holland, J., and Tross, S. (1985). "The psychosocial and neuropsychiatric sequelae of the acquired immunodeficiency syndrome and related disorders." *Annals of Internal Medicine*, 103(5), 760-764.

Holman, R. (1998). "The Slow Discovery of the Importance of ω3 Essential Fatty Acids in Human Health." *The Journal of Nutrition*, 128(2), 427S-433S.

Holman, R. T., Johnson, S. B., and Hatch, T. F. (1982). "A case of human linolenic acid deficiency involving neurological abnormalities." *Am J Clin Nutr*, 35(3), 617-23.

Hornstra, G. (2003). "Essential fatty acids in women and their neonates." *American Journal of Clinical Nutrition* 71(5 suppl), 1262S-1269S

Ickovics, J., Hamburger, M., Vlahov, D., Schoenbaum, E., Schuman, P., Boland, R., and Moore, J. (2001). "Mortality, CD4 cell count decline, and depressive symptoms among HIV-seropositive women: Longitudinal analysis from the HIV Epidemiology Research Study." *JAMA*, 285(11), 1466-1474.

Innis, S., and Friesen, R. (2008). "Essential n-3 fatty acids in pregnant women and early visual acuity maturation in term infants." *American Journal of Clinical Nutrition*, 87, 548-557.

Isaac, R., Jacobson, D., Wanke, C., Hendricks, K., Knox, T. A., and Wilson, I. B. (2008). "Declines in dietary macronutrient intake in persons with HIV infection who develop depression." *Public health nutrition*, 11(02), 124-131.

Jamieson, J. (2004). "Analysis of covariance (ANCOVA) with difference scores." *International Journal of Psychophysiology*, 52(3), 277-283.

Jans, L., Giltay, E., and Willem Van der Does, A. (2010). "The efficacy of n-3 fatty acids DHA and EPA (fish oil) for perinatal depression. Systematic Review." *British Journal of Nutrition*, 104(11), 1577-1585.

Jones, P., and Papamandjaris, A. (2001). "Lipid: Cellular Metabolism in Present Knowledge in Nutrition", B. Bowman and R. Russell, (eds.), *Present Knowledge in Nutrition*. City: ILSI Press: Washington, DC, pp. 104-114.

Kaaya, S. F., Mbwambo, J. K., Kilonzo, G. P., Van Den Borne, H., Leshabari, M. T., Fawzi, M. C., and Schaalma, H. (2010). "Socio-economic and partner relationship factors associated with antenatal depressive morbidity among

pregnant women in Dar es Salaam, Tanzania." *Tanzan J Health Res*, 12(1), 23-35.

Kagee, A., Nel, A., and Saal, W. (2014). "Factor structure of the Beck Depression Inventory-II among South Africans receiving antiretroviral therapy." *AIDS Care*, 26(2), 257-62.

Kammerer, M., Taylor, A., and Glover, V. (2006a). "The HPA axis and perinatal depression: a hypothesis." *Archives of women's mental health*, 9(4), 187-196.

Kammerer, M., Taylor, A., and Glover, V. (2006b). "The HPA axis and perinatal depression: a hypothesis." *Archive of Womens Menta Health*

Kapembwa, M. S., Fleming, S. C., Griffin, G. E., Caun, K., Pinching, A. J., and Harris, J. R. (1990). "Fat absorption and exocrine pancreatic function in human immunodeficiency virus infection." *Q J Med*, 74(273), 49-56.

Kapetanovic, S., Christensen, S., Karim, R., Lin, F., Mack, W. J., Operskalski, E., Frederick, T., Spencer, L., Stek, A., and Kramer, F. (2009). "Correlates of perinatal depression in HIV-infected women." *AIDS patient care and STDs*, 23(2), 101-108.

Kilts, C. D., Wade, A. G., Andersen, H. F., and Schlaepfer, T. E. (2009). "Baseline severity of depression predicts antidepressant drug response relative to escitalopram." *Expert Opin Pharmacother*, 10(6), 927-36.

Kim, M. H., Mazenga, A. C., Devandra, A., Ahmed, S., Kazembe, P. N., Yu, X., Nguyen, C., and Sharp, C. (2014). "Prevalence of depression and validation of the Beck Depression Inventory-II and the Children's Depression Inventory-Short amongst HIV-positive adolescents in Malawi." *J Int AIDS Soc*, 17(1).

Klingler, M., Demmelmair, H., Koletzko, B., and Glaser, C. (2011). "Fatty acid status determination by cheek cell sampling combined with methanol-based ultrasound extraction of glycerophospholipids." *Lipids*, 46(10), 981-90.

Kris-Etherton, P. M., Grieger, J. A., and Etherton, T. D. (2009). "Dietary reference intakes for DHA and EPA." *Prostaglandins Leukot Essent Fatty Acids*, 81(2-3), 99-104.

Kwalombota, M. (2002). "The effect of pregnancy in HIV-infected women." *AIDS Care*, 14(3), 431-433.

Lancaster, C. A., Gold, K. J., Flynn, H. A., Yoo, H., Marcus, S. M., and Davis, M. M. (2010). "Risk factors for depressive symptoms during pregnancy: a systematic review." *American Journal of Obstetrics and Gynecology*, 202(1), 5-14.

Lapillonne, A., DeMar, J. C., Nannegari, V., and Heird, W. C. (2002). "The fatty acid profile of buccal cheek cell phospholipids is a noninvasive marker of long-chain polyunsaturated Fatty Acid status in piglets." *J Nutr*, 132(8), 2319-23.

Lesperance, F., Frasure-Smith, N., St-Andre, E., Turecki, G., Lesperance, P., and Wisniewski, S. R. (2011). "The efficacy of omega-3 supplementation for major depression: a randomized controlled trial." *J Clin Psychiatry*, 72(8), 1054-62.

Lespérance, F., Frasure-Smith, N., Turecki, G., Lespérance, P., and Wisniewski, S. R. (2010). "The efficacy of omega-3 supplementation for major depression: a randomized controlled trial." *Journal of Clinical Psychiatry*.

Leung, B., and Kaplan, B. (2009). " Perinatal Depression: Prevalence, Risks, and the Nutrition Link-A Review of the Literature." *Journal of American Dietetians Association*, 109, 1566-1575.

Lipps, G., Lowe, G., De La Haye, W., Longman-Mills, S., Clarke, T., Barton, E., and Bain, B. (2010a). "Validation of the Beck Depression Inventory II in HIV-positive patients." *The West Indian medical journal*, 59(4), 374-9.

Lipps, G. E., Lowe, G. A., De La Haye, W., Longman-Mills, S., Clarke, T. R., Barton, E. N., and Bain, B. (2010b). "Validation of the Beck Depression Inventory II in HIV-positive patients." *West Indian Med J*, 59(4), 374-9.

Llorente, A., Jensen, C., Voigt, R., Fraley, J., Berretta, M., and Heird, W. (2003). "Effect of maternal docosahexaenoic acid supplementation on postpartum depression and information processing." *American journal of obstetrics and gynecologys* 188, 1348-53.

Logan, A. (2004). "Omega-3 fatty acids and major depression: A primer for the mental health professional." *Lipids in Health and Disease*, 3(25), 1-8.

Makrides, M., Gibson, R., McPhee, A., Yelland, L., Quinlivan, J., Ryan, P., and Team, D. I. (2010). "Effect of DHA Supplementation During Pregnancy on Maternal Depression and Neurodevelopment of Young Children A Randomized Controlled Trial." *JAMA.*, 304(15), 1675-1683.

Manfredi, R., Calza, L., and Chiodo, F. (2004). "A case-control study of HIV-associated pancreatic abnormalities during HAART era. Focus on emerging risk factors and specific management." *Eur J Med Res*, 9(12), 537-44.

Manikkam, L., and Burns, J. K. (2012). "Antenatal depression and its risk factors: an urban prevalence study in KwaZulu-Natal." *S Afr Med J*, 102(12), 940-4.

Marangell, L., Martinez, J., Zboyan, H., Kertz, B., Seung Kim, F., and Puryear, L. (2003). "A Double-Blind, Placebo-Controlled Study of the Omega-3 Fatty Acid Docosahexaenoic Acid in the Treatment of Major Depression"*American Journal of Psychiatry*. City, pp. 996-99.

Marcus, M., Yasamy, T., Mark van Ommeren, Chisholm, D., and Saxena, S. (2012). *DEPRESSION: A Global Crisis. World Mental Health Day, October 10 2012*. WHO.

Marcus, S., Flynn, H., Blow, F., and Barry, K. (2004). "Depressive Symptoms among Pregnant Women Screened in Obstetrics Settings." *Journal of Women's Health.*, 12(4), 373-380.

Maundu, P., Ngugi, G., and Kabuye, C. (1999). *Traditional food plants of Kenya*, Nairobi, Kenya: Kenya Resource Centre for Indigenous Knowledge, National Museums of Kenya.

McCarney, R., Warner, J., Iliffe, S., van Haselen, R., Griffin, M., and Fisher, P. (2007). "The Hawthorne Effect: a randomised, controlled trial." *BMC Med Res Methodol*, 7, 30.

McMurchi, E., Margetts, B., Beilin, L., Croft, K., Vandongen, R., and Armstrong, B. (1984). "Dietary-induced changes in the fatty acid composition of human cheek cell phospholipids: correlation with changes in the dietary polyunsaturated/saturated fat ratio." *American Journal of Clinical Nutrition*, 39, 975-980.

Moher, D., Hopewell, S., Schulz, K. F., Montori, V., Gøtzsche, P. C., Devereaux, P., Elbourne, D., Egger, M., and Altman, D. G. (2010). "CONSORT 2010 explanation and elaboration: updated guidelines for reporting parallel group randomised trials." *Journal of clinical epidemiology*, 63(8), e1-e37.

Moradi, Y., Bakar, J., Motalebi, A. A., Syed Muhamad, S. H., and Che Man, Y. (2011). "A Review on Fish Lipid: Composition and Changes During Cooking Methods." *Journal of Aquatic Food Product Technology*, 20(4), 379-390.

Morrison, E., and Lift, H. (1990). "Health Maintenance organizations environment in the 1980s and beyond." *Health Care Financ Rev*, 12, 81 - 90.

Mouratidou, T., Ford, F., Prountzou, F., and Fraser, R. (2006). "Dietary assessment of a population of pregnant women in Sheffield, UK." *Br J Nutr*, 96(5), 929-35.

Mozurkewich, E. L., Clinton, C. M., Chilimigras, J. L., Hamilton, S. E., Allbaugh, L. J., Berman, D. R., Marcus, S. M., Romero, V. C., Treadwell, M. C., Keeton, K. L., Vahratian, A. M., Schrader, R. M., Ren, J., and Djuric, Z. (2013). "The mothers, omega-3, and mental health study: a double-blind, randomized controlled trial". City: USA, pp. 313-e9.

Murphy, S. P., and Vorster, H. H. (2007). "Methods for using nutrient intake values (NIVs) to assess or plan nutrient intakes." *Food & Nutrition Bulletin*, 28(Supplement 1), 51S-60S.

National AIDS and STI Control Programme, N. (2014). *Kenya AIDS Indicator Survey 2012: Final Report.*: NASCOP, Nairobi.

Ndetei, D. M., Khasakhala, L. I., Mutiso, V., and Mbwayo, A. W. (2010). "Suicidality and depression among adult patients admitted in general medical facilities in Kenya." *Ann Gen Psychiatry*, 9, 7.

Nochera, C. L., Goossen, L. H., Brutus, A. R., Cristales, M., and Eastman, B. (2011). "Consumption of DHA + EPA by low-income women during pregnancy and lactation." *Nutr Clin Pract*, 26(4), 445-50.

Nutrisurvey.de. (2007). "Nutrition Surveys and Calculations". City.

Obel, C., Hedegaard, M., Henriksen, T. B., Secher, N. J., Olsen, J., and Levine, S. (2005). "Stress and salivary cortisol during pregnancy." *Psychoneuroendocrinology*, 30(7), 647-656.

Oliveira, J. M., and Rondo, P. H. C. (2011). "Omega-3 fatty acids and hypertriglyceridemia in HIV-infected subjects on antiretroviral therapy: systematic review and meta-analysis." *HIV clinical trials*, 12(5), 268-74.

Opperman, M. (2013). "What health professionals should know about omega-3 fatty acid supplements." *South African Journal of Clinical Nutrition*, 26(2), 6-11.

Panagiotakos, D., Mamplekou, E., Pitsavos, C., Kalogeropoulos, N., Kastorini, C., Papageorgiou, C., Papadimitriou, G., and Stefanadis, C. (2010). "Fatty acids intake and depressive symptomatology in a Greek sample: an epidemiological analysis." *Journal of the American College of Nutrition*, 29(6), 586-94.

Paulsrud, J., Pensler, L., Whitten, C., Stewart, S., and Holman, R. (1972). "Essential fatty acid deficiency in infants induced by fat-free intravenous feeding." *American Journal of Clinical Nutrition*, 25(9), 897-904.

Pawlosky, R. J., Hibbeln, J. R., Novotny, J. A., and Salem, N., Jr. (2001). "Physiological compartmental analysis of alpha-linolenic acid metabolism in adult humans." *J Lipid Res*, 42(8), 1257-65.

Petersen, I. (2013). "Closing the treatment gap for depression co-morbid with HIV in South Africa: Voices of afflicted women." *Health Health*, 05(03), 557-566.

Pinto, E., Barros, H., and dos Santos Silva, I. (2009). "Dietary intake and nutritional adequacy prior to conception and during pregnancy: a follow-up study in the north of Portugal." *Public Health Nutr*, 12(7), 922-31.

Piwoz, E., and Preble, E. (2000). *HIV/AIDS and nutrition: a review of the literature and recommendations for nutritional care and support in sub-Saharan Africa*. . United States Agency for International Development.

Psaros, C., Geller, P. A., and Aaron, E. (2009). "The importance of identifying and treating depression in HIV infected, pregnant women: a review." *J Psychosom Obstet Gynaecol*, 30(4), 275-81.

Puri, B. K., Counsell, S. J., Hamilton, G., Richardson, A. J., and Horrobin, D. F. (2001). "Eicosapentaenoic acid in treatment-resistant depression associated with symptom remission, structural brain changes and reduced neuronal phospholipid turnover." *Int J Clin Pract*, 55(8), 560-3.

Ranieri, R. (2007). "[Effect of omega-3 fatty acids on the lipid structure of HIV-positive patients on antiretroviral therapy: personal observation of 20 subjects]." *Infez Med*, 15(4), 237-41.

Ratnayake, W. M., and Galli, C. (2009). "Fat and fatty acid terminology, methods of analysis and fat digestion and metabolism: a background review paper." *Ann Nutr Metab*, 55(1-3), 8-43.

Rees, A.-M., Austin, M.-P., Owen, C., and Parker, G. (2009). "Omega-3 deficiency associated with perinatal depression: case control study." *Psychiatry research*, 166(2-3), 254-9.

Rees, A., Austin, M., and Parker, G. (2008). "Omega-3 fatty acids as a treatment for perinatal depression: randomized double-blind placebo-controlled trial." *Australia New Zealand Journal of Psychiatry*(42), 199-205.

Rochat, T. J., Richter, L. M., Doll, H. A., Buthelezi, N. P., Tomkins, A., and Stein, A. (2006). "Depression among pregnant rural South African women undergoing HIV testing." *Jama*, 295(12), 1376-8.

Ross, R., Sawatphanit, W., and Zeller, R. (2009). "Depressive symptoms among HIV-positive pregnant women in Thailand." *J Nurs Scholarsh*, 41(4), 344-50.

Samuel, T. M., Thomas, T., Finkelstein, J., Bosch, R., Rajendran, R., Virtanen, S. M., Srinivasan, K., Kurpad, A. V., and Duggan, C. (2013). "Correlates of anaemia in pregnant urban South Indian women: a possible role of dietary intake of nutrients that inhibit iron absorption." *Public Health Nutr*, 16(2), 316-24.

Sandstrom, B. (2001). "Micronutrient interactions: effects on absorption and bioavailability." *Br J Nutr*, 85 Suppl 2, S181-5.

Santoro, K., and Peabody, H. (2010). *Identifying and Treating Maternal Depression: Strategies & Considerations for Health Plans NIHCM Foundation Issue Brief June 2010*. The NIHCM Foundation.

Santos, J. R. A. (1999). "Cronbach's alpha: A tool for assessing the reliability of scales." *Journal of extension*, 37(2), 1-5.

Schlechtriem, C., Henderson, R., and Tocher, D. (2008). "A critical assessment of different transmethylation procedures commonly employed in the fatty acid analysis of aquatic organisms " *Limnol. Oceanogr* (Methods 6), 523-531.

Schulz, K., Altman, D., and Moher, D. (2010). "CONSORT 2010 statement: Updated guidelines for reporting parallel group randomised trials." *Journal of Pharmacology and Pharmacotherapy*, 1(2), 100-7.

Schulz, K. F., and Grimes, D. A. (2002). "Allocation concealment in randomised trials: defending against deciphering." *The Lancet*, 359(9306), 614-618.

Sehmi, J. K. (1993). *National food composition tables and the planning of satisfactory diets in Kenya*, Nairobi, Kenya: Govt. Printer.

Shrivastava, A., and Gupta, V. B. (2011). "Methods for the determination of limit of detection and limit of quantitation of the analytical methods." *Chronicles of Young Scientists*, 2(1), 21.

Singh, M., Jain , S., and Maya Choudhary, M. (2009). "Dietary Adequacy of Pregnant Women of Four District of Rajasthan." *Journal of Human Ecology* 25(3), 161-165.

Smith Fawzi, M., Kaaya, S., Mbwambo, J., Msamanga, G., Antelman, G., Wei, R., Hunter, D., and Fawzi, W. (2007). "Multivitamin supplementation in HIV-positive pregnant women: impact on depression and quality of life in a resource-poor setting." *HIV Medicine* 8, 203-212.

Stata, C. (2009). "Stata Statistical Software "*Release 11*. City: College Station, Texas,USA.

Steyn, N., Parker, W., Nel, J., Ayah R, and Mbithe, D. (2012a). "The Nutrition Transition and Adequacy of the Diet of Pregnant Women in Kenya." *International Proceedings of Chemical, Biological and Environmental Engineering (IPCBEE)*, 39(2012 International Conference on Nutrition and Food Sciences).

Steyn, N. P., Nel, J. H., Parker, W., Ayah, R., and Mbithe, D. (2012b). "Urbanisation and the nutrition transition: a comparison of diet and weight status of South African and Kenyan women." *Scand J Public Health*, 40(3), 229-38.

Stoll, A. (2001). *The Omega-3 connection.*, New York.: Simon and Schuster.

Su, K., Huang, S., Chiu, T., Huang, K., Huang, C., Chang, H., and Pariante, C. (2008). "Omega-3 fatty acids for major depressive disorder during pregnancy: results from a randomized, double-blind, placebo-controlled trial." *J Clin Psychiatry*, 69(4), 644-51.

Thase, M. (2011). "The role of neurobiologic processes in symptoms of depression." *Journal of Clinical Psychiatry.*, 72(2), e08.

The National Academies. (1998). *Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6,Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline (1998);* .

The National Academies. (2001). *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium and Zinc* New York, National Academies.: The National Academies Press.

The National Academies. (2005). *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients)*, New York: , National Academies.: The National Academies Press.

The National Agricultural Library. (2011). "USDA National Nutrient Database for Standard Reference ". City: http://fnic.nal.usda.gov/food-composition: Beltsville, USA.

Turchini, G. M., Nichols, P. D., Barrow, C., and Sinclair, A. J. (2011). "Jumping on the Omega-3 Bandwagon: Distinguishing the Role of Long-Chain and Short-Chain Omega-3 Fatty Acids." *Critical Reviews in Food Science and Nutrition*, 52(9), 795-803.

Wang, D., Lees, B., and Bakhai, A. (2006). "Trial profile." *Clinical trials: a practical guide to design, analysis and reporting*, 377-84.

Warner, R., Appleby, L., Whitton, A., and Faragher, B. (1996). "Demographic and obstetric risk factors for postnatal psychiatric morbidity. ." *British Journal of Psychiatry*, 168, 607-611.

WHO. (2008). *The Global Burden of Disease, 2004 Update*, Geneva.

WHO. (2010). "Antiretroviral therapy for HIV infection in adults and adolescents: recommendations for a public health approach-2010 revision."

WHO. (2012). *Kenya Commemorates World Mental Health Day 2012*. World Health Organization, Nairobi.

WHO/FAO. (2004). "Vitamin and mineral requirements in human nutrition [report of a joint FAO/WHO expert consultation, Bangkok, Thailand, 21-30 September 1998."

Wohl, D., Tien, H., Busby, M., Cunningham, C., MacIntosh, B., Napravnik, S., Danan, E., Donovan, K., Hossenipour, M., and Simpson, J. R. (2005). "Randomized Study of the Safety and Efficacy of Fish Oil (Omega-3 Fatty Acid) Supplementation with Dietary and Exercise Counseling for the Treatment of Antiretroviral Therapy Associated Hypertriglyceridemia." *Clinical Infectious Diseases* 41, 1498-504.

World Health Organization/UNAIDS/UNICEF. (2007). *Towards Universal Access: Scaling up priority HIV/AIDS interventions in the health sector. Progress Report, April 2007*.: World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland.

APPENDICES

Appendix 1: Amended Participant Informed Consent Explanation and Forms

Informed Consent Explanation and Consent Form for Study Participants (English)

I, **Ms. Rose O. Opiyo**, from School of Public Health, University of Nairobi, am conducting a research on "Role *of Fish Oil Omega-3 Fatty Acids on Depression among HIV-Seropositive Pregnant Women in Nairobi*". Fish oil omega-3 fatty acids are some of the nutrients important for health, especially in the brains, eyes, heart, and skin. In pregnancy, adequate intake of these nutrients is important since the growing foetus also needs them for normal growth, development and maturation of many organs.

What is the Purpose of this study?
This research is my doctorate degree work at the University of Nairobi. It seeks to establish the role of fish oil omega-3 supplements in reduction of symptoms of depression among HIV-positive pregnant women.

Who will Participate?:
If you are pregnant HIV-positive, in week 13-27 (second trimester) of pregnancy and with known CD4 cell count of less than 500 cells/mm^3 and have:
Symptoms of depression with scores of 14 or more on BDI-II Scoring tool;
Not been under weight or overweight just before getting pregnant;
Given consent to be in the study by signing the acceptance form.

However, those who will not qualify to be included in the study after screening will be women:
Who were underweight or overweight just before getting pregnant as measured by mid-upper arm circumference of (MUAC: less than 21cm or more than 33 cm);
Taking antidepressant medications;
On anti-clotting medication (have liver disease, varicose veins, peptic ulcers); or Vitamin K;
On diabetic medication. Omega-3 may increase their blood sugar;
Who refuse to answer all depression symptom questions;
Currently taking omega-3 nutritional supplement;
Who will not be willing to participate in the study by not signing the acceptance form;
Without symptoms of depression- BDI-II score are less than 14.

What will be the procedure for data Collection and intervention?
Data collection will be at 4 levels/stages - 1) screening; 2) baseline at recruitment and randomization; 3) monitoring and 4) end of intervention. The data collection will involve individual interviews on socio-demographic information, dietary intake, and morbidity and depression symptoms. Your weight, mid-upper arm circumference (MUAC) measurements will also be taken. Your blood pressure and CD4 readings will also be recorded from your Mother and Child Health Booklet. You will then rinse your mouth with clean water and spit it into the glass for collection to the laboratory at the University of Nairobi for analysis of your omega-3 status.

On the first visit, you will be randomly givenomega-3 supplement from Innovix Pharma Inc (http://www.omegavia.com). Each recruited mother has an equal chance of

receiving any of the two supplements provided. You will be given three (3) soft gels (capsules) to swallow whole without breaking it per day with food. You will take one in the morning, mid-day and evening alongside your ARVs and other usual medicine. Swallowing these supplements with food helps improve their absorption. You will be in the study for eight (8) weeks from the time you consent to participate. There will be monitoring after every two (2) weeks or as necessary. During the bi-weekly monitoring, you will get the same supplement soft gels (capsules) and porridge flour to last for the next 2 weeks.

The Omegavia fish oil omega-3 soft gel samples will only be used for purposes of the study and will not be stored for future use. I will be responsible for disposal of the remaining samples as per the approved bio safety rules.

Reimbursements – Who will pay transport cost and other expenses?
During each visit, you will be given KSh. 150.00 to cover your transport costs and buy at least a packet of milk. The transport cost calculation is based on the fact that mothers recruited from this health facility reside within a distance where they pay up to KSh. 50.00 for public transport. You will also receive150 g/day of millet porridge flour to carry home for breakfast while taking the omega-3 supplement and other medication.
Can someone be forced to participate in this study? - Voluntarism
Participation in this study is completely voluntary. If you decide not to participate, there is no penalty for such decision. If you agree to take part in the study, you are also free to withdraw from participating any time. If you decide to participate, you are also free not to answer questions that you are not comfortable with.

What are the Benefits of participating in this study?
You will have free access to fish-oil omega-3 supplements and this has several health benefits both for the mother and baby:
They are essential nutrients needed to maintain health;
They facilitate regulation of many functions of the body e.g. brain, eyes;
They help reduce the amount of other types of bad fat circulating in the blood
Research has shown that dietary intake of omega-3 fatty acids increased CD4 levels;
Since the modern diet is inadequate in long chain omega-3, the supplement(capsule) form offers you the most convenient way of meeting omega-3 daily needs for the body;
Omega-3 supplements reduce possibility of pre-mature births and it is important for the infants' brain development

What are the Risks in participating in this study?
There are no major risks for participating in this study. The procedures for taking weight, MUAC and mouth rinse are painless. The dosage of omega-3 fish oil capsule that you will receive of 3 g/day (3 soft gels of *Omegavia* EPA-rich) fish oil omega-3 supplement has been found to be safe with no major side effects except that some mothers may feel the following mild symptoms:

- Fishy taste;
- Stomach upset;
- Nose bleeding;
- Loose stools.

Warning!

Taking more than four (4) soft gels of omegavia per day is dangerous for you and can:
Cause bleeding; Increase symptoms of depression; Lower blood pressure and increase risks of diabetes.

What happens in case of any adverse events related to the study?
I will closely monitor any symptoms of side effects or adverse events related to the study throughout the study period. In case of any, it will be reported immediately to the doctor to take action as necessary.

Is there any Confidentiality in this study?
The responses you provide will be kept confidential and anonymous. Names will not appear in the questionnaires. You will just be assigned codes. The data collected for this study is only for education purposes. In case of publication your names will not appear on the papers. Confidentiality of your responses will therefore be strictly adhered to.

What if there is any question/concern/complaint? What is the Contact address?
If you have any question regarding what I have explained to you, you are free to ask now. You are also free to contact me any time or day incise of any questions/concern or complaints about the study on the mobile: 0722 473122, or The Secretary, Kenyatta National Hospital / University of Nairobi – Ethics and Research Committee – (Tel: 726300-9 or P.O Box 20773, Nairobi.)

Consent Form for Study Participants
I ……. (initials), have read or been explained what the study entails and I have also had some chance to ask questions, and I hereby agree/ not agree to participate in the study.

Study participant: ..…… Date: ……..
Signature

Researcher …… Date: ……
Signature

Appendix 2: Kiswahili Translation of Consent Explanation and Form

Maelezo Kamili ya kuridhia ushiriki wa utafiti (Ya Akina mama washiriki)

Mimi, **Bi Rose O. Opiyo**, kutoka Shule ya Public Health, Chuo Kikuu cha Nairobi, nafanya utafiti kuhusu"
'*Umihimu wa aina ya mafuta ya Omega-3 inayopatikana kwa samaki na vile inaweza kusaidia na shida ya shindikizo ya mawazo (depression) kati ya wanawake wanaoishi na virusi vya ukimwi na niwajawazito"*. Mafuta hii ya omega-3 ni muhimu kwa afya, hasa katika akili, macho, moyo, ngozi na kadhalika. Kwa mama mjamzito, ni muhimu apate haya madini ya kutosha ili viungo anayo pia ipate ya kutosha.

Je, nini Lengo (*purpose*) la tafiti hii?
Tafiti hii ni kazi yangu ya shahada katika Chuo Kikuu cha Nairobi ambayo. Itaangalia kama hii aina ya mafuta ya Omega-3 inayopatikana kwa samaki inaweza kupunguza dalilil za shindikizo ya mawazo (depression) kati ya wanawake wanaoishi na virusi vya ukimwi na niwajawazito.

Je, akina nani watashiriki?
Mama wajawazito, walioambukizwa na virusi, na CD4 count yao iko chini ya 500 na mimba yao inaanzia wiki 13 hadi 27. Mama hawa wataulizwa maswali fulani kujua kama wako na dalili za shindikizo za mawazo. Wale wataopatikana na hizi dalili kupita kiasi ya alama 14, na wamekubali kuweka sahihi ndiyo watashiriki katika hi utafiti. Tena hawa washiriki lazima wasiwe wanatumia dawa za diabetes, ulcers, vericose veins, ama omega-3 wakati huo.

Ni nini itakuwa ikiendelea kwa hii tafiti?
Kila mama mshiriki atahojiwa binafsi juu ya kijamii lishe bora (nutrition), dalili za ugonjwa na shindikizo ya mawazo.Utapimwa uzito, mdwara wa mkono (mid-upper arm circumference). CD4 count na Blood pressure zako pia zitachukuliwa kutoka kitabu chako na mtoto (Mother and Child Health Booklet). Utasafisha mdomo wako na maji kidogo safi halafu uteme hiyo maji kwa chupa ambayo itapelekwa kwa laboratory ili kujua kiasi ya madini ya omega-3 mwilini mwako.

Kwanza, utakapomaliza kujibu maswali na kupeana chupa ya maji ya mdomo wako, utapewa aina ya omega-3 ya utafiti huu kutoka **Innovix Pharma Inc (http://omegavia.com).** Kutakuwa na aina mbili na kila moja wenu atakuwa na nafasi sawa ya kupokea yoyote ya hizo mbili zinazotolewa. Utapewa vidonge (capsules) 3 kumeza nzima bila kuvunja kwa siku na chakula. Utapewa za kumeza kwa wiki mbili (2), moja asubuhi, mchana na jioni pamoja na ARVs yako na dawa zako zingine za kawaida. Ni lazima uendelee na hizo dawa zako za kawaida bila kukosa kwa sababu hizi unapata kwa hii tafiti siyo dawa.Ujaribu umeze hizi vidonge na chakula ndio ziingie kwa mwili. Utashiriki katika tafiti hii kwa muda wa wiki nane (miezi miwili) kuanzia siku utakapokubali kuwa mshiriki. Kutakuwa na ufuatiliaji baada ya kila wiki mbili (2) au kama ni muhimu. Wakati huo, utapata hizo vidonge za omega-3 na unga ya uji pia za kutumia kwa wiki mbili. Hizi vidonge za omega-3 ni za sampuli na zitatumika tu kwa madhumuni ya utafiti na wala kuhifadhiwa kwa ajili ya matumizi ya baadaye. Mimi kama mwenye tafiti hii, nitahakikisha ya kwamba zile vidonge zitakazobaki zimetupwa ama kuwekwa kulingana na sheria ya Biosafety.

Gharama za usafiri nani atalipa?
Utapewa Ksh. 150.00 ya gharama za usafiri na kununua angalau maziwa paketi moja kila wakati utakapokuja hapa kuhusu mambo ya hi tafiti. Hesabu ya hi malipo ni kwa vile magari za *matatu* zinalipisha kutoka mahali wengi wenu mnaishi kufika hapa kituo cha afya. Utapewa pia unga wa mtama uji 1Kg kila wiki (150 g / kila siku) ya kunyua kila asubuhi ndiyo upate kumeza vidonge za omega-3 pamoja na dawa zako zingine.

Je, mama anaweza lazimisha kushiriki katika huu utafiti? *(Voluntarism*)
Kushiriki katika utafiti huu ni hiari kabisa. Sio lazima.Ukiamua kushiriki katika utafiti huu, hakuna adhabu kwa uamuzi huo. Uko huru kushiriki na tena kuacha kushiriki katika utafiti. . Kama kuamua kushiriki, ukohuru pia kutojibu maswali ambazo hujafurahiya.

Kuna faida gani kushiriki katika utafiti hii?
Vvidonge vya omega-3, zitakuwa bila malipo, na omega-3 ina faida nyingi za afya kwa mama na mtoto kama ifwatavyo:

- Ni moja ya zile madini zilizo kwa chakula ambazo mwili inahitaji ili kudumisha afya;
- zinasaidia viungo nyingi za mwili kufanya kazi, kwa mfano ubongo, macho;
- Husaidia kupunguza kiasi cha aina ya mafuta mbaya kuzunguka na damu mwilini
- Omega-3 husaidia kuongeza CD4 cells;
- Kwa vile hizi madini za omega-3 hazipatikani kwa kutosha kwa mlo ya kisasa, hizi vidonge (supplement), zitakuwezesha kutimiza mahitaji yako ya omega-3
- Mtoto hawezi zaliwa kabla hajakomaa, na pia muhimu kwa ubongo ya mtoto mchanga

Matokeo ya utafiti huu itatumika kupanga miradi inayoweza saidia wale wako na shida ya shindikizo ya mawazo na ni wajawazito ama wanaoishi na virusi vya ukimwi katika siku zijazo.

Je, Kuna Hatari (Risks) kwa utafiti hii?
Ukishiriki na hii utafiti, hakuna hatari kubwa vile. Kupimwa uzito, mikono na kujiosha mdomo iliuteme mate kwa chupa hazina uchungu wote. Kiasi ya vidonge vya omega-3 amabazo utapata kutoka kampuni ya *Omegavia* haina madhara. Labda tu ni ladha ya samaki ndiyo inaweza sumbua wa mama wengine, sio kila mtu. Wa mama wengine pia waweza kusumbuliwa na tumbo kidogo, kwa wengine pua kutokwa na damu kidogo, ama choo iwe nyepesi.

Onyo!
Kumeza zaidi ya vidonge nne(4) ya *Omegavia* omega-3 kwa siku moja ni hatari na inaweza kusababisha kutokwa na damu; kuongeza dalili za *depression*, kupeleka *blood pressure* chini na hata kuongeza hatari ya ugonjwa wa kisukari*(diabetes*).

Je, itakuaje kama kutatokezea madhara yoyote kuhusu utafiti hii?
Mimi binafsi nitafuatilia wakati wote wa utafiti hii. Ikitokezea madhara yoyote, nitajulisha daktari ambaye atachukua hatua ya matibabu inayotakikana.

Usiri (Confidentiality)

kutoa majibu kuwa siri na bila majina wala kuonekana katika dodoso. Utafiti washiriki tu kupewa namba. Takwimu zilizokusanywa kwa ajili ya utafiti huu ni kwa madhumuni ya elimu. Kwa upande wa uchapishaji, hakuna washiriki majina itaonekana kwenye magazeti. Siri ya majibu kwa hiyo madhubuti kuzingatiwa.

Je, kama kuna swali ama jambo lolote kuhusu utafiti huu itakuwa aje? - Wasiliana (*Contact*)
Kama una swali kuhusu nini nilivyoeleza kwako, uko huru kuuliza. Tena, kama una wasiwasi au malalamiko kuhusu utafiti huu uko huru kuwasiliana nami kwa simu wakati wote: 0722 473122, au Katibu, Kenyatta National Hospital / Chuo Kikuu cha Nairobi - Kamati ya Maadili na Utafiti - (Tel: 726,300-9 au P.O. Box 20,773, Nairobi.)

Idhini Fomu ya mama mshiriki wa utafiti (Consent Form for Participant)

Mimi, ... (Initials), nimesoma au imeelezwa utafiti unahusu nini na mimi pia nilikuwa na nafasi ya kuuliza maswali, nakubaliana / si kukubali kushiriki katika utafiti.

Utafiti mshiriki: ... ... Tarehe: ... ...
Sahihi

Mtafiti ... ... Tarehe: ... ...
Sahihi

Appendix 3: Randomization

No of Blocks	4
No. of Treatments	2
No. of Combinations	6

Possible combinations

BBAA BABA BAAB ABBA ABAB

Kayole	Riruta	Kariobangi	Mathare
A,B,A,B	B,B,A,A	A,B,B,A	A,B,A,B
B,A,A,B	A,B,B,A	A,B,A,B	A,B,B,A
B,A,B,A	B,A,B,A	A,B,B,A	A,B,A,B
B,B,A,A	A,B,A,B	B,A,A,B	B,A,B,A
B,B,A,A	A,A,B,B	B,A,B,A	A,A,B,B
A,A,B,B	A,B,B,A	B,B,A,A	B,B,A,A
A,B,B,A	A,B,B,A	A,B,A,B	A,B,A,B
A,B,A,B	B,A,A,B	A,B,B,A	A,B,A,B
A,A,B,B	B,A,A,B	A,B,B,A	A,A,B,B
A,B,A,B	B,B,A,A	B,A,B,A	A,A,B,B
B,B,A,A	A,B,B,A	A,A,B,B	A,A,B,B
A,B,B,A	A,B,A,B	B,A,B,A	A,B,A,B
B,A,B,A	B,A,B,A	A,B,B,A	B,A,A,B
A,B,B,A	A,A,B,B	A,B,B,A	A,B,B,A
B,B,A,A	B,A,B,A	A,B,A,B	B,A,B,A
A,A,B,B	B,A,A,B	A,A,B,B	A,A,B,B
B,A,A,B	A,B,A,B	B,B,A,A	B,A,A,B
A,B,B,A	A,A,B,B	A,A,B,B	A,B,A,B
A,A,B,B	B,A,B,A	A,B,A,B	B,A,B,A
B,A,B,A	A,A,B,B	B,B,A,A	A,B,A,B
B,B,A,A	B,B,A,A	B,A,B,A	B,A,A,B
B,A,A,B	A,A,B,B	B,A,B,A	A,A,B,B
B,A,B,A	A,A,B,B	A,B,A,B	A,A,B,B
A,B,B,A	A,B,A,B	B,B,A,A	A,B,A,B
A,B,B,A	B,A,B,A	A,B,A,B	B,A,B,A

Appendix 4: Training Curriculum for Research Assistants

This was a 7-day training curriculum from Saturday to the following Saturday to allow for pre-test and omega-3 safety test among sampling frame at the health facility.

Day 1: Level 1 of Data Collection – Screening participants for Recruitment

1. Introduction to the study, research objectives and duration of data collection;
2. Screening study participants for recruitment
    - Inclusion and exclusion criteria – Researcher;
    - Pre-pregnancy BMI Calculation – Researcher;
    - Morbidity history and medication;
    - Depression screening - BDI-II scoring tool.

Day 2: Level 2 - Socio-demographic, food intake, EPDS and other data collection

1. Socio-demographic and morbidity questionnaire;
2. Food intake and use of nutrition supplements;
3. Edinburgh Postnatal Depression Scale (EPDS);
4. Recording other data from health records (e.g. Blood pressure and CD4 cell counts).

Day 3: Anthropometry, mouth-wash, cheek cell samples and safety test of trial samples

1. Pregnancy weight measurements;
2. Mouth wash for cheek cell sample collection;
3. Packaging and storing cheek cell samples for transportation to the laboratory;
4. Safety test of omega-3 supplement and placebo as per randomization design.

Day 4: Pretest of Study tools

1. Pre-pregnancy BMI Calculation;
2. Morbidity history and medication;
3. Depression screening - Beck Depression Inventory Second Edition (BDI-II) scale.

Day 5: Pre-test of Study tools

1. Socio-demographic and morbidity questionnaire;
2. Food intake and use of nutrition supplements;
3. Edinburgh Postnatal Depression Scale (EPDS);
4. Recording other data from health records (e.g. Blood pressure and CD4 cell counts).

Day 6: Pre-test of study tools and omega-3 safety test

1. Pregnancy weight measurements;
2. Mouth- wash for cheek cell sample collection;
3. Packaging and storing cheek cell samples for transportation to the laboratory;
4. Distribution of omega-3 supplement and placebo for safety test.

Day 7: Review of pre-test by the whole research team

1. Sharing of experiences from pre-test and improvement of data collection tools;
2. Program and Logistics of data collection.

Appendix 5: List of locally available foods and their serving sizes

Commonly consumed foods rich in selected nutrients (B-Complex vitamins, Vit. C, Vit. E, Iron, Calcium, Selenium, Omega-3, Folate & Zinc)

Case Study of Mathare North and Kawangware residential estates

Food Item	Description of Commonly used serving sizes in residential estates in Nairobi				
	Cost (Ksh)/ Description	Serving Equipment (spoon, cup, bowl)	Actual Size	Quantity (g)	Other descriptions
Cabbage	10	Serving spoon	½	50	Cooked
		Serving spoon	¾	78	Cooked
		Serving spoon	1	90	level
		Serving spoon	1	103	Heaped
		Tea spoon	½	12	Cooked
		Table spoon	1	33	Cooked
		Large Table spoon	1	39	Cooked
Carrots	Cooked chopped	Serving spoon	½	41	Cooked
		Serving spoon	¾	56	Cooked
		Serving spoon	1	85	Cooked
		Table spoon	½	8	Cooked
		Table spoon	¾	13	Cooked
		Table spoon	1	18	Cooked
	Raw Grated carrots	Serving spoon	½	7	Raw
		Serving spoon	¾	10	Raw
		Serving spoon	1	19	Raw
		Table spoon	½	43	Raw
		Table spoon	¾	55	Raw
		Table spoon	1	79	Raw
	Raw whole carrot	Whole	Small	43	Raw
		Whole	Medium	55	Raw
Chicken	Chicken drumstick		Medium	85	cooked
	Chicken breast		Medium	89	cooked
	Chicken liver	1 large size	medium	33	cooked
	Chicken wing	1 wing	medium	29	cooked
Eggs boiled		1	medium	52 g	Boiled
Eggs Fried		1	medium	51 g	Fried
Fish: Nile perch	Muscles :	*Helicopter(20/=)* *Helicopter (15/=)*	78g 66g	78g 66g	Stewed
	Head +gills (Edible parts)	Large size (20/=) Medium size (15/=)	80g 67g	80g 67g	Stewed
Fish: *Omena*	250g Blue band tin	Serving spoon	3/4	100 g	Stewed
		Table spoon	1	67 g	Stewed
Fish: Sea fish (specify)	None reported				
Fish: Tilapia cooked	Small (100/=)				
	Medium (200/=)				
	Large (300/=)				
Fruits (Avocado)	5/=	Whole	1	145	Small size
	10	Whole	1	185	Medium size
Fruits (Mangoes)	20	Whole	1	552	Large
	15	Whole	1	416	Medium size
Fruits (Orange)	5	Whole	1	124	Medium
Fruits(Ripe bananas)	5	Whole	1	140	Medium
Other Green vegetables	Same quantities as for Kale **(***Skuma Wiki)*				
Green vegetables (Sukuma Wiki)	Chopped	Serving spoon	½	46	Cooked
		Serving spoon	¾	78	Cooked
		Serving spoon	1	130	Cooked
		Table spoon	½	13	Cooked
		Table spoon	1	25	Cooked
Green vegetables (Spinach)	Same quantities as for Kale **(***Skuma Wiki)*				
Green vegetables (Terere)	Same quantities as for Kale **(***Skuma Wiki)*				
Sukuma and spinach (mixed same ratio)	10	Serving spoon	1	71	Cooked
		Table spoon	1	27	Cooked

			Large table spoon	1	36	Cooked
	Legumes (Green grams/lentils)		Plate (3 serving spoons)	3	209g	stewed
	Legumes (Beans cooked)	Per cup	Cup	1	150 g	Cooked
	Legumes (Njahi cooked)	15/= per cup	Cup	1	177	Cooked
	Legumes (Soya bean flour)	Flour for porridge	Table Spoon heaped	1	15 grams flour	1Heaped T. spoons make 250 ml Porridge
	Liver	Serving spoon	1 serving spoon is 7 pieces	1Serving spoon	100 g	Fried
	Meat (Beef)	One medium piece =12 g	1 serving spoon is 10 pieces	1Serving spoon	85g	Stewed beef
	Meat (Goat)	One medium piece =12 g	1 serving spoon is 10 pieces	1Serving spoon	85g	Stewed beef
	Meat (Mutton)	One medium piece =12 g	1 serving spoon is 10 pieces	1Serving spoon	85g	Stewed mutton
	Meat (Pork)	One medium piece =12 g	1 serving spoon is 10 pieces	1 SSp	85g	Stewed pork
	Milk	If milk was used in tea, the quantity (milliliters) used in tea was divided by the number of cups made and participants asked to state how many cups the mother took				
	Nuts (Peanuts, Macademia)	A handful	One handful	1	26 g	Roasted
	Oils (Vegetable fat -solid)	One Table spoon	Table spoon	1	25 g	Vegetable cooking fat
	Oils (Margarine)	Half table spoon	Table spoon	1/2	10 g	Margarine
	Liquid vegetable Oil	Table Spoon	1	1 Table Spoon	20 g	Liquid cooking oil
	Pasta/noodles	One Plate	Two serving spoons per plate	2 Serving Spoons	160 g	Cooked
	Potatoes (Irish)	20	Bunch	4	247	Cooked medium size pieces
	Potatoes (Sweet, *ngwachi*)		Medium size	1	60 g	Cooked
	Seeds (sesame, sunflower,etc)	Roasted in sugar syrup	Small ball-shaped	1		Roasted
	Whole grain cereals (Brown chapatti/bread)	Cooked chapatti or baked bread	Normal, medium size *Chapati* *1 Slice brown bread*	One Chapati One slice bread	29 g	
	Whole grain cereals (Maize meal)	Flour	1 cup water=250ml	Medium tea cup	200g flour	Makes 427 g of Ugali
	Whole grain cereals (Millet, sorghum)	Flour	Table Spoon heaped	1	15 grams flour	2 Heaped T. spoons make 509 ml Porridge

Appendix 6: Data Collection tools

Date…… Health Facility…… Centre Code….. Participant's Code……..…

Role of Fish Oil Omega-3 Fatty Acids on Depression among HIV-Seropositive Pregnant Women
Enumerator …… Date……
Level 1A: Inclusion Screening Questionnaire

	Participant's Age	……Years (Must be 15 -49 years)
1-1	Gestation period *(circle the most applicable period) If pregnancy is* ***Less than 13 weeks or more than 27 weeks do not proceed*** *with interview*. Thank participant and release her) ***(Check from her Health Booklet, do not calculate manually)***	0= Less than 13 weeks or more than 27 weeks – Not applicable– ***Do not proceed with interview*** 1 = 13-27 weeks (Record gestational age.....weeks)
1-2	CD4 Count (Record from her Mother & Child Health Booklet).	CD4 = …… *(Should be 500 and below to be in the study, if more than 500, release the mother, do not continue with interview)*
1-3	Mid-Upper arm circumference (MUAC) measurement	1 = MUAC Less than 21 cm *(Under nutrition*) 2 = MUAC 22cm – 33 cm 3 = MUAC More than 33cm *(Over nutrition)* *(If MUAC is Less than 21 cm for under-nutrition or more than 33 cm for over-nutrition, DO NOT Proceed with interview. Thanks her and release her)*
1-4	**Medical History*: Have you been diagnosed with any of the following conditions in the last 2 weeks and are on medication?***	
	Diabetes	1= Yes (Medication……..……) 2= No
	Vericose Veins	1= Yes (Medication……..……) 2=No
	Peptic Ulcers	1= Yes (Medicati……..……) 2=No
	Liver Problem	1= Yes (Medication…….……) 2=No
	Using Vitamin K supplement	1= Yes (Medication…….) 2=No
	Depression (Mood disorder)	1= Yes (Medication…… ……..……..……) 2=No
If the answer to ANY question in 1-3 is YES, *DONOT proceed* *with interview. Thank participant and release her.*		
1-5	*Explain the study to participant and read out the consent explanation form to her. Allow her to ask any question about the research, and then ask her to sign the form.(If mother cannot write ask for thumb print)*	**Consent form signed:** 0 = No (Give reason if No and do not proceed with interview. Thank and release participant) 1 =Yes
After signing the Consent Form, tell participant that you will now ask her some questions on depressive symptoms to determine if she qualifies to participate in this study.		

Date…… Health Facility…… Centre Code….. Participant's Code……

LEVEL 1B: Inclusion Screening for Depression – BDI-II

1-5: BDI-II: Screening for depressive symptoms - Below is a list of some of the ways you may have felt or behaved in the past Two weeks (14 days). Please tell me exactly how you felt in terms of each of the following: **(*Allow participant to explain how she felt. Repeat what she said before scoring, then score)***		
Depressive Symptom	**NO=0 YES = 1**	**Score the options as 0, 1, 2, and 3 on how the participant felt by selecting <u>one option only</u> from choices given for each depressive symptom.**
<u>1-5-1: Sadness:</u> Have you felt sad in the last 14 days? *(Umehisi huzuni siku 14 zilizopita?)*		0. I do not feel sad (Mimi Sihisi huzuni) 1. I feel sad much of the time (Mimi huwa na huzuni mara nyingi) 2. I am sad all the time (Mimi huwa na huzuni wakati wote) 3. I am so sad or unhappy that I can't stand it(Nina huzunisana hadi siwezikuistahimili)
<u>1-5-2: Pessimism:</u> How do you view your life? *(Unaona maisha yako kuwa namna gani?)*		0. I am not discouraged about my future (Sijafiwa moyo na maisha yangu ya mbele) 1. I feel more discouraged about my future than I should be (Nimekufa moyo kuhusu maisha yangu ya mbele kupita kiasi 2. I do not expect things to work out for me(Situmaini vitu viende ninavyotarajia) 3. I feel my future is hopeless and will only get worse(Sitaraji mazuri wala situmaini kuwa maisha yangu itaimarika)
<u>1-5-3: Past Failure</u> Do you feel like you have failed in life*? (Je, unahisi kuwa umefeli maishani?)*		0. I do not feel like a failure(Sijihisi kuwa nimefeli) 1. I have failed more than I should have(Nimefeli kupita kiasi kilichotarajiwa) 2. As I look back, I see a lot of failures(Nikiangalia yaliyopita, naona kutofaulu kwingi) 3. I feel I am a failure as a person(nahisi kuwa nimefeli kibinafsi)
<u>1-5-4: Anhedonia (Loss of Pleasure)</u> Do you feel like you enjoy doing things and being with people*? (unahisi kuwa unafuraha ukifanya vitu na kushirikiana na wengine?*		0. I get as much pleasure as I ever did from what I enjoy(Napata kiwango sawa cha radhi kutokana na vitu nilivyokuwa nikifurahia awali) 1. I don't enjoy things as much as I used to(Siku hizi, sifurahi vitu vile nilivyokuwa nikifurahia hapo awali) 2. I get very little pleasure from the things I used to enjoy(Napata radhi kidogo sana kutokana na vitu nilivyokuwa nafurahia) 3. I can't get any pleasure from the things I used to enjoy(Siwezi pata radhi yoyote kutokana navitu nilivyokuwa nikifurahia)
<u>1-5-5: Guilty Feelings</u> Have you had guilty feelings in the last 14 days*? (Je, umepata hisia zozote zahatia katika mudawa wiki mbili zilizopita?*		0. I don't feel particularly guilty(La, sihisi hatia hata kidogo) 1. I feel guilty over many things I have done or should have done(Ndio, nahisi hatia kutokana na matendo mengi niliyofanya na niliyostahili kufanya) 2. I feel quite guilty most of the time(Nahisi ninahatia wakati mwingi) 3. I feel guilty all the time(Nahisi kuwa nina hatia kila wakati)
<u>1-5-6:Punishment Feelings</u> Have you felt like being punished?*(Je,umehisi kuwa unastahili kuadhibiwa?)*		0. I don't feel I am being punished (La, sihisi kuwa ninaadhibiwa) 1. I feel I may be punished(Ninahisi kuwa kuna uwezekano wa kuadhibiwa) 2. I expect to be punished(Nina tarajia kuadhibiwa) 3. I feel I am being punished(Ninahisi kuwa ninadhibiwa sasa hive)
<u>1-5-7: Self-Dislike</u> Have you felt like you don't like yourself?*(Je, kunawakati umejichukia?)*		0. I feel the same about my future myself as ever (Nahisi vile vile kuhusu maisha ya nguya usoni) 1. I have lost confidence in myself(Sijiamini) 2. I am disappointed in myself(Nimekufa moyo) 3. I dislike myself(Najichukia)
<u>1-5-8: Self-criticalness</u> Have		0. I don't criticize or blame myself more than usual (Sijakuwa nikijilaumu kupita kiasi cha kawaida)

you been blaming yourself? *(Umekuwa ukijilaumu?)*		1. I am more critical of myself than I used to be(Nimekuwa nikijilaumu kupita kiasi) 2. I blame myself for all my faults(Ninajilaumu kwa ajili ya makosa yangu yote) 3. I blame myself for everything that happens(Mimi hujilaumu kwa ajili ya kilakitu kinachofanyika)
1-5-9: Suicidal thoughts or Wishes Have you ever felt like you want to kill yourself? *(Kuna wakati umehisi kujiua?)*		0. I don't have any thought of killing myself(La, sinamawazo yoyote yakujiua) 1. I have thoughts of killing myself but I would not carry them out(Ninafikira ya kujiua lakini siwezi tenda kitendo chenyewe) 2. I would like to kill myself(Ningependa kujiua) 3. I would like to kill myself if I had the chance(Ningejiua kama ningeweza)
1-5-10: Crying: **Have you been crying**? (Je, umekuwaukilia?)		0. I don't cry any more than I used to(Sijakuwa nikilia kuzidisha nilivyokuwa nikilia hapo awali) 1. I cry more than I used to(Nimekuwa nikilia kupita kiasi cha awali) 2. I cry over every little thing(Karibu kila kitu hunisababisha nilie) 3. I feel like crying, but I can't(Nimekuwa nikihisi kulia lakini siwezi)
1-5-11: Agitation Have you been feeling agitated or restless? *(Je, umehisi kuwa hauwezitulia?)*		0. I am no more restless and would up than usual(Sina hali yakutulia kupita kiwango cha kawaida) 1. I feel more restless and would up than usual(Ninahisikutotuliakupitakiwangonilichokuwanikishuhudiaawali) 2. I am so restless or agitated that it's hard to stay still(Siwezitulia na hupata ugumu ninapojaribu kutulia) 3. I am so restless or agitated that I have to keep moving or doing something(Siwezitulia kamwe!)
1-5-12: Loss of Interest Have you lost interest in people/ things/ activities? *(Umekosa kujisikia kuwa na wengine au kufanya vitu wale smugly?)*		0. I have no interest in other people or activities(Sinahaja na watu wengine ama vitu virgin) 1. I am less interested in other people or things than before(HajayangunawatuwengineamavituvingineimedidimianikilinganishanaawaIi) 2. I have lost most of my interest in other people or things(Nimepoteza haja na watu wengine na vitu virgin) 3. Its' hard to get interested in anything(Ni ngumu kuwa na haja na chochote)
1-5-13: Indecisiveness Have you been unable to make decisions? (*Je,umekuwa na uwezo kukata kauli?)*		0. I make decisions about as well as ever(Ninakata kauli kama kawaida) 1. I find it more difficult to make decisions than usual(Ninapatwa na shida ninapojaribu kukata kauli) 2. I have much more difficulty to make decisions than I used to(Nimepatashida sana ninapojaribu kukata kauli) 3. I have no trouble making any decisions(Sinashida ninapokata kauli)
1-5-14: Worthlessness Have you felt worthless? *(Umejihisi hauna dhamani ama kuwa haufai?)*		0. I do not feel I am worthless(Sihisi kuwa sinadhamani) 1. I don't consider myself as worthwhile and useful as I used to(Sijihisi ninadhamani kama nilivyokuwa nayo awali) 2. I feel more worthless as compared to other people(Najihisi sinadhamani nikijilinganisha na watu wengine) 3. I feel utterly useless(Ninajihisi sinadhamani kabala)
1-5-15: Loss of Energy Have you been feeling less energetic? *(Umekuwa ukijihisi umekosa nguvu?*)		0. I have as much energy as ever(Nina nguvu kama kawaida) 1. I have less energy than I used to have(Nguvu yangu imedidimia ikilinganishwa na kitambo) 2. I don't have enough energy to do very much(Sina nguvu yaku fanya mengi) 3. I don't have enough energy to do anything(Sina nguvu ya kufanya chochote)
1-5-16: Changes in Sleeping Pattern How has your sleeping pattern been in the last 2 weeks? *(Usingizi wako umekuwa namna gani kwenye wiki mbili zilizopita)*		0= I have not experienced any change in my sleeping pattern(Sijashuhudia mabadiliko yoyote) 1a= I sleep somewhat more than usual(Nalala kwa muda mrefu ikilinganishwa na kawaida yangu) 1b= I sleep somewhat less than usual(Nalala kwa muda mfupi ikilinganishwa na kawaida yangu) 2a= I sleep a lot more than usual(Nalala kwa muda mrefu sana ikilinganishwa na kawaida yangu) 2b= I sleep a lot less than usual(Nalala kwa muda mfupi sana ikilinganishwa na kawaida yangu) 3a=I sleep most of the day(Nalala sana china) 3b=When I wake up in the night, I can't get back to sleep(Ninapoamka usiku, siwezi rejea usingizini)

Date…… Health Facility…… Centre Code….. Participant's Code……

1-5-17: Irritability Have you been irritable? *(Umekuwa ukikasirika haraka wiki mbili zilizopita?)*		0. I am no more irritable than usual(Sijashuhudia mabadiliko yoyote) 1. I am more irritable than usual(Ninakasirishwa haraka ikilinganishwa na kawaida yangu) 2. I am much more irritable than usual(Ninakasirishwa haraka sana ikiliganishwa na kawaida yangu) 3. I am irritable all the time(Ninakasirishwa haraka sana kila wakati)
1-5-18: Changes in appetite How has your appetite been in the last 2 weeks? (*Je unaona kwamba kukula kwako kumebadilika?)*		0= I have not experienced any change in my appetite(Sijashuhudia mabadiliko yoyote wa kola kwingi) 1a=My appetite is somewhat less than usual(Kukula kwingi kumedidimia nikilinganisha na kawaida yangu) 1b=My appetite is somewhat more than usual(Kukula kwingi kumeimarika nikilinganisha na kawaida yangu) 2a=My appetite is much less than before(Kukula kwingi kumedidimia sana nikilinganisha na kawaida yangu) 2b=My appetite is much greater than before(Kukula kwingi kumeirika sana nikilinganisha na kawaida yangu) 3a=I have no appetite at all(Sinahamu ya kola) 3b=I crave food all the time(Ninahamu ya kula kila wakati)
1-5-19:Concentration difficulty Have you had any difficulties concentrating in the last 14 days*? (Umekosa kuwa makini wiki mbili zilizopita?)*		0. I can concentrate as well as ever (Niko makini sijashuhudia mabadiliko yoyote) 1. I can't concentrate as well as usual(Siwezi kuwa makini kama nilivyokuwa awali) 2. It's hard to keep my mind on anything for very long(Siwezitilia makini kwa kitu kimoja kwa muda mrefu) 3. I find I can't concentrate on anything(Siwezi tilia makini kwa kitu chochote)
1-5-20: Tiredness or Fatigue Have you been feeling tired/fatigued? *(Je, Umekuwa na uchovu?)*		0. I am no more tired or fatigued than usual (Sinauchovu, sijashuhudia mabadiliko yoyote) 1. I get more tired or fatigued more easily than usual(Nina uchovu mwingi ni kulinganisha na kiwango cha uchovu uliokawaida yangu) 2. I am too tired or fatigued to do a lot of the things I used to do(Ninauchovu mwingi wala siwezifanya vitu nilivyokuwa nimezoea) 3. I am too tired or fatigued to do most of the things I used to do(Nina uchovu mwingi wala siwezi fanya vitu nilivyo kuwa nimezoea)
1-5-21: Loss of Interest in Sex In the last 14 days, have you lost interest in sex? *(Umepoteza hamu ya noon?)*		0. I have not noticed any recent change in my interest in sex(Sijashuhudia mabadiliko yoyote kulingana na hamu ya noon) 1. I am less interested in sex than I used to be(Hamu yangu ya ngono imedidimia) 2. I am much less interested in sex now(Hamu yangu ya ngono imedidimia sana) 3. I have lost interest in sex completely(Sinahamu yoyote ya noon)
TOTAL SCORES:		**ADD ALL SCORES =.....** *(Maximum Scores=63)* *(If more than 5 items are missing, the total scores should not be added. Probe for missing items till completed);*

SUMMARY RECOMMENDATION AFTER SCREENING:

TOTAL SCORES	*RECOMMENDATION*
1= SCORES LESS THAN 14	*Explain to her that she does not have any symptoms of depression, hence does not qualify to participate in the study. Give transport reimbursement and release her.*
2=SCORES MORE THAN 14	*Explain to her that she has symptoms of depression and therefore qualifies to participate in the study. Continue with the next set of questions with her.*

LEVEL 2: RECRUITMENT AND RANDOMIZATIONQUESTIONNAIRE
(Check again Questions LA 1-1 up to 1-5 to confirm if to continue with participant)
L2A: Demographic and Socio-economic Data

1	Participant's Age	……Years
2	Parity Status *(How many pregnancies)*	No of times pregnant……
3	Duration with HIV infection (since the time of confirming serostatus).	1= Less than 6 months 2= 6months – 1 year; 3= More than 1 year
4	Did you know your HIV status before or after you knew you were pregnant?	1= Before confirming pregnancy 2=After Confirming pregnant
5	If you knew your HIV status before being pregnant, did you plan to be pregnant or not	1= Yes, pregnancy planned for 2= No, pregnancy not planned for
6	Disclosure status (Have you told anyone that you have HIV virus?)	1= Yes; 2=No
7	Marital Status	1= Never married/Never lived together 2= Married/Living together 3= Divorced/separated 4=Widowed
8	Formal Education level	1= None 2= Primary school level 3=Secondary school level 4= Post-secondary level
9	Religion	1= Christian 2= Muslim 3=others
10	Your Occupation	0=Not employed;1= Housewife; 2=student 3= Salaried employment 4= Business (self-employed) 5= Labourer (wage earner) 6=Retired 7=others
11	If married, what is husband's employment status	1=Not employed; 2=student; 3= Salaried employment 4= Business (self-employed) 5= Labourer (wage earner) 6=Retired; 7=others
12	On average, what is the total household income/ month normally, *(from all sources - Probe)*	Indicate amount……KSh
13	How many times have you attended counselling sessions?	1= None; 2=1-2 times 3=3-4 times 4= 5 times and more
14	How many times have you attended the Mother-to-Mother support group meetings?	1= None; 2=1-2 times 3=3-4 times 4= 5 times and more
15	Have you experienced **any specific stressful event** in the **last two (2) weeks**	0=No 1=Yes (Very briefly specify the event……

Date…… Health Facility…… Centre Code….. Participant's Code……

L2B: Dietary Intake Data – 24-Hour Recall:

Intake of B-Complex vitamins, Vit. C, Vit. E, Iron, Calcium, Selenium, Omega-3, Folate& Zinc foods (E*stimate amounts using standard serving sizes if ANY was eaten in the last 24 hour*

	Food Item	**Normally Eaten***(Tick)*		**How often***(Tick the reported option*				**Estimated Quantity by Meal if was Eaten in last 24 hours***(Indicate amounts estimated)*				
		No=0	**Yes=1**	Once in a while	Daily	At least 2 times per week	More than Twice per week	Snack	Lunch	Snack	Dinner	B/Fast
1	Cabbage											
2	Carrots											
3	Cheese											
4	Chicken											
5	Eggs boiled											
6	Eggs Fried											
7	Fish: Nile perch*(Mbuta)*											
8	Fish: *Omena*											
9	Fish: Sea fish (specify)											
10	Fish: Tilapia cooked											
11	Fruits (Avocado)											
12	Fruits (Lemon)											
13	Fruits (Mangoes)											
14	Fruits (Orange)											
15	Fruits (others - indicate),											
16	Fruits (Pawpaw											
17	Fruits(Ripe bananas)											
18	Green vegetables (Others)											
19	Green vegetables (Kales)											
20	Green vegetables (Spinach)											
21	Green vegetables (Terere)											
22	Legumes (Green grams/lentils)											
23	Legumes (Beans cooked)											
24	Legumes (Njahi cooked)											
25	Legumes (Soya bean flour)											
26	Legumes (Soya chunks)											
27	Liver											
28	Meat (Beef)											
29	Meat (Goat)											

30	Meat (Mutton)											
31	Meat (Pork)											
32	Milk											
33	Nuts (Specify)											
34	Oils (Vegetable oil - solid)											
35	Oils (Margarine)											
36	Cooking oil–Liquid (type)											
37	Pasta/noodles											
38	Potatoes (Irish)											
39	Potatoes (Sweet, *ngwachi*)											
40	Seeds(sesame, sunflower,)											
41	Whole grain cereals (Br/chapo)											
42	Whole grain cereals (Specify – Maize, millet or sorghum)											
43	Whole grain cereals (Weetabix)											
44	Yoghurt											

L2C: Use of multivitamins and other nutritional supplements

(Probe for any supplements used in the last 2 weeks)

	Have you been taking any nutrition supplements and multivitamins in the last 2 weeks? - Probe for iron and folic acid given at the health facility.	**0= No** **1=Yes**
	If Yes, please tell me if you have been taking any of the following nutrition supplements and multivitamins in the last 2 weeks: Please bring its container in next visit for checking on nutrient content.	
1	Vitamin C (Ascorbic Acid)	No=0 1= Yes
2	Thiamin (Vit. B1)	No=0 1= Yes
3	Riboflavin (Vit. B2)	No=0 1= Yes
4	Niacin (Vit. B3)	No=0 1= Yes
5	Pyridoxine (B6)	No=0 1= Yes
6	Vitamin B12	No=0 1= Yes
7	Vit. E,	No=0 1= Yes
8	Calcium	No=0 1= Yes
9	Selenium	No=0 1= Yes
10	Zinc	No=0 1= Yes
11	Omega-3	No=0 1= Yes

L2D: Weight, Blood pressure, CD4 cell count, Cheek cell samples and study supplement dose

1	Monthly weight gain (Kg) (*Take weight*)	1= Previous weight (PW)-------kg 2=Current weight (CW)…….kg 3=Weight gain (WG=CW-PW)……kg
2	Mid-Upper arm circumference(MUAC)	MUAC = ……cm
3	Blood pressure (Record from her Mother & Child Health Booklet)	BP = ……
4	CD4 Count (Record from her Mother & Child Health Booklet)	CD4 = ……
5	Please tell me if in the **last two (2) weeks** you have taken your ARV medication dose as advised every day?	0=No (Give reasons ……..……) 1=Yes
6	Mouth-wash water collected for cheek cells *(Indicate participants' code number on sample)*	0= No (Indicate reasons)…….. 1=Yes (Put participant's Code Number on sample…)
7	Study supplement dose given for the next 2 weeks	0= No (Reasons……) 1= Yes
8	Number written at the bottom of supplement bottle given***(Check bottom of bottle to see the number)***	Bottle Number……..
9	Porridge flour given for the next 2 weeks (daily ration of 150 g/day)	0=No (Reasons…….) 1=Yes

Summary after recruitment:

DATE RECRUITED……..

NEXT VISIT (Two weeks from recruitment date) …….

2ND VISIT QUESTIONNAIRE FOR FOLLOW-UP – Date:……

The following information will be obtained from each participant after the first contact (visit) and distribution of the study supplements:

F1	Have you completed your last dose of the study supplements?	0 = No (Reasons……) 1= Yes
F2	If you missed to take the study supplement as advised at any one time, what were the reasons?	Reasons for missing dose ---------- ----------
F3	Please tell me any of the bad feelings(side effects) that you experienced while taking the study *supplements:*	List Side effects of study supplements experienced 1.…… 2……
F4	Have you developed/experienced any health	1 = No

	problem since you started taking these study *supplements?*	2= Yes
F5	If you experienced any health problems, please list and describe	**Health problems experienced:** **1**…… 2……
F6	Please tell me if in the **last two (2) weeks** you have taken your ARV medication dose as advised every day?	0=No (Give reasons ……..……) 1=Yes
F7	Study supplement dose given for the next 2 weeks	0=No (Reasons……) 1= Yes
F8	Number written at the bottom of supplement bottle given***(Check bottom of bottle to see the number)***	Bottle Number……..
F9	Porridge flour given for the next 2 weeks (daily ration of 50 g/day)	0=No (Reasons…….) 1=Yes
A two week dose for the study supplement and porridge flour will then be distributed.		

Summary after follow-up visit:

Recruitment and Follow-up	Appointment Date	Comments
Recruitment (1st visit)		
1st Follow-up (2nd visit)		
2nd Follow-up (3rd visit- mid-data collection)		
3rd Follow-up (4th visit)		
4th follow-up (5th visit-Final Data Collection)		
Other Follow-up visits		

Enumerator Name…… Sign……Date……..

Date…… Health Facility…… Centre Code….. Participant's Code……

Key Informant Interview Checklist ***(For health workers – nurses, clinical officers and Mother-to-Mother support group workers):***

A: Records of HIV positive pregnant women with depression

1. Do you see many pregnant HIV positive women who present with depression symptoms?

2. How do the records for the women who have symptoms of depression help you in your day-to-day work when dealing with HIV positive pregnant women?

B: Symptoms and causes of depression in HIV and pregnancy

3. What do you see as the common signs and symptoms of depression among these HIV positive pregnant women? Are the signs the same as those you see in other pregnant women who are not HIV infected?

4. What do you think are the possible causes of depression among HIV positive pregnant women?

C: Assessment of depressive symptoms in ANCs

5. How do you assess for depressive symptoms among your clients?

6. Are there any standard tools/guidelines that you use for screening for depression?

7. If you do not use any tool/guideline to screen for depression, what is your opinion on use of a standardized one by psychiatry nurses?

End of Study Data Collection - (5th Visit)

Beck Depression Inventory Second Edition (BDI-II) Scale

BDI-II: Screening for depressive symptoms - Below is a list of some of the ways you may have felt or behaved in the past Two weeks (14 days). Please tell me exactly how you felt in terms of each of the following: (***Allow participant to explain how she felt. Repeat what she said before scoring, then score)***		
Depressive Symptom	**NO=0 YES = 1**	**Score the options as 0, 1, 2, and 3 on how the participant felt by selecting <u>one option only</u> from choices given for each depressive symptom.**
<u>Final-Sadness:</u> Have you felt sad in the last 14 days? *(Umehisi huzuni siku 14 zilizopita?)*		4. I do not feel sad (Mimi Sihisi huzuni) 5. I feel sad much of the time (Mimi huwa na huzuni mara nyingi) 6. I am sad all the time (Mimi huwa na huzuni wakati wote) 7. I am so sad or unhappy that I can't stand it (Nina huzuni sana hadi siwezi kuistahimili)
<u>Final-Pessimism:</u> How do you view your life? *(Unaona maisha yako kuwa namna gani?)*		4. I am not discouraged about my future (Sijafiwa moyo na maisha yangu ya mbele) 5. I feel more discouraged about my future than I should be (Nimekufa moyo kuhusu maisha yangu ya mbele kupita kiasi 6. I do not expect things to work out for me (Situmaini vitu viende ninavyotarajia) 7. I feel my future is hopeless and will only get worse (Sitaraji mazuri wala situmaini kuwa maisha yangu itaimarika)
<u>Final-Past Failure</u> Do you feel like you have failed in life*? (Je, unahisi kuwa umefeli maishani?)*		4. I do not feel like a failure (Sijihisi kuwa nimefeli) 5. I have failed more than I should have (Nimefeli kupita kiasi kilichotarajiwa) 6. As I look back, I see a lot of failures (Nikiangalia yaliyopita, naona kutofaulu kwingi) 7. I feel I am a failure as a person (nahisi kuwa nimefeli kibinafsi)
<u>Final-Anhedonia(Loss of Pleasure)</u> Do you feel like you enjoy doing things and being with people*? (unahisi kuwa unafuraha ukifanya vitu na kushirikiana na wengine?*		4. I get as much pleasure as I ever did from what I enjoy (Napata kiwango sawa cha radhi kutokana na vitu nilivyokuwa nikifurahia awali) 5. I don't enjoy things as much as I used to (Siku hizi, sifurahi vitu vile nilivyokuwa nikifurahia hapo awali) 6. I get very little pleasure from the things I used to enjoy (Napata radhi kidogo sana kutokana na vitu nilivyokuwa nafurahia) 7. I can't get any pleasure from the things I used to enjoy (Siwezi pata radhi yoyote kutokana na vitu nilivyokuwa nikifurahia)
<u>Final-Guilty Feelings</u> Have you had guilty feelings in the last 14 days*? (Je, umepata hisia zozote zahatia kutoka muda wa wiki mbili zilizopita?*		4. I don't feel particularly guilty (La, sihisi hatia hata kidogo) 5. I feel guilty over many things I have done or should have done (Ndio, nahisi hatia kutokana na matendo mengi niliyofanya na niliyostahili kufanya) 6. I feel quite guilty most of the time (Nahisi ninahatia wakati mwingi) 7. I feel guilty all the time (Nahisi kuwa nina hatia kila wakati)
<u>Final-Punishment Feelings</u> Have you felt like being punished?*(Je,umehisi kuwa unastahili kuadhibiwa?)*		4. I don't feel I am being punished (La, sihisi kuwa ninaadhibiwa) 5. I feel I may be punished (Ninahisi kuwa kuna uwezekano wakuadhibiwa) 6. I expect to be punished (Ninatarajia kuadhibiwa) 7. I feel I am being punished (Ninahisi kuwa ninadhibiwa salsa hive)

Final-Self-Dislike Have you felt like you don't like yourself? *(Je, kuna wakati umejichukia?)*		4. I feel the same about my future myself as ever (Nahisi vile vile kuhusu maisha yangu ya usoni) 5. I have lost confidence in myself (Sijiamini) 6. I am disappointed in myself (Nimekufa moyo) 7. I dislike myself (Najichukia)
Final-Self-criticalness Have you been blaming yourself? *(Umekuwa ukijilaumu?)*		4. I don't criticize or blame myself more than usual (Sijakuwa nikijilaumu kupita kiasi cha kawaida) 5. I am more critical of myself than I used to be (Nimekuwa nikijilaumu kupita kiasi) 6. I blame myself for all my faults (Ninajilaumu wa ajili ya makosa yangu yote) 7. I blame myself for everything that happens (Mimi hujilaumu wa ajili ya kila kitu kinachofanyika)
Final-Suicidal thoughts or Wishes Have you ever felt like you want to kill yourself? *(Kuna wakati umehisi kujiua?)*		4. I don't have any thought of killing myself (La, sina mawazo yoyote yakujiua) 5. I have thoughts of killing myself but I would not carry them out (Ninafikira ya kujiua lakini siwezi tenda kitendo chenyewe) 6. I would like to kill myself (Ningependa kujiua) 7. I would like to kill myself if I had the chance (Ningejiua kama ningeweza)
Final-Crying: **Have you been crying**? (Je, umekuwa ukilia?)		4. I don't cry any more than I used to (Sijakuwa nikilia kuzidisha nilivyokuwa nikilia hapo awali) 5. I cry more than I used to (Nimekuwa nikilia kupita kiasi cha awali) 6. I cry over every little thing (Karibu kila kitu hunisababisha nilie) 7. I feel like crying, but I can't (Nimekuwa nikihisi kulia lakini siwezi)
Final-Agitation Have you been feeling agitated or restless? *(Je, umehisi kuwa hauwezitulia?)*		4. I am no more restless and would up than usual (Sina hali yakutulia kupita kiwango cha kawaida) 5. I feel more restless and would up than usual (Ninahisi kutotulia kupita kiwango nilichokuwa nikishuhudia awali) 6. I am so restless or agitated that it's hard to stay still (Siwezi tulia na hupata ugumu ninapo jaribu kutulia) 7. I am so restless or agitated that I have to keep moving or doing something (Siwezi tulia kamwe!)
Final-Loss of Interest Have you lost interest in people/ things/ activities? *(Umekosa kujisikia kuwa na wengine au kufanya vitu wala shuguli?)*		4. I have not lost interest in other people or activities (Sijapoteza haja na watu wengine ama vitu virgin) 5. I am less interested in other people or things than before (Haja yangu na watu wengine ama vitu virgin imedidimia nikilinganisha na awali) 6. I have lost most of my interest in other people or things (Nimepoteza haja na watu wengine na vitu virgin) 7. Its' hard to get interested in anything (Ni ngumu kuwa na haja na chochote)
Final-Indecisiveness Have you been unable to make decisions? (*Je,umekuwa na uwwezo kukata kauli?)*		4. I make decisions about as well as ever (Ninakata kauli kama kawaida) 5. I find it more difficult to make decisions than usual (Ninapatwa na shida ninapo jaribu kukata kauli) 6. I have much more difficulty to make decisions than I used to (Nimepata shida sana ninapo jaribu kukata kauli) 7. I have trouble making any decisions (Nina shida ninapo kata kauli)
Final-Worthlessness Have you felt worthless? *(Umejihisi hauna dhamani ama kuwa haufai?)*		4. I do not feel I am worthless (Sihisi kuwa sina dhamani) 5. I don't consider myself as worthwhile and useful as I used to (Sijihisi ninadhamani kama nilivyokuwa nayo awali) 6. I feel more worthless as compared to other people (Najihisi sina dhamani nikijilinganisha nawatu wengine) 7. I feel utterly useless (Ninajihisi sina dhamani kabala)
Final-Loss of Energy Have you been feeling less energetic? *(Umekuwa ukijihisi umekosa nguvu?*)		4. I have as much energy as ever (Nina nguvu kama kawaida) 5. I have less energy than I used to have (Nguvu yangu imedidimia ikilinganishwa na kitambo) 6. I don't have enough energy to do very much (Sina nguvu ya kufanya mengi) 7. I don't have enough energy to do anything (Sina nguvu ya kufanyachochote)
Final-Changes in Sleeping		0= I have not experienced any change in my sleeping pattern (Sijashuhudia mabadiliko yoyote)

Pattern How has your sleeping pattern been in the last 2 weeks? *(Usingizi wako umekuwa namna gani kwenye wiki mbili zilizopita)*		1a= I sleep somewhat more than usual (Nalala wa muda mrefu ikilinganishwa na kawaida yangu) 1b= I sleep somewhat less than usual (Nalala wa muda mfupi ikilinganishwa na kawaida yangu) 2a= I sleep a lot more than usual (Nalala wa muda mrefu sana ikilinganishwa na kawaida yangu) 2b= I sleep a lot less than usual (Nalala wa muda mfupi sana ikilinganishwa na kawaida yangu) 3a=I sleep most of the day (Nalala sana china) 3b=When I wake up in the night, I can't get back to sleep (Ninapoamka usiku, siwezi rejea usingizini)
Final-Irritability Have you been irritable? *(Umekuwa ukikasirika haraka wiki mbili zilizopita?)*		4. I am no more irritable than usual (Sijashuhudia mabadiliko yoyote) 5. I am more irritable than usual (Ninakasirishwa haraka ikilinganishwa na kawaida yangu) 6. I am much more irritable than usual (Ninakasirishwa haraka sana ikiliganishwa na kawaida yangu) 7. I am irritable all the time (Ninakasirishwa haraka sana kila wakati)
Final-Changes in appetite How has your appetite been in the last 2 weeks? (*Je unaona kwamba kukula kwako kumebadilika?)*		0= I have not experienced any change in my appetite (Sijashuhudia mabadiliko yoyote wa kola kwingi) 1a=My appetite is somewhat less than usual (Kukula kwingi kumedidimia nikilinganisha na kawaida yangu) 1b=My appetite is somewhat more than usual (Kukula kwingi kumeimarika nikilinganisha na kawaida yangu) 2a=My appetite is much less than before (Kukula kwingi kumedidimia sana nikilinganisha na kawaida yangu) 2b=My appetite is much greater than before (Kukula kwingi kumeirika sana nikilinganisha na kawaida yangu) 3a=I have no appetite at all (Sina hamu ya kola) 3b=I crave food all the time (Nina hamu ya kola kila wakati)
Final-Concentration difficulty Have you had any difficulties concentrating in the last 14 days*? (Umekosa kuwa makini wiki mbili zilizopita?)*		4. I can concentrate as well as ever (Niko makini sijashuhudia mabadiliko yoyote) 5. I can't concentrate as well as usual (Siwezikuwa makini kama nilivyokuwa awali) 6. It's hard to keep my mind on anything for very long (Siwezi tilia makini wa kitu kimoja wa muda mrefu) 7. I find I can't concentrate on anything (Siwezi tilia makini wa kitu chochote)
Final-Tiredness or Fatigue Have you been feeling tired/fatigued? *(Je, Umekuwa na uchovu?)*		4. I am no more tired or fatigued than usual (Sina uchovu, sijashuhudia mabadiliko yoyote) 5. I get more tired or fatigued more easily than usual (Ninauchovu mwingini kulinganisha na kiwango cha uchovu ulio kawaida yangu) 6. I am too tired or fatigued to do a lot of the things I used to do (Ninauchovu mwingi wala siwezi fanya vitu nilivyokuwa nimezoea) 7. I am too tired or fatigued to do most of the things I used to do (Nina uchovu mwingi wala siwezi fanya vitu nilivyo kuwa nimezoea)
Final-Loss of Interest in Sex In the last 14 days, have you lost interest in sex? *(Umepoteza hamu ya noon?)*		4. I have not noticed any recent change in my interest in sex (Sijashuhudia mabadiliko yoyote kulingana na hamu ya noon) 5. I am less interested in sex than I used to be (Hamu yangu ya noon imedidimia) 6. I am much less interested in sex now (Hamu yangu ya noon imedidimia sana) 7. I have lost interest in sex completely(Sina hamu yoyote ya noon)
FINAL-TOTAL SCORES:.....		**ADD ALL SCORES =....**..... *(Maximum Scores=63)* *(If more than 5 items are missing, the total scores should not be added. Probe for missing items till completed);*

F1	Participants Age in Years	…….Years
	Parity Status *(How many pregnancies)*	No of times pregnant……
	Gestation Age	Weeks…….
F2	Intervention Bottle number	Bottle Number----------
F3	Have you completed your last dose of the study supplements?	0 = No (Reasons……) 1= Yes
F4	If you missed to take the study supplement as advised at any one time, what were the reasons?	Reasons for missing dose ---------- ----------
F5	Please tell me any of the bad feelings (side effects) that you experienced while taking the study *supplements:*	List Side effects of study supplements experienced 1..…… 2……
F6	Have you developed/experienced any health problem since you started taking these study *supplements?*	1 = No 2= Yes
F7	If you experienced any health problems, please list and describe	Health problems experienced: 1…… 2……
F8	How did you feel generally during the period while you took the omega-3	1= Felt calm and less disturbed emotionally 2= Skin became soft and smooth 3= Felt healthy 4= Generally I felt good. 5= Any other feeling (specify exactly)
F9	Please tell me if in the **last two (2) weeks** you have taken your ARV medication dose as advised everyday?	0=No (Give reasons ……) 1=Yes
F10	How many times have you attended counseling sessions?	1= None; 2=1-2 times 3=3-4 times 4= 5 times and more
F11	How many times have you attended the Mother-to-Mother support group meetings?	1= None; 2=1-2 times 3=3-4 times 4= 5 times and more
F12	Have you experienced **any specific stressful event** in the **last two (2) weeks**	0=No 1=Yes (Very briefly specify the event……
F13	Duration with HIV infection (since the time of confirming serostatus).	1= Less than 6 months 2= 6months – 1 year;

		3= More than 1 year
F14	Did you know your HIV status before or after you knew you were pregnant?	1= Before confirming pregnancy 2=After Confirming pregnant
F15	If you knew your HIV status before being pregnant, did you plan to be pregnant or not	1= Yes, pregnancy planned for 2= No, pregnancy not planned for
F16	Disclosure status (Have you told anyone that you have HIV virus?)	1= Yes; 2=No
F17	Marital Status	1= Never married/Never lived together 2= Married/Living together 3= Divorced/separated 4=Widowed
F18	Formal Education level	1= None 2= Primary school level 3=Secondary school level 4= Post-secondary level
F19	Your Occupation	0=Not employed;1= Housewife; 2=student 3= Salaried employment 4= Business (self-employed) 5= Labourer (wage earner) 6=Retired 7=others
F20	If married, what is husband's employment status	1=Not employed; 2=student; 3= Salaried employment 4= Business (self-employed) 5= Labourer (wage earner) 6=Retired; 7=others
F21	On average, what is the total household income/ month normally, *(from all sources - Probe)*	Indicate amount……KSh

F23	**Please tell me if you have been taking any of the following nutrition supplements and multivitamins in the last 2 weeks:***(Probe for any supplements used in the last 2 weeks)*	
1	Vitamin C (Ascorbic Acid)	No=0 1= Yes
2	Thiamin (Vit. B1)	No=0 1= Yes
3	Riboflavin (Vit. B2)	No=0 1= Yes
4	Niacin (Vit. B3)	No=0 1= Yes
5	Pyridoxine (B6)	No=0 1= Yes
6	Vitamin B12	No=0 1= Yes
7	Vit. E,	No=0 1= Yes
8	Calcium	No=0 1= Yes

9	Selenium	No=0	1= Yes
10	Zinc	No=0	1= Yes
11	Iron	No=0	1= Yes
12	Folic Acid	No=0	1= Yes

F24	Monthly weight gain (Kg) (*Take weight*)	1= Previous study weight (PW)-------……..kg
		2=Current study weight (CW)……..kg
		3=Weight gain (WG=CW-PW)……kg
F25	Mid-Upper arm circumference(MUAC)	Current MUAC = …….……....cm
F26	Blood pressure *(Record current from her Mother & Child Health Booklet-if no current, send her to take)*	BP = ……
F27	CD4 Count (*Taken in the last 2 weeks)*	CD4 = ……
F28	Mouth-wash water collected for cheek cells *(Indicate participants' code number on sample)*	0= No (Indicate reasons)…….. 1=Yes (Put participant's Number on sample)
F29	What are the common signs and symptoms of depression in HIV positive pregnant women?	
F30	What do you think are the possible causes of depression among HIV positive pregnant women?	

Appendix 7: List of Standard Operating Procedures (SOPs)

Introduction

The Standard operating procedures (SOPs) in this document are step-by-step guidelines in the implementation of the research activities in the study on the "Role of Fish Oil Omega-3 Fatty Acids on Depression among HIV-Seropositive Pregnant Women in Nairobi: A Randomized Double-blind Controlled Trial". The SOPs are a set of procedures, based on the methodology chapter of the research proposal/protocol.

SOP1: Sampling - Number of Participants by Study Health Facility

Health Facility*	**ANC clients HIV-positive**	**ANC clients given preventive ARVs (Aug-Oct 2011)**	**Minimum Number of mothers to be in the sample****	**Number of Mothers to be screened**
Kariobangi Health Centre	59	43	36	81
Riruta Health Centre	54	54	48	104
Mathare North H/Centre	76	66	56	126
Kayole	74	74	60	140
Total	**263**	**237**	**200**	**450**

** Nairobi City Council health facilities that reported the highest number of HIV positive pregnant women who received preventive ARVs in the period August to October 2011.*

***The minimum number of mothers sampled is a proportion of the ANC clients per health facilities given ARVs multiplied by the total sample size required.*

SOP 2: Procedure for Recruitment of participants into the study

- Invite mothers who meet the following criteria to attend a screening and recruitment session:
- Pregnant in 2nd trimester (14 weeks to 27 weeks)
- HIV-seropositive
- Already on Preventive ARVs
- CD4 cell count of not more than 500.
- Explain the study to the mothers as per the informed consent explanation form
- Those willing to participate should provide their details – names, gestation age, cellphone number and estate for further communication about the study.
-

Health Centre.....: Invite mothers for screening and recruitment

Recruit Code#	Date	Name of Mother	Gestation Age	Cell Phone No.	CD4 cell count	Residence

SOP 3: Schedule of Participant Bi-weekly Visits
Since all admissions for participants in the study is within the second trimester, the total number of possible groups by gestation age at each health facility will be 14.

Definition of a week: In this study, a week was considered as the completed week if exceeded by 3 days, or the next completed week if exceeded by 4 or more days.

	Gestation Age in Weeks				
Entry Group	1st Visit Enrolment (*Screening stage)*	2nd visit (Monitoring & re-supply)	3rd visit (Mid-Study Data collection & re-supply)	4th Visit(Monitoring & re-supply)	5th Visit (Monitoring &End of Intervention data collection)
Group 1	14	16	18	20	22
Group 2	15	17	19	21	23
Group 3	16	18	20	22	24
Group 4	17	19	21	23	25
Group 5	18	20	22	24	26
Group 6	19	21	23	25	27
Group 7	20	22	24	26	28
Group 8	21	23	25	27	29
Group 9	22	24	26	28	30
Group 10	23	25	27	29	31
Group 11	24	26	28	30	32
Group 12	25	27	29	31	33
Group 13	26	28	30	32	34
Group 14	27	29	31	33	35

In this study, The minimum gestation age at entry into the study (enrolment) will be 14 weeks *(13 weeks, 4 days to 14 weeks, 3 days). The Maximum age at entry* into the study (enrolment) will be 27 weeks *(26 weeks, 4 days to 27weeks, 3 days).*

SOP 4: Data Collection Procedure

- This SOP is a **MUST-READ** for all those involved in data collection. During each visit:
- Read each question and instructions carefully;
- Fill in ALL SPACES in the questionnaire/form;
- Ask & record questions systematically as they appear on the question paper.

Screening for Recruitment
Screening for enrolment into the study is a 2-step process based on the study inclusion and exclusion criteria. The first step requires participants to meet the study inclusion criteria of gestation age (14-27), CD4 cell count (not more than 500), not using blood thinning medication for a list of health conditions and consent to participate in the study (signing of consent form after explanation). Only participants who meet these inclusion criteria can proceed to the next screening step which involves assessment of depression symptoms using "Beck Depression Inventory Second Edition (BDI-II)" scoring scale. Further interview and enrolment in the study for participants will depend on how many points they score on the BDI-II screening questionnaire.

Levels or visits as follows:

Visit Number	**Week**	**Activities**
1st visit	0	Screening for recruitment; Recruitment if meets inclusion criteria Baseline data collection questionnaire Randomization Intervention (a 2 – week dose)
2nd Visit	2	Monitoring compliance and side effects Re-supply of bi-weekly intervention dose
3rd Visit	4	Monitoring compliance and side effects Mid-study data collection Re-supply of bi-weekly intervention dose

4th Visit	6	Monitoring compliance and side effects; Re-supply of bi-weekly intervention dose
5th Visit	8	Monitoring compliance and side effects; Mid-study data collection questionnaire; Re-supply of bi-weekly intervention dose.

SOP 5: Mouth wash procedure for cheek cell collection

Objective of SOP 5: To collect participant's cheek cells samples for analysis of omega-3 fatty acid status.

When to carry out the procedure: At the end of questionnaire administration during baseline data collection, after four weeks and after eight weeks

Procedure:

1. This should be done at the end of the first visit interview for those enrolled in the study before they receive the intervention and porridge flour to take home.
2. Give each participant a cup and a 300ml bottle of distilled water to rinse her mouth and spit away the first rinse.
3. Ask her to take about 30-35ml of the water to **vigorously rinse** her mouths and expectorate the water into the cup two times after the first wash.
4. Transfer the mouthwash into the centrifuge tube.
5. Tightly close the lid of the tube and remember to LABEL the participant's CODE, HEALTH CENTRE, DATE and Visit Number on the tube.
6. Place the tube with the water in the coolbox with a cold icepack for transportation to the laboratory for refrigeration at minus 20 ^{0}C temperature conditions, awaiting extraction and analysis of omega-3 fatty acids.

SOP 6: Randomization and Blinding Procedures

Randomization:

1. Randomization sequence will be generated using permuted block design in MS Excel.
2. Participants will be stratified by health facility site with a 1:1 allocation ratio using a block size of 4. That means that for every 6 participants, 3 would be allocated to each arm of the trial- omega-3 or placebo.
3. The randomization sequence will be confidentially kept by the 3rd party who is not involved in the recruitment, enrolment or data management until end of trial.

Blinding

The study will be double-blinded so that both the study participants and those administering the interventionsincluding the principal investigator are unaware of the intervention/treatment allocation. Specifically, the following will be blinded from the study intervention:

- Principal investigator,
- Study participants,
- Health centre personnel including the Mother2Mother mentors
- Research assistants/trial monitors involved in trial activities;
- Data clerk handling the study data

SOP 7: Packaging and Allocation Concealment of the Interventions

The purpose of allocation concealment is to keep those involved in the study, including study participants, unaware of intervention allocation. The packaging and allocation concealment of the study interventions in this study is done by someone who is not involved in the data collection, nor is in direct contact with the study participants or teams. The interventions will be sealed in sequentially numbered, opaque identical bottles according to the allocation sequence. The following step-by-step guidelines are used in the packaging and allocation concealment:

- Label the clean, dry opaque bottles with dosage instructions. Ensure the bottle is clean and completely dry inside before use.
- Label each bottle sequentially as per the randomized allocation codes and list provided by the statistician.
- Count 42 soft-gels (capsules) of omega-3 and placebo; and place in each bottle as per the allocation codes provided for each study site;
- Cover each of the bottles containing the 42 soft-gels with cap and FIRMLY PRESS the cap for the bottle's security seal to hold. Ensure the bottle is securely closed.
- Store safely in the appropriately labelled study site space in the cabinet.
- Lock the cabinet and keep the keys with the School of Public Health (SPH) Secretary –pick it from her when you need to do further packaging. Throughout the study period**,** *Only You and the SPH office secretary will have access*ion the interventions cabinet.
- In addition, you will also be expected to take stocks of omega-3 and placebo and update the stock records daily.

SOP 8: Monitoring and Reporting Adverse Events

Symptoms of both known and unknown side effects will be closely monitored throughout the study period. Specifically, the following will be closely monitored:

i. Persistent stomach upset;
ii. Severe nose bleeding;
iii. Intestinal discomfort /stomach pain;
iv. Loose stools;
v. Any unknown symptoms;
vi. Any unanticipated adverse effect not related to the study supplements.

If any of the above symptoms occur among any study participant, it should be reported immediately to me for immediate consultation with the physician responsible for this study related medical decision: Such adverse events, if they occur, will also be reported to the Data Monitoring committee (DMC) and KNH/UoN–Ethics Research Committee.

The following information should be recorded at the study site for each participant reporting adverse events/side effects:

Date of Reporting					
Name of Health facility					
Participants Code and Name,	Code:…… Name:……				
Participants Contact	Phone number…….				
Symptom/side effect reported*(Clearly Tick and describe the reported symptom*)	Symptom	Description	Date Started	For how long	Any prior Action taken
	Persistent stomach upset				
	Severe nose bleeding				
	Intestinal discomfort /stomach pain				
	Loose stools				
	Any other symptoms not listed above				
Duration completed in the study (weeks)	Total Weeks completed in the study at the time of reporting symptom ----				

SOP 9: Guidelines for withdrawing participants from the study

A participant may withdraw from the study at any time during this study if:

1. She feels and decides that she wishes to withdraw after enrolment;
2. She develops any of the following side effect of fish oil omega-3 supplements:
    - Persistent stomach upset,
    - Severe nose bleeding
    - Intestinal discomfort /stomach pain
    - Persistent Loose stools
3. She develops any unanticipated adverse effect not related to the study supplements;
4. Develops an unknown placebo effect;
5. If she fails to come for re-supply of the intervention dose within a period of three days;
6. She gives birth before the end of the intervention;
7. During follow up and monitoring, a participant reports that she has been diagnosed with a medical condition in the last two weeks after enrolment in the study and the doctor gave her blood thinning medication for any of the following conditions:
    - Diabetes
    - Varicose Veins
    - Peptic Ulcers
    - Liver Problem
    - Using Vitamin K supplement
    - Depression (Mood disorder)

The following information should be recorded for each participant withdrawing from the study:

Participants Code, name and contact	Code:…… Name:…… Contact: ……..
Date of withdrawal	
Name of Health facility	
Duration completed in the study (weeks)	Weeks completed ----------
Reasons for withdrawal (*Clearly indicate specific reason for withdrawal)*	

SOP 10: Monitoring and Quality Control

Quality assurance before the intervention

- Training of research assistants/trial monitors on data collection tools and principles of good clinical practices (GCP) online course at East African Consortium for Clinical Research (EACCR) website at http://www.eaccr.org/nodes/training/ and get a certificate of course completion.
- Allocation concealment using securely sealed bottles, done by a third party not involved in the study
- Randomization sequence done and kept by a third party not involved in the study until the end of the trial;
- Calibration of research equipment–weighing scales and laboratory equipment.

Monitoring Compliance:

Calling participants through their contact cell phone numbers to remind them to take their omega-3 and to come for the next scheduled visit as indicated on the container.

Monitoring research progress to ensure quality

- The Data Monitoring Committee (DMC)and academic research supervisors to closely monitor the progress of the trial and advise accordingly.
- The academic supervisors will be responsible for ensuring that the research meets the required University of Nairobi PhD academic standards.

- The DMC will be responsible for monitoring the safety and efficacy of study supplements. Specifically, the DMC will be:
    i. Monitoring the protocol adherence;
    ii. Monitoring participants withdrawal from the study;
    iii. Monitoring the statistical analysis of efficacy evaluation as outlined in the study protocol monitoring guidelines related to efficacy;
    iv. Making recommendations on further study conduct based on the results of monitoring activities, e.g. continuing or terminating the study or making modifications.

Data Handling and record keeping

- All efforts will be made to ensure that data is collected and managed as accurately as possible as per protocol
- Check all completed questionnaires /CRFs for completeness and accuracy before entry in the computer.
- Safe storage of original copies of data (questionnaires and CRFs) for later/future reference
- Inclusion of only valid cases (who completed the study) in final data analysis.

SOP 11: Guidelines for Research Assistants

The research assistant in each study site is the main focal research person in that facility and will be specifically in-charge of the following tasks:

1. Screening participants for inclusion in the study;
2. Enrolment of participants who meet the inclusion criteria;
3. Conducting interviews with study participants using the study tools;
4. Accurate recording of information during interviews with participants;
5. Collection of cheek cell samples (mouth wash) from participants;
6. Distribution of intervention supplements;
7. Counselling participants on the actual dosage as indicated on supplement bottles;
8. Monitoring and follow-up of study participants during the 8-week study period through bi-weekly face-to-face visits.

The following documents are therefore a MUST have and read for each research assistant:

1. SOP 2: Invitation of mothers to participate in the study;
2. SOP 3: Guidelines to enrolment and follow-up gestation weeks;
3. SOP 4: Summary guidelines on data collection procedure;
4. SOP 5: Mouth wash procedure for cheek cell collection;
5. SOP 8: Monitoring and reporting of adverse events;
6. SOP 9: Guidelines for withdrawing participants from the study;
7. SOP 13: Data collection questionnaire submission monitoring form
8. Participant explanation and consent forms (English and Kiswahili versions);
9. Questionnaire and other relevant data collection forms;
10. MUAC Tape (for adults);
11. Centrifuge bottles for collecting samples;
12. Intervention supplements- clearly labelled and numbered bottles (NOTE:*The bottle should NEVER be OPENED, except by the recipient participant herself, and it should NEVER be EXCHANGED by the participant after receiving and opening).*

In addition to the above documents, each research assistant must have also read the following documents:

1. ICH GCP basic course materials*(Test and certificate at www. Eaccr.org);*
2. Glossary of clinical terms;
3. Health benefits of omega-3.

While at the study site, each research assistant will be expected to lias with the mother2mother (*m2m)* mentors in PMTCTs. These *m2m* mentors have been assisting the principal investigator with identification and recruitment of study participants.

SOP 12: Guidelines for Research Site Facility support Staff

The Health facility Nurse-in-Charge is the main contact person in the health facilities where the study is conducted as shown in table below:

Name of Site	Physical Address	Contact Details	Contact Person
Riruta Health Centre	At Kawangware along Naivasha Road	The Nurse-In-Charge	Teresa Kimita DMOH: Dr. Maundu-0721370362
Kariobangi North Health Centre	At Kariobangi North near Kariobangi North C/Council Market	The Nurse-In-Charge	Rosemary Maina (0722364528)
Mathare North Health Centre	Mathare North estate near Bus terminus 29/30	The Nurse-In-Charge	Florence Gataka 0722245019)
Kayole Health Centre	Kayole II estate near the Dos/Chiefs Office	The Nurse-In-Charge	Lilian Muiruri (0721991638)
Westlands Health Centre	Near Sarit Centre	The Nurse-In-Charge	Rosemary Kimani

Mother2mother (*m2m*) mentors in PMTCTs have been assisting the principal investigator to identify and recruit study participants. The *m2m* mentors are therefore the links with the study participants. The Nurse-in-charge of the health facility will however be regularly updated on the research activities although she is not directly involved in the work

Name of Site	List of *m2m* Mentors	Contact
Riruta Health Centre	Carol Faith Muthoni; Elizabeth Rahab	M2M Office: 0714-428183 Faith: 0717215502
Kariobangi North Health Centre	Maurine Olaka Angela Florence Catherine	M2M Office: 0714 428181 Maurine: 0722 935055
Mathare North Health Centre	Jane Njoki Margret Odera Charity Julie Obiero Kimanzi (couple)	M2M Office: 0712 768502 Njoki: 0722924787 Julie: 0701 447257
Kayole Health Centre	Irene Awuor Damaris (Couple) Lydia Jackline Idaki	M2M Office: 0712768503 Irene: 0724 912542

During data collection, the M2M mentors in the centres will be responsible for:

- Continuing with referrals for recruitment until the required participant enrolment number per centre is achieved;
- Storage of data collection tools – electronic weighing scale for use by the researcher assistants in data collection;
- Storage of bottled water, disposable cups

SOP 13-a: Data Collection Questionnaire Submission Monitoring Form

This SOP is useful in monitoring the data collection forms that have been completed by the research team members. It is also useful in processing the payment for the research teams that are based on the type and number of forms completed. *Each data collection team member must fill this form in duplicate, submit one copy to accompany the completed questionnaires and keep one copy for reference.*

Research Site (Health Centre) ----------

Date of Data collection----------

Code Screened	Code EPDS	Code Enrolled	Enrolled Supplement Bottle Code (See *Bottle bottom*)	Enrolled phone number	Enrolled Participants Name	BDI-II Scores if Enrolled	Any Comments on screened or enrolled Participant

Form Completed and Submitted by:_____Sign_____Date_____

SOP 13-b: Follow-Up Visit Data Monitoring Submission Form

This SOP is useful in monitoring the data collection forms that have been completed by the research team members during follow-up visits. It is also useful in processing the payment for the research teams that are based on the type and number of forms completed.*Each data collection team member must fill this form in duplicate, submit one copy to accompany the completed questionnaires and keep one copy for reference.*

Research Site (Health Centre) ----------

Date of Data collection----------

Participants Details			Last Dose Compliance		Any Reported Side Effects	Any Reported Health problems	Supplement Bottle Code	Comments
Code	Name	Phone No.	Yes	No (List Reasons)				

Form Completed and Submitted by:_____Sign_____Date_____

SOP 13-c: Enrolled Participant's signing Form Consent - Interventions & Incentives

Each Research Team member must double check on each of the following items for each participant, then let the participant sign against her Code/name
Research Site (Health Centre) ----------

Date	Participant code	Participant Name	Visit No. (1st, 2nd, 3rd, 4th)	Consent Form signed	Cheek-Cell code labelled	Supplement Bottle Code labeled	Porridge flour-*1kg*	Transport (K*Sh.)*	Phone No.	Participant Signature	Any Comment (next visit date)

Form Completed and Submitted by:_____ Sign_____ Date _____

SOP 13-d: Transport reimbursement for Only Screened participants
Each Research Team member must double check on each of the following items for each participant, then let the participant sign against her Code/name

Research Site (Health Centre) ----------

Date	Participants code	Participants Name	Consent Form signed (Yes/No)	Transport *(KSh. Per guideline*)	Short Comment on why not enrolled	Participants Signature

Form Completed and Submitted by:_____Sign_____Date_____

SOP 13-e: Monitoring for Recruitment, Enrolment & Follow-ups

This form must be completed by a m2m mentor representative in PMTCT from each Study site(facility) in duplicate, submit one copy to accompany completed questionnaires and keep a copy.

Research Site (Health Centre) ----------
Date of Data collection----------

	Participants Name			Enrolled Participants phone number	Any Comments on Participant
	Screened only	Screened and Enrolled	For Follow-up visit		

Name of Health Facility Staff (m2m) _____Date_____

SOP 14: Cleaning Bottles for Packaging the interventions

1. Use warm water and Liquid soap to clean the bottles;
2. Clean bottles and lids separately;
3. Clean the lids carefully; making sure the security seal is not broken;
4. Do not try to close the lids on the bottles *(usifunike chupa na vifuniko)* during washing or storage;
5. Spread all the bottles and lids separately on the rack to dry;
6. Store away, carefully making sure the lid seal is not broken.

SOP 15: Participants Transport Reimbursement Guidelines

Reimbursement for transport will be paid to participants as follows:

If:	*KSh.*	*Justification for payment*
Gestation and CD4 criteria NOT met	-	*Does not meet criteria to be screened*
All criteria on Page one of screening form met but NOT willing to sign consent form	-	*Not willing to participate*
Gestation and CD4 criteria met but Q1-2 and/or Q1-3 not met	*100*	*Participant came willingly to participate but inclusion criteria for enrolment not met*
Meets all criteria on page 1, signed consent form, but BDI-II Less than 14 scores	*150*	*Was willing to participate, has taken time to respond to the BDI-II questions, but has no depressive symptoms.*
Meets all criteria on page 1, signed consent form, and BDI-II is 14 or more scores	*150*	*Was willing to participate, has taken time to respond to all the questions and has symptoms of depression.*

Principal Investigator Sign_____Date_____-

SOP 16: Payment Guidelines for Research Teams

1. **Research Assistants:**

During data collection the mode of remuneration for the data collection team members will be as per the questionnaire completed for different sections as follows:

Purpose for payment	Amount (KSh.) per participant	Comment
Screening page 1-4 of questionnaire	Complete screening – 100 1st page only - 50	If only 1st page is completed up to Q1-4 on participant explanation consent payment will be half, 50/= per participant
Enrolment, collection of cheek cell samples, and distribution of intervention products	250	The 250/= is in addition to the 100/=, hence total for screening and enrollment is 350/= per participant
Bi-weekly visit, forms appropriately completed and intervention products given to participants	200	The questions are only 8 on half page, and NO Cheek cell collection this time.

2. **Payment for m2m mentors in PMTCT support in recruitment and follow-ups**

Rose O. Opiyo
School of Public Health
University of Nairobi,
Cell Phone: +254 722 473122

June 2012.

Dear Mentor Mothers,

Re: Your support in the Omega-3 and Depression study

I wish to thank you for your support in recruitment of participants in this study on omega-3 and depression among HIV-positive pregnant women. We are now starting the data collection and the omega-3 intervention at the same time and I wish to request that you continue supporting the study, specifically in the following areas:

1. Constitute recruitment of HIV positive *pregnant mother who are in 2nd trimester (14 - 27 weeks), CD4 not more than 500 and are on* ARV, for screening for enrolment into the study until the required participant number per centre is achieved;
2. Storage of electronic weighing scale for use by the research assistant in data collection
3. Storage of a few bottled water, disposable cups and centrifuge bottles for samples
4. Storage of a few packets of porridge flour (for distribution to study participants).

During the study period, some incentives will be made available to the mentor mothers' office *(not individuals)* as an appreciation for the support as follows:

Purpose for Incentive Payment	Explanation	Amount (KSh.)
Recruitment for Screening	For every pregnant women in 14-27 weeks, CD4 below 500 and on ARVrecruited and screened only	50
Enrolment into the study	For every participant who meets the above criteria, recruited, screened and enrolled	100
Follow-up visits	For every enrolled participant who comes back to the facility for follow-up visits as scheduled	100

Mentor mothers site coordinator will be expected to complete the form provided on SOP-13e for monitoring recruitment, enrolment and follow-up visits and submit to the principal investigator, Rose Opiyo, for the above incentives to be made. In addition, each Mentor Mother will get 1 kg of millet porridge flour once every two weeks during data collection visits to carry home for their porridge.

Sign_____Date_____ Sign_____Date_____
Principal Research Investigator Mentor mother site coordinator

Appendix 8: Compliance in the trial

Compliance in the trial was determined by completion of taking the fish oil and soybean oil soft gels. At week 4 of the intervention, the proportion of participants who completed taking the soft gels were similar in both the Fish oil and Soybean oil study arms. By week 8, the proportions of participants who did not complete taking the soft gels from fish group was higher (11.6%) than from the soybean oil group (6.3%) (Table a-8-1). This difference in completion was however not significant at week -8 (Chi2(1) = 1.64; p-value=0.20), and did not significantly influence the change in BDI-II depressive symptom scores at the end of the study as seen from the regression coefficients estimates for in fish oil group (0.41(95% CI: -4.49 - 5.32), p=0.87) and soybean oil group (-3.21 (95% CI: -13.18 - 6.76), p=0.52).

Table A-8-1: **Completion and reasons for non-completion of taking the soft gels**

Completion of taking soft gels	**Fish oil group (n(%)**		**Soybean oil group (n(%)**	
	Week 4(N=92)	**Week 8(N=86)**	**Week 4(N=98)**	**Week 8(N=96)**
Completed taking the last supply of soft gels	82 (89.1)	76(88.3)	88(89.8)	90(93.7)
Did not complete taking bi-weekly supply of soft gels	10(10.9)	10(11.6)	10(10.2)	6(6.3)

The main reasons for not taking the trial products as advised was forgotingt (Table A-8-2).

Table A-8-2: Reasons for non-completion

Reasons	**Fish oil Group (n(%)**		**Soybean oil Group (n(%)**	
	Week 4(N=10)	**Week 8(N=10)**	**Week 4(N=10)**	**Week 8(N=6)**
Forgot	9(90.0)	9(90.0)	9(90.0)	5(83.3)
Was sick, and forgot	1(10.0)	0(0.0)	1(10.0)	1(16.7)
Was at work	-	1	-	0(0.0)

N=participants who did not complete taking the bi-weekly supply of soft gels

Unpleasant feelings

Some participants reported that they experienced unpleasant feelings (Table A-8-3). These experiences were reported by participants from Fish oil (at week 4=16.3% of participants; at week 8=11.6%) and Soybean oil (at week 4=11.2% of participants; at week 8=5.2%) groups.

Among the feelings experienced during the first four weeks of taking the soft gels were nausea (Fish oil=6.5%; Soybean oil=3.1%), vomiting (Fish oil=3.2%; Soybean oil=3.1%), heartburn (Fish oil=1.1%; Soybean oil=2.0%), bloated stomach (Fish oil=2.2%; Soybean oil=0.0%), loose stool (Fish oil=0.0%; Soybean oil=1.1%) and itchy skin (Fish oil=2.2%; Soybean oil=1.0%). Other unpleasant feeling reported by only one participant from each group were nose bleeding (Fish oil=1.1%; Soybean oil=0.0%).

The feelings reported at the end of the study were mainly related to gastrointestinal disorders and included nausea, with fishy-after-taste (Fish oil = 4.6%; Soybean oil=2.1%), vomiting (Fish oil = 3.5%; Soybean oil=0.0%), heart-burn (Fish oil = 2.3%; Soybean oil=3.1%).

Table A-8-3: **Participants reporting unpleasant feelings by study arm and period**

Unpleasant feeling	**Fish oil (n(%)**		**Soybean oil (n(%)**	
	Week 4(N=92)	**Week 8 (N=86)**	**Week 4(N=98)**	**Week 8(N=96)**
None	77 (83.7)	76(88.4)	87(88.8)	91(94.8)
Nauseated, fishy-after-taste	6(6.5)	4(4.6)	3(3.1)	2 (2.1)
Vomiting	3(3.2)	3(3.5)	3(3.1)	0(0.0)
Heartburn	1(1.1)	2(2.3)	2(2.0)	3(3.1)
Bloated stomach	2(2.2)	-	0(0.0)	-
Loose stool	0(0.0)	-	1(1.1)	-
Itchy skin	2(2.2)	-	1(1.0)	-
Nose Bleeding	1(1.1)	-	0(0.0)	-

Appendix 9: Instruments and analytical conditions for GC analysis

The fatty acid methyl esters from cheek cell samples were analyzed using *Shimadzu GC-2014* gas chromatograph with a flame ionization detector (FID). The analytical column used was HP-88 60m by 0.25mm by 0.20µm (*Agilent Technologies - Part No. 112-8867).*

The column was conditioned at 260 ^{0}C and carrier gas, helium maintained at a flow rate of 2.4 ml per minute until a flat baseline was obtained. Marine oil FAME Mix standard *(RESTEK - catalogue number 35066)* was injected in the GC at programmed oven conditions until complete seperation of the compounds in the standard FAME Mix was achieved. The conditions which were considered as the method for analysis of omega-3 eicosapentaenoic (EPA, C20:5n-3) and docosahexaenoic (DHA, C22:6n-3) fatty acids in this study were: injector temperature of 250 ^{0}C with helium carrier gas at a programmed pressure of 248.8 kPa with a total flow of 42.2 mL per minute, oven temperature initially set at 150 and increased at a rate of 5.0 ^{o}C/min to190^{o}C and held for 5 min and then further raised to 240 ^{o}C at a rate of 6 ^{0}C per minute, then held for 2 minutes giving a total analysis time of 23.67 minutes per sample injected. The FID detector temperature was 240 ^{0}C. Carrier gas flow rate was maintained at 3.0 mL per minute. The make-up gases were hydrogen and air for ignition at a split ratio of 25:1. One micrlolitre of the sample was injected at a time. The analytical conditions are summarized in Box 1.

Box 1: Gas Chromatography (GC) analytical conditions

Instrument: *Shimadzu GC-2014* gas chromatogram;
GC detector: Flame ionization detector (FID);
GC column: *Agilent Technologies* capillary column HP-88 (60m x 0.25mm x 0.20µm)
Carrier gas: Helium (248.8 kPa);
Conditioning temperature: 260 ^{0}C;
Carrier gas flow rate: Start 2.4 ml per minute; maintained at 3.0 mL per minute ;
Chemical Standard: Marine oil FAME Mix standard *(RESTEK - catalogue number 35066);*
Carrier gas analysis conditions: 248.8 kPa with a total flow of 42.2 mL per minute;
Oven temperatures: 150-190^{o}C at 5.0 ^{o}C per minute to 240 ^{o}C at 6 ^{0}C per minute; hold for 2 minutes.
Total program/analysis time: 23.67 minutes per sample injected;
FID detector temperature: 240 ^{0}C;.
Make-up gases: Hydrogen and air for ignition;
Mode of injection: Split at a ratio of 25:1;
Sample injection method: One micrlolitre injected at a time.

Appendix 10: Manufacturer's Certificates of Analysis for trial products

3994 Leighton Point Road, Calabasas, CA 91301
Phone: 818.207.4969 - Fax: 818.914.6403 - Email: info@omegavia.com

CERTIFICATE OF ANALYSIS

INNOVIX OMEGAVIA PRODUCT CODE: F1103 (1250 MG)
LOT # 12A12846

CHARACTERISTIC	SPECIFICATION	METHOD	Result
CAPSULE CONTENT			
FISH OIL	1250 mg		1252.2 mg
Total Capsule weight	1760 mg		1769.2 mg
Date of Manufacture	January 2012		
Expiry Date:	January 2015	QC-193C (GOED Modified	
Total Omega-3	1060 mg		1132.3 mg
EPA (EE)	750 mg		765.8 mg
DHA (EE)	250 mg		259.9 mg
PHYSICAL PROPERTIES			
Appearance	Clear color oblong shaped gelatin softgel with clear light yellow fill material		
Capsule size	24 OBLONG		
Gelatin source	BSE-free bovine gelatin		
Capsule content	85% Omega-3 ethyl ester		
HEAVY METALS			
Lead	LT 0.1 mg/kg	GOED Monograph	
Mercury	LT 0.1 mg/kg	GOED Monograph	
Arsenic	LT 0.1 mg/kg	GOED Monograph	
Cadmium	LT 0.1 mg/kg	GOED Monograph	
MICROBIOLOGICAL			
Total Plate Count	< 3,000 cfu/g	USP Version 31 - Section 61	
Yeast and Mold	< 300 cfu/g	USP Version 31 - Section 61	
Escherichia Coli	Negative	USP Version 31 - Section 61	
Salmonella	Negative	USP Version 31 - Section 61	
Staphylococcus Aureus	Negative	USP Version 31 - Section 61	

QUALITY ASSURANCE
Cert date: 3-Feb-2012

Corporate Office:
Innovix Pharma Inc. 3994 Leighton Point Road, Calabasas, CA 91301, USA. 800-270-4010.

3994 Leighton Point Road, Calabasas, CA 91301
Phone: 818.207.4969 - Fax: 818.914.6403 - Email: info@omegavia.com

CERTIFICATE OF ANALYSIS

INNOVIX SOYBEAN OIL PLACEBO
LOT # S2B009

CHARACTERISTIC	SPECIFICATION	METHOD	Result
CAPSULE CONTENT			
SOYBEAN OIL	1200 mg		1230.3 mg
Total Capsule weight	1650 mg		1612.1 mg
Date of Manufacture	February 2012		
Expiry Date:	February 2015	QC-193C (GOED Modified	
Saturated fatty acids	190 mg		180.3 mg
Monounsaturated fatty acids	270 mg		265.4 mg
Polyunsturated fatty acids	700 mg		642.9 mg
PHYSICAL PROPERTIES			
Appearance	Clear color oblong shaped gelatin softgel with clear light yellow fill material		
Capsule size	22 OBLONG		
Gelatin source	BSE-free bovine gelatin		
Capsule content	Soybean oil		
HEAVY METALS			
Lead	LT 0.1 mg/kg	GOED Monograph	
Mercury	LT 0.1 mg/kg	GOED Monograph	
Arsenic	LT 0.1 mg/kg	GOED Monograph	
Cadmium	LT 0.1 mg/kg	GOED Monograph	
MICROBIOLOGICAL			
Total Plate Count	< 3,000 cfu/g	USP Version 31 - Section 61	
Yeast and Mold	< 300 cfu/g	USP Version 31 - Section 61	
Escherichia coli	Negative	USP Version 31 - Section 61	
Salmonella	Negative	USP Version 31 - Section 61	
Staphylococcus aureus	Negative	USP Version 31 - Section 61	

QUALITY ASSURANCE
Cert date: 24-Feb-2012

Corporate Office:
Innovix Pharma Inc. 3994 Leighton Point Road, Calabasas, CA 91301, USA. 800-270-4010.

Appendix 11: Ethical clearance letters

KENYATTA NATIONAL HOSPITAL
Hospital Rd. along, Ngong Rd.
P.O. Box 20723, Nairobi.
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP", Nairobi.
Email: KNHplan@Ken.Healthnet.org

7th September 2011

Ref: KNH-ERC/ A/236

Rose Okoyo Opiyo
Dept. of Food Technology and Nutrition
College of Agriculture and Veterinary Sciences
University of Nairobi

Dear Rose

Research proposal: "Role of Fish Oil Omega-3 Fatty Acids on Depression among HIV-Seropositive Pregnant Women in Nairobi: A Randomized Double-blind Controlled Trial" (P266/6/2011)

This is to inform you that the KNH/UON-Ethics & Research Committee has reviewed and **approved** your above revised research proposal. The approval periods are 7th September 2011 6th September 2012.

You will be required to request for a renewal of the approval if you intend to continue with the study beyond the deadline given. Clearance for export of biological specimens must also be obtained from KNH/UON-Ethics & Research Committee for each batch.

On behalf of the Committee, I wish you a fruitful research and look forward to receiving a summary of the research findings upon completion of the study.

This information will form part of the data base that will be consulted in future when processing related research study so as to minimize chances of study duplication.

Yours sincerely

PROF A N GUANTAI
SECRETARY, KNH/UON-ERC

c.c. The Deputy Director CS, KNH
The Chairman, Dept. of Food Technology and Nutrition, UON
The HOD, Medical Records, KNH
Supervisors: Prof. Wambui Kogi-Makau, Dept. of Food Technology and Nutrition, UON
Prof. Kamau R. Dept.of Obs/Gynae, UON
Dr.Obondo Anne, Dept.of Psychiatry, UON
Dr. Ogoyi Dorington, Dept. of Biochemistry, UON

UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
P O BOX 19676 Code 00202
Telegrams: varsity
(254-020) 2726300 Ext 44355

KNH/UON-ERC
Email: uonknh_erc@uonbi.ac.ke
Website: www.uonbi.ac.ke
Link:www.uonbi.ac.ke/activities/KNHUoN

KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

Ref: KNH-ERC/ MOD/117

15th May 2012

Rose Okoyo Opiyo
School of Public Health
University of Nairobi

Dear Rose

Re: Request for approval of modifications study titled 'Role of fish oil Omega-3 Fatty Acids on Depression among HIV-Seropositive Pregnant Women in Nairobi: A randomized double-blind controlled Trial" (P266/06/2011)

Your communication of 2nd April 2012 refers.

The KNH/UON-ERC has reviewed and approved modifications as contained in the revised consent documents.

1. Informed consent explanation and consent form for study participants (English version 2: 3rd April 2012)
2. Informed consent explanation and consent form for study participants (Kiswahili version 2: 3rd April 2012)
3. Data collection tools/Case Report Forms(English-Kiswahili version 2.3rd April 2012)

The above documents replace the earlier documents approved 07 Sept 2011.

Provide the updated protocol incorporating the changes.

Yours sincerely

PROF.A.N. GUANTAI
SECRETARY, KNH/UON-ERC

c.c. The Deputy Director CS, KNH
The Principal, College of Health Sciences, UoN
The Director, School of Public Health, UON

Protect to Discover

REPUBLIC OF KENYA

MINISTRY OF MEDICAL SERVICES

PHARMACY AND POISONS BOARD

Telegram: "MINHEALTH" Nairobi
Telephone: 020-2716905/6, 020-3562107
Cellphone: 0733-884411/0720 608811
Fax: 2713409
E-mail: info@pharmacyboardkenya.org

When replying please quote

PHARMACY AND POISONS BOARD HOUSE
LENANA ROAD
P.O Box 27663-00506
NAIROBI

Ref. No. PPB/ECCT/12/03/01/2012(71) **22nd May 2012**

Principal Investigator, ECCT/12/03/01
School of Public Health
University of Nairobi
P. O. Box 19676-00202
Nairobi.
Attn: Ms Rose Okoyo Opiyo

RE: ECCT/12/03/01: Role of Fish Oil Omega-3 Fatty Acids on Depression Among HIV-Seropositive Pregnant Women in Nairobi: A Randomized Double-blind Controlled Trial.

Reference is made to the above study.

We acknowledge receipt of your responses to the issues raised during the initial review.

Upon review of the submission, the Pharmacy and Poisons Board's Expert Committee on Clinical Trials is satisfied and grants approval to the research protocol: **Role of Fish Oil Omega-3 Fatty Acids on Depression Among HIV-Seropositive Pregnant Women in Nairobi: A Randomized Double-blind Controlled Trial (ECCT/12/03/01)**

In case the study extends beyond one year, you are required to file with us the continuous review approval letter from the ERC on record for our acknowledgement before proceeding.

Please take note that it is your responsibility to inform the Pharmacy and Poisons Board of any changes to protocol, research design and procedures that could introduce new or more than minimum risk to the human subjects.

The Pharmacy and Poisons Board requires you to **provide regular updates and half yearly reports**, especially on Suspected Unexpected Serious Adverse Reactions (SUSARS) from the study, for monitoring purposes and involve the PPB where necessary.

Yours faithfully,

Dr. Edward Abwao
Division of Medicines Information and Pharmacovigilance
For Registrar

CITY COUNCIL OF NAIROBI

TOWN CLERK
FAX: 2217704
TELEPHONE: 2224281
Web: www.citycouncilofnairobi.go.ke

CITY HALL
P.O. BOX 30075 - 00100
NAIROBI
KENYA.

EXT.....

Ref No. PHD/MOH/R.1/101/2011/ac

14TH OCTOBER, 2011

ROSE OKOYO OPIYO
UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
NAIROBI

Dear Madam,

RE: RESEARCH AUTHORIZATION AMONG HIV – POSITIVE PREGNANT WOMEN AT HEALTH FACILITIES WITH PMTCT CENTRES IN NAIROBI FROM OCTOBER 2011 TO SEPTEMBER 2014

Reference is made to your letter dated 13th October, 2011 on the matter above.

This letter serves to grant your request to be allowed at the Nairobi City Council Health facilities with PMTCT centre's from October 2011 to September 2014 but the following will be observed.

- You will be expected to adhere to the rules and regulations pertaining to the City Council of Nairobi.
- That during the period of research there will be no cost devolving to the Council.
- That you undertake to indemnify the Council against any claim that may arise from the research.
- A copy of the finding to be submitted to the office of the undersigned.
-

By a copy of this letter the DMOH's and the facility in charges of the respective districts are requested to accord you the necessary assistance.

DR. ROBERT K. AYISI HSC
FOR: TOWN CLERK

CC – DMOH AND FACILITY INCHARGES

REPUBLIC OF KENYA

NATIONAL COUNCIL FOR SCIENCE AND TECHNOLOGY

Telegrams: "SCIENCETECH", Nairobi
Telephone: 254-020-241349, 2213102
254-020-310571, 2213123.
Fax: 254-020-2213215, 318245, 318249
When replying please quote

P.O. Box 30623-00100
NAIROBI-KENYA
Website: www.ncst.go.ke

Our Ref: NCST/RRI/12/1/MED-011/167/4

Date: 7th October, 2011

Rose Okoyo Opiyo
University of Nairobi
School of Public Health
P. O. Box 19676-00202
NAIROBI

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on "***Role of fish oil Omega-3 fatty acids on depression among HIV-Seropositive pregnant women in Nairobi: A randomized double-blind controlled trial***" I am pleased to inform you that you have been authorized to undertake research **in Nairobi district** for a period ending ***30th September 2014.***

You are advised to report to **the Provincial Commissioner & the Provincial Director of Medical Services, Nairobi Province** before embarking on the research project.

On completion of the research, you are expected to submit **one hard copy and one soft copy** of the research report/thesis to our office.

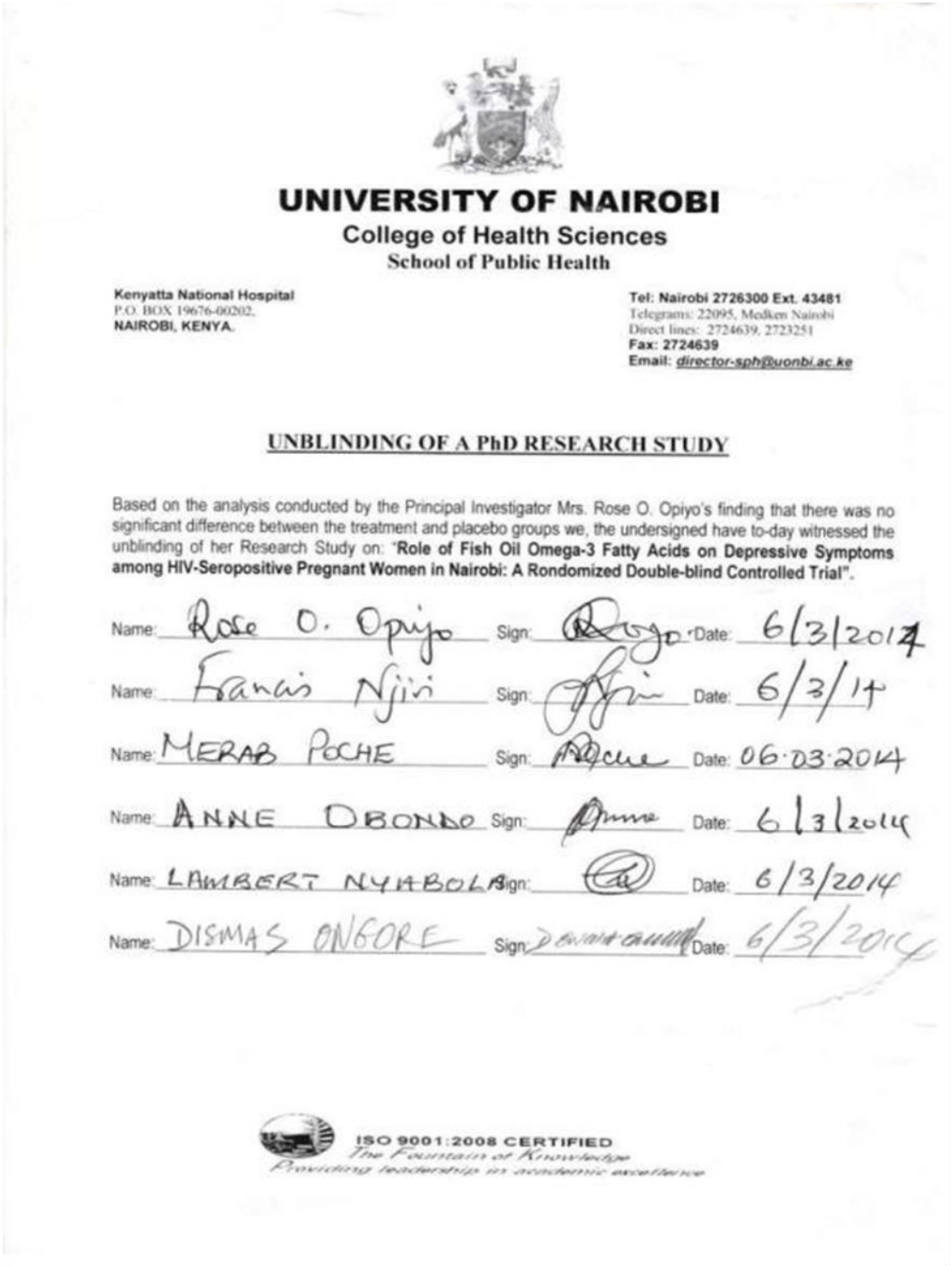
P. N. NYAKUNDI
FOR: SECRETARY/CEO

Copy to:

The Provincial Commissioner
Nairobi Province

PROVINCIAL COMMISS...
RECEIVED
13 OCT 2011
NAIROBI AREA

Appendix 12: Witnesses for unblinding of the trial codes



UNIVERSITY OF NAIROBI

College of Health Sciences

School of Public Health

Kenyatta National Hospital
P.O. BOX 19676-00202,
NAIROBI, KENYA.

Tel: Nairobi 2726300 Ext. 43481
Telegrams: 22095, Medken Nairobi
Direct lines: 2724639, 2723251
Fax: 2724639
Email: director-sph@uonbi.ac.ke

UNBLINDING OF A PhD RESEARCH STUDY

Based on the analysis conducted by the Principal Investigator Mrs. Rose O. Opiyo's finding that there was no significant difference between the treatment and placebo groups we, the undersigned have to-day witnessed the unblinding of her Research Study on: "**Role of Fish Oil Omega-3 Fatty Acids on Depressive Symptoms among HIV-Seropositive Pregnant Women in Nairobi: A Rondomized Double-blind Controlled Trial**".

Name: Rose O. Opiyo Sign: Date: 6/3/2014

Name: Francis Njiri Sign: Date: 6/3/14

Name: MERAB POCHE Sign: Date: 06.03.2014

Name: ANNE OBONDO Sign: Date: 6/3/2014

Name: LAMBERT NYAMBOLA Sign: Date: 6/3/2014

Name: DISMAS ONGORE Sign: Date: 6/3/2014

ISO 9001:2008 CERTIFIED
The Fountain of Knowledge
Providing leadership in academic excellence