Effect of Amaranth (*Amaranthus cruentus L.*) Supplementation on Nutritional Status and Body Composition of HIV Infected Lactating Mothers

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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DEDICATION

To my husband Sam and beloved daughter Joy

for their great support and encouragement.

ACKNOWLEDGEMENT

By God's sufficient grace and provision, I was able to complete this work.

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and modu-health organizations who supported the study with raw materials for preparing intervention media.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS AND ACRONYMS

AFASS	Acceptable, Feasible, Affordable, Sustainable, Safe.
ANOVA	Analysis of Variance
ASAL	Arid and Semi Arid Lands
AIDS	Acquired Immune Deficiency Syndrome
ART	Antiretroviral Therapy
BIA	Bioelectrical Impedance Analysis
BFFMI	Body Fat Free Mass Index
BFMI	Body Fat Mass Index
BMI	Body Mass Index
CD_4	HIV receptor glycoproteins
CPHR	Centre for Public Health Research
D_2O	Deuterium Isotopes
EBF	Exclusive Breast feeding
FFM	Fat Free Mass
FM	Fat Mass
FTIR	Fourier Transform Infrared Spectrometry
HIV	Human Immunodeficiency Virus
ID	Participant identification number
KEMRI	Kenya Medical Research Institute
KDHS	Kenya Demographic and Health Survey
Kg	Kilogram

MTCT	Mother To Child Transmission
MUAC	Mid Upper Arm Circumference
MBF	Mixed Breast Feeding
PMTCT	Prevention of Mother To Child Transmission
PLWHA	People Living With HIV / AIDS
SPSS	Statistical Package for Social Sciences
WHO	World Health Organization

ABSTRACT

The combined effect of lactation and HIV infection results in increased energy demand for the lactating HIV infected women which culminate into increased rates of malnutrition. A suitable food supplement needs to be identified to meet this demand. *Amaranthus cruentus L.* flour is said to improve nutritional status markedly especially for people living with HIV. However, there is little information on how A*maranthus cruentus L.* supplementation affects body composition and nutritional status of HIV infected mothers who are breastfeeding.

The objective of this study was to assess the effect of A*maranthus cruentus L.* supplementation on nutritional status and body composition of lactating HIV infected mothers attending Nyambene District Hospital.

HIV positive women who opted to breastfeed for six months were recruited into the study. Eighty women were randomly assigned to the experimental group while eighty nine were randomly assigned to the control group. The experimental group received whole amaranth flour while the control group received maize/ wheat composite flour for a period of six months. For both groups, daily porridge rations were prepared using 300g of flour in three litres of water and were served in the morning, mid-morning and at four o'clock in the evening. Each serving provided 370 k/cal in the amaranth group and 362 k/cal in the maize composite group. Follow-up was done on the mothers to ascertain adherence to the feeding

instructions. Stable Isotope technique was used to assess body composition at baseline and at three and six months post intervention. Anthropometric measurements (Body Mass Index and Mid- Upper Arm Circumference) were also done at similar intervals.

Based on BMI results, about half (47.3 %) of the women were undernourished at baseline. The results on nutritional status assessment revealed baseline equivalence on each of the indicators for every treatment arm. There was a significant positive relationship between fat free mass and Body Mass Index (Correlation coefficient=0.459, $P<0.001$), implying that fat free mass had significant contribution to Body Mass Index. Similarly there was a significant positive relationship between fat free mass and Mid –Upper Arm Circumference (Correlation coefficient=0.479, $P<0.001$). The relationship was much stronger compared to Body Mass Index and fat free mass. Mean Body Mass Index and Mid Upper Arm Circumference were significantly higher in the experimental group ($P < 0.05$) and a higher proportion of women increased in Fat Free Mass in the experimental group in comparison to the control group.

Notably, all the variables had a higher mean in experimental group compared to control at both mid point and end point. Mean change in Body Mass Index and Mid-upper Arm Circumference was statistically significant ($P<0.05$) in experimental group. Mean change in Fat Free Mass was not statistically significant ($P>0.05$).

However, the change was higher in experimental group compared to control.

In conclusion, the *Amaranthus cruentus* flour had a statistically significant positive effect on nutritional status of lactating HIV positive women compared to maize which had no statistically significant positive effect. In addition, the results revealed that *Amaranthus cruentus* is about three times more likely to improve the nutritional status of lactating HIV positive women than maize. This finding demonstrates that *Amaranthus cruentus* is more efficacious than maize in improving the nutritional status of lactating HIV positive women.

CHAPTER 1: INTRODUCTION

1.1 Background

Malnutrition is widespread in Kenya and other developing countries. It is a major direct and indirect cause of infant and childhood mortality and morbidity. Besides children, other vulnerable individuals such as People Living with HIV and AIDS (PLWHA) have high nutrient requirements.

The poor are unable to access adequate amounts of nutrient-rich foods to meet dietary requirements and this is the major reason for the high prevalence of malnutrition. For instance, according to preliminary report of Kenya Demographic Health Survey (KDHS) 2008/9, the prevalence of anemia among women of reproductive age in Kenya is 55%. Statistics from other countries in sub-Saharan Africa and South East Asia are not much better (Lartey, 2008).

There is therefore a need to identify nutrient-rich foods that can be produced inexpensively to meet the nutrient requirements for these vulnerable groups.

1.2 Problem Statement

HIV infection has debilitating effects on nutritional status of infected patients worldwide. These effects are worsened among infected mothers who are breastfeeding their infants.

The high energy demands accompanying disease management and the demand to sustain lactation calls for nutrition interventions that are both accessible and sustainable among populations in resource-poor settings. A suitable food supplement needs to be identified to meet these demands.

Amaranthus cruentus L. flour is gaining popularity in Kenya as a nutritious product, believed to significantly improve nutritional status of people living with HIV/AIDS. However, there has been no science-based information on how *Amaranthus cruentus L.* supplementation affects body composition and nutritional status of HIV infected mothers who are lactating.

1.3 Study justification

HIV infection and lactation elevate daily energy requirements by approximately 642 and 500 kcal respectively. For this reason, World Health Organization (WHO) recommends support for PLWHA and particularly HIV infected mothers. Supporting the breastfeeding mother to improve their nutritional status which in effect will improve their lactation performance using an efficacious product can be a remarkable intervention.

Maize is the staple food for most Kenyan communities. On the other hand, *Amaranthus cruentus L.* is believed to be highly nutritious, but how it would impact on the nutritional status and body composition of breastfeeding HIV positive

mothers is not exactly known. In addition, whether *Amaranthus cruentus L.* is more efficacious than *Zea mays L.* in improving nutritional status of these infected mothers is not known.

Grain amaranth has the potential to contribute to addressing the nutritional needs of vulnerable people because of its high protein content, superior protein quality, high content of essential fatty acids and micronutrients (Escudero et al., 1999). Feeding trials on the product will assist in generating evidence-based information that will be used in the development of policy and hence promote utilization of the hardy and locally grown *Amaranthus cruentus L*.

This study has provided evidence based information that will help promote utilization of grain Amaranth which is a hardy crop and yet nutritious. In addition, it will contribute to adoption of grain amaranth as a staple crop in the local communities and this will go a long way in addressing household food insecurity even in communities classified as Arid and Semi arid lands.

Moreover, this study will lead to increased awareness on grain amaranth and hence trigger economic revolution among communities who choose large scale production of the same for income generation.

1.4 Study Objectives

1.4.1 Main Objective

The main objective of this study was to assess the effect of *Amaranthus cruentus L.* supplementation on nutritional status of lactating HIV infected mothers attending Nyambene District Hospital.

1.4.2 Specific Objectives

The specific objectives were to:

1. Determine the baseline nutritional status of participants.
2. Determine dietary habits of participants
3. Provide amaranthus supplementation to participants
4. Determine the nutritional status of participants post intervention (that is at three and six months).

1.5 Hypothesis

Amaranthus supplementation has significant effect on nutritional status in comparison to maize/wheat composite among lactating HIV infected mothers.

1.6 Research Questions

1. Are lactating mothers infected with HIV in Nyambene malnourished?
2. Do HIV infected lactating women show improvement in nutritional status when supplemented with *Amaranthus cruentus L.* flour?

3. How does *amaranthus* supplementation compare with maize/wheat composite supplementation in affecting nutritional status of lactating HIV infected mothers?

CHAPTER 2: LITERATURE REVIEW

2.1: Overview of Mother to Child Transmission of HIV

Prevention of mother to child transmission of HIV has become an important intervention in the prevention and control of HIV/AIDS in developing countries with the main goal being to improve maternal and child health survival (Debbie et al, 2006).

In recent years, MTCT rates have fallen to as low as 2 to 5% of births among HIV-infected mothers in developed countries (Kevin et al, 2000). This reduction in transmission was made possible by the introduction of comprehensive services including HIV counseling and testing, antiretroviral therapy, elective caesarean section delivery and the safe use of infant feeding formula instead of breastfeeding (UNAIDS, 2002). These interventions have generally not been available in Africa and prolonged breastfeeding is the norm. Approximately 25-35% of HIV infected mothers pass on HIV to their infants (Coutsoudis, 2001).

The prevalence of HIV infection among pregnant women in Kenya is currently estimated at 13% (KAIS, 2008). This rate of infection in pregnant women aged 15-49 (the reproductive age group) coupled with high birth rates translates into an estimated 50,000-60,000 children under five years of age infected with HIV per annum (UNAIDS, 2006). The severity of MTCT problem in Sub-Saharan Africa is

due to high rates of HIV infection in women of reproductive age, high birth rates, and the lack of effective MTCT prevention interventions (Latham and Preble, 2000).

MTCT prevention requires more than provision of drugs and commodities. The World Health Organization (WHO) recommends support for the HIV infected mother, both during pregnancy and after delivery (WHO,2007). Several infant feeding options have been recommended for the HIV infected mothers. These options are replacement feeding and breast milk options such as heat treating, wet nursing, breast milk banks, or exclusive breast feeding (Peggy, 2004). Replacement feeding is feeding infants who are receiving no breast milk with a diet that provides most of the nutrients that infants need until the age at which they can be fully fed on family foods. Unlike breastfeeding, it does not provide immune protection against other diseases. There are two types of breast milk substitutes namely commercial infant formula and home modified formula with micronutrient supplements. Both of these have one major disadvantage of cost to HIV infected mothers living in poor resource settings, thus making their affordability difficult.

There is evidence that infants breast fed by HIV infected mothers stand a risk of 5%-15% of HIV infection through breast feeding (Peggy, 2004). However, there is insufficient information on how much of this risk is attributable to mixed feeding while breastfeeding (Latham and Preble et al, 2000). While the dangers associated with mixed feeding are known, it is probable that high transmission rates during

breastfeeding can be blamed on mixed feeding, which increases the likelihood of sub clinical mastitis-a condition associated with increased shedding of HIV virus in breast milk and hence increased transmission of HIV through breast milk (Latham and Preble ,2000).

2.2 Breastfeeding in the context of HIV/AIDS

According to UNAIDS, 2004, approximately 17 million women worldwide between the ages of 15 and 49 years are HIV positive. Most (77%) live in sub-Saharan Africa. In some regions, such as Botswana and Swaziland, the HIV prevalence in women attending antenatal clinics is as high as 40% (UNAIDS, 2004). Despite the many women of reproductive age who are HIV positive, few studies have investigated the relationship between HIV infection during pregnancy or lactation with a focus on maternal nutritional status and health (Saadeh,2005). In most trials with HIV positive pregnant women, the primary focus has been the effect on the infant rather than the HIV positive mother. In antiretroviral therapy trials to date, the intervention uses the woman to deliver prevention to the infant rather than being for her benefit (WHO, 2001).

Although the World Health Organization (WHO) recommends that HIV positive mothers avoid breastfeeding "when replacement milk is acceptable, feasible, affordable, sustainable and safe" (WHO, 2001) breastfeeding is a cultural norm in

many parts of the world for both HIV positive and negative mothers. Anecdotal reports suggest that considerable stigma is associated with not breastfeeding.

Despite concerns about the HIV transmission risk to the infant from breastfeeding and the possible effect of breastfeeding on the health and nutritional status of HIV positive mothers (Nduati et al, 2001), use of replacement milk is largely considered unacceptable, unaffordable or unsafe (Latham and Preble et al, 2000). Therefore, breastfeeding will likely remain the norm for HIV positive mothers in most parts of Africa irrespective of the effect of lactation on the health of the HIV positive mother. Leroy et al, (2004), concur with this suggestion and indicates that infected mothers who opt to breastfeed need support in order to sustain exclusive breastfeeding and avoid tendency towards mixed breastfeeding which increases the risk of MTCT.

2.3 Grain amaranth

Amaranths, which comprise the genus Amaranth, are widely distributed, short-lived herbs, occurring in temperate and tropical regions. There are about 60 amaranth species, several of which are cultivated as leaf vegetables, grains or ornamental plants, while others are weeds (Kauffman and Weber, 1990). The main species grown as vegetables are *A. tricolor, A. dubius, A. lividus, A. creuntus , A. palmeri* and *A. hybridus* while *A. hypochondriacus, A. cruentus* and *A. caudatus* are the main grain species (Teutonico and Knorr, 1985).

Amaranth produces a large amount of biomass in a short period of time (Kauffman and Weber, 1990) and therefore has the potential to contribute to a substantial increase in world food production to enhance food security. Grain yield of up to 5,000 kg/ha has been reported (Stallknecht and Schulz-Schaeffer, 1993).

Figure 2.1: Amaranth plant (head loaded with seeds)

Amaranth is one of the few plants whose leaves are eaten as a vegetable while the seeds (Figure 2.2) are used in the same way as cereals; there is no distinct separation between the vegetable and grain types since the leaves of young plants grown for grain can be eaten as both human and animal food. When the leaves are harvested in

moderation, the grain yield is unaltered. Vegetable amaranth species are utilized for food in different parts of the world.

Grain amaranth can be used as seeds or flour to make products such as cookies, cakes, pancakes, bread muffins, crackers, pasta and other bakery products (Teutonico and Knorr, 1985). Kauffman and Weber (1990) provided a description of the variety of products made from amaranth in different parts of the world. These include soups and stews from whole grain; *alegria*, a confection made from popped amaranth in Mexico; *atolea*, a fermented Mexican drink made from roasted amaranth flour; *chichi*, which is a form of beer made from amaranth in Peru; *sattoo*, a gruel consumed in Nepal, and *chapatti* made in different parts of Asia.

2.3.1 Nutrition composition of grain amaranth

Amaranthus cruentus L. seeds are 13 to 15 percent protein, among the highest for any grain. *Amaranthus cruentus L.* seeds are also high in fiber, calcium, iron, potassium, phosphorus, zinc, and vitamins A and C. Leaves are also edible, containing more calcium, phosphorus, and vitamin C than spinach, in addition to the high levels of folate and other nutrients present in the seeds (Kauffman and Weber 1990).

Table 2.1: Nutritional value of *Amaranthus cruentus L.*compared with other popular grains.

	Amaranth 100g	**Rice 100g**	**Wheat 100g**	**Corn 100g**
Protein	14.7 g	6.5 g	10.7 g	9.4 g
Fiber	9.3 g	2.8 g	12.7 g	7.3 g
Fat	6.5 g	0.5 g	2.0 g	4.7 g
Carbohydrate	66.2 g	79.2 g	75.4 g	74.0 g
Calcium	153.0 mg	3.0 mg	34.0 mg	7.0 mg
Iron	7.6 g	4.2 g	5.4 mg	2.7 mg
Zinc	3.1 mg	-	-	-
Calories	3742 kcal	358 kcal	340 kcal	365 kcal

(**Source**: Puente, 2008)

Amaranth grains are also known to contain substantial amounts of vitamins and minerals (Table 2.1). Amaranth grains contain twice the level of calcium found in milk, five times the level of iron in wheat, and higher sodium, potassium and vitamins A, E, C and folic acid than cereal grains (Becker *et al*., 1981).

Table 2. 2: Content of selected vitamins and minerals in Amaranth

NUTRIENT	CONTENT (mg/g)
Iron	17.4
Zinc	3.7
Sodium	31
Potassium	290
Calcium	175
Vitamin C	4.5
Niacin	1.45
Riboflavin	0.23
Thiamine	0.1

Source: www.eap.mcgill.ca/CPAT_1.htm: (2008.) Lorenz and Wright (1984)

Grain amaranth has higher levels of protein than most grains and its protein is of higher quality than that of most cereals and pulses. Reports from Uganda show that the varieties grown there have been found to have protein content of 12-13%, which is higher than that of most cereal grains and other common staples. Grain amaranth proteins contain substantial amounts of the essential amino acids that tend to be marginal in common cereals and pulses (Table 2.3).

The level of lysine in both varieties grown in Uganda was found to be above the FAO/WHO reference and more than double the level reported for maize. Methionine levels in the amaranth grains, though slightly lower than FAO/WHO recommended level, is about 3 times the levels in beans. However, amaranth has lower levels of threonine and phenylalanine than the FAO/WHO reference protein and marginal

levels of leucine and valine. It should be noted however, that the essential amino acids that are low in amaranth are quite abundant in most diets.

Table 2.3: Amino acid composition of grain amaranth in comparison to that of maize and beans

	Content (g/100 g protein)		
Amino Acid	**Amaranth**	**Maize**	**Beans**
Aspartic Acid	7.4	7.4	10.6
Glutamic Acid	19.7	18.3	13.2
Serine	6.0	4.5	4.8
Glycine	8.7	3.8	3.3
Histidine	2.9	5.5	2.2
Arginine	10.2	5.5	4.9
Threonine*	2.0	3.6	4.0
Alanine	4.4	5.7	3.7
Proline	4.8	6.4	3.2
Tyrosine	4.1	4.0	2.9
Valine*	4.7	4.5	5.4
Methionine*	2.5	1.7	1.0
Cysteine	0.1	2.3	0.0
Isoleucine*	4.3	4.2	4.0
Leucine*	6.1	13.7	7.1
Phenylalanine*	4.5	3.6	4.9
Lysine*	6.6	3.4	5.8

(**Source**: Becker et al., 1981) * Essential Amino Acids

The protein digestibility of grain amaranth grown in Uganda was found to be around 72% but roasting and popping, the two commonly-used preparation methods, were found to reduce digestibility to 60.6% and 52.5%, respectively. The level of tannins, an anti-nutrient known to reduce protein digestibility, in the grain amaranth varieties grown in Uganda was found to range from 0.11% catechin equivalent to 0.42%, which is higher than the levels in other grains like millet and sorghum. The level varied with the geographical area where the amaranth was grown. The levels of other nutrient inhibitors such as hemagglutinin, trypsin inhibitor and saponins in amaranth have been reported to be within the non-critical range (Escudero *et al*., 1999).

The carbohydrates in amaranth grain consist primarily of starch made up of both glutinous and nonglutinous fractions. Amaranth starch granules are much smaller (1-3 μm) than those found in other cereal grains (Teutonico and Knorr, 1985). Due to the unique size and composition of amaranth starch, the starch may exhibit distinctive characteristics which could be of benefit to the food industry (Lehman, 1988). Amaranth starch seems to have potential for use in the preparation of custards, pastes and salad dressing (Singhal and Kulkarni, 1990a, b).

Amaranth grain obtained from farmers in Kamuli, Uganda was found to contain 6.9-7.4% oil and the oil was made up predominantly of unsaturated fatty acids, with high levels of the essential fatty acid linoleic acid (Table 2.4). Based on its fatty acid profile, it can be concluded that grain amaranth is reasonably safe for consumption

by individuals that are at high risk of chronic non-communicable diseases such as coronary heart disease and diabetes. Its high content of linoleic acid, an essential fatty acid, makes it suitable for consumption by children since they need essential fatty acids for proper growth and development.

Table 2.4: Fatty acids profile for grain amaranth

Fatty Acid	Amaranth (mg/g)
Palmitic Acid	2.31
Stearic Acid	0.21
Oleic Acid	1.92
Linoleic Acid	2.41

(**Source**: Escudero et al., 1999)

2.3.2 Nutrition and health benefits of grain amaranth consumption

Consumption of grain amaranth is reported to have nutritional and health benefits, ranging from a general improvement in well-being to prevention and improvement of specific ailments and symptoms including recovery of severely malnourished children and an increase in the body mass index of people formerly wasted by HIV/AIDS (SRLP, 2005). Tagwira *et al*., (2006) documented perceived benefits of consuming grain amaranth among communities in Zimbabwe**.** The communities claimed that eating grain amaranth made them feel healthier and they noticed improvements in the health of their children. Specific health improvements noted included improvement in appetite, fast healing of mouth sores and herpes zoster, and

weight gain for PLWHAs. Amaranth consumption was also associated with higher milk production among breast feeding mothers.

Other studies show that the integration of only a small amount of *amaranthus cruentus L.* grain into the daily diet can help children recover from states of malnutrition. For instance, in a study performed by San Miguel de Proyectos Agropecuarios(2007), one thousand children eating an equivalent of only 20 grams of amaranth grain daily for one year recovered at a rate of 61.70 % while the control group only recovered at a rate of 15.33 %. Such results prove the grain's viability in fighting malnutrition around the world.

The improvements in general well-being and health reported by people who included grain amaranth in their diets are generally explainable by its high nutritional value. Some specific nutritional and health benefits of amaranth consumption have been elucidated. Amaranth oil has been shown, in animal studies, to lower total serum triglycerides and levels of low density lipoproteins (LDL) (Escudero *et al*., 2006). Similar effects have been reported in humans (Martirosyan *et al*., 2007). High levels of serum LDL are associated with coronary heart disease. The serum LDL lowering effect of amaranth has been attributed to the tocotrienols (unsaturated forms of vitamin E) and squalene in amaranth oil. These compounds affect cholesterol biosynthesis in humans (Martirosyan *et al*., 2007). They are also believed to have

anti-tumor and antioxidative activity (Kim *et al*., 2006a), pointing to potential anti-cancer effects.

Supplementation of patients with coronary heart disease with amaranth oil has been shown to contribute to a decrease or disappearance of headaches, weakness, increased fatigability, shortness of breath during a physical activity, edema of the legs towards the evening hours and feeling of intermission of heart function in most patients (Martirosyan *et al*., 2007). In addition, decrease in body weight has also been reported. Consumption of grain amaranth has also been shown to have potential benefits to diabetics. Studies suggest that supplementation of diets with amaranth grain and amaranth oil improves glucose and lipid metabolism in diabetic rats (Kim *et al*., 2006b). The fasting serum glucose levels and the glucose tolerance of the diabetic rats were both improved.

2.4 Increased energy needs due to HIV & Lactation

Both lactation and HIV increase the energy needs of infected mothers. Amaranth's high nutritional value could perhaps meet the extra energy demands for such mothers. More clinical trials are therefore needed to elucidate this suggestion. A comparison between energy needs for lactating HIV infected and non infected mothers is described in Table 2.5 (FANTA, 2004).

Table 2.5: Increased energy needs due to HIV and Lactation

HIV status	RDI (kcal)	+++ HIV	+++ Lactation	Total (kcal)
Non- infected	**2140**	**_**	**+ 500**	**2640**
HIV infected	**2140**	**+ 642**	**+ 500**	**3282**

(**Source**: WHO, FANTA, 2004)

2.5 Nutritional assessment indices

Body mass index (BMI) is defined as the individual's body weight divided by the square of his or her height (Eknoyan and Garabed,2008). The formulae universally used in nutritional assessments produce a unit of measure of kg/m^2. Thus; BMI = Weight (kg) / (Height (m)) $^{2.}$ On the other hand, Mid-Upper Arm Circumference (MUAC) is the circumference of the upper arm at that same midpoint, measured with a non-stretchable tape measure (Michele et al., 2004) .

The anthropometric measures of the upper arm are divided into principal measures, which are measured directly, and derived measures, which are derived from the principal measures using specific formulae and empirically-derived corrections. The derived measures attempt to provide better indicators of body composition and nutritional status than the principal measures, by accounting for the fact that external

measurements of the arm necessarily compound measurements of bone, fat, and muscle (Roy, 1991). The three principal anthropometric measures of the upper arm are the upper arm length; the triceps skin fold (TSF), and the mid-upper arm circumference (MUAC).

Because the BMI formula depends only upon weight and height, its assumptions about the distribution between lean mass and adipose tissue are not always exact. BMI sometimes overestimates adiposity on those with more lean body mass (e.g., athletes) while greatly under-estimating excess adiposity on those with less lean body mass.

A study in June, 2008 by Romero-Corral et al. examined 13,601 subjects from the United States' Third National Health and Nutrition Examination Survey (NHANES III) and found that BMI-defined obesity was present in 21% of men and 31% of women. Using body fat percentages (BF%), however, BF%-defined obesity was found in 50% of men and 62% of women. While BMI-defined obesity showed high specificity (95% of men and 99% of women presenting BMI-defined obesity also presented BF%-defined obesity), BMI showed poor sensitivity (BMI only identified 36% of the men and 49% of the women who presented BF%-defined obesity).

In an analysis of 40 studies involving 250,000 people, patients with coronary artery disease with "normal" BMIs were at higher risk of death from cardiovascular disease than people whose BMIs put them in the "overweight" range (BMI 25–

29.9),(Romeral –Corral et al., 2006). In the "overweight", or intermediate, range of BMI (25–29.9) the study found that BMI failed to discriminate between body fat percentage and lean mass. The study concluded that "the accuracy of BMI in diagnosing obesity is limited, particularly for individuals in the intermediate BMI ranges, in men and in the elderly. These results may help to explain the unexpected better survival in overweight/mild obese patients (Romeral- Corral et al., 2008). Eknoyan and Garabed, 2008 notes a further limitation which relates to loss of height through aging. In this situation, BMI will increase without any corresponding increase in weight.

As a possible alternative to BMI, the concepts fat-free mass index (FFMI) and fat mass index (FMI) were introduced in the early 1990s by studies on body composition (VanItallie et al., 1990).Body Fat Free Mass Index (BFFMI) is obtained by dividing body fat free mass in kilograms by height in meters squared, the unit of measure being kg/m^2 . Similarly, Body Fat Mass Index (BFMI) is obtained by dividing body fat mass in kilograms by height in meters squared.

The most common approach in body composition assessment is to divide body mass into two compartments, fat mass and fat-free mass. The three commonly recognized primary body composition assessment techniques are densitometry, elemental analysis and the measurement of total body water. Densitometry involves the estimation of body density which has conventionally been made by underwater weighing.

More recently, air displacement plethysmography has provided a simpler alternative. Both densitometry approaches are laboratory-based and therefore not suitable for use in field settings. Elemental analysis techniques, including total body in vivo neutron activation analysis and total body potassium analysis, are also limited in terms of wider application. Dual-energy x-ray absorptiometry is a widely used body composition method although not commonly used in field studies. The third primary body composition measurement technique is the assessment of total body water. The technique is based on the assumption that the water content of fat free mass is relatively constant (approximately 73.2% in adults) and that a negligible amount of water is associated with fat in adipose tissue.

Total body water assessment using stable isotope labels is the criterion method of body composition analysis and ideally suited for nutrition applications in field settings. Less exacting techniques, including anthropometry and bioelectrical impedance have been used in large nutrition interventions and population studies with validation against total body water in a representative sample (Moore et al., 2007).

Stable, that is, non-radioactive isotopes of an element and the ability to measure these isotopes by mass spectrometry were first recognized in the 1920s (Aston, 1927). Following a long history of use in research, stable isotopes are increasingly being used in the wider nutritional context (Ettyang et al., 2003).

There is a wide range of stable isotope techniques used in nutrition (Cisse et al., 2002), however, the scope of this review is limited to an overview of three of the most widely used techniques with particular relevance to the development and monitoring of nutritional interventions globally. These techniques include the doubly labelled water technique of deuterium (2H) and oxygen-18 (18O) to assess total energy expenditure, the use of 2H for the estimate of total body water and assessment of body composition as well as the deuterium oxide "dose-to-mother" technique to assess human milk intake in breastfed infants.

The stable isotopes of hydrogen (2H) and oxygen (18O) are present in the body, food and water; about 0.015% of all hydrogen is deuterium while approximately 0.20% of all oxygen is 18O. Thus, an adult man weighing 70 kg with 40 kg of body water contains almost 80 g 18O water and about 6 g deuterium. Consequently, body cells are accustomed to molecules containing 2H and 18O at natural abundance levels.

These unique characteristics of the stable isotope techniques make the methods highly suitable for development and evaluation of interventions to address the urgent need to improve nutrition throughout the life cycle. These techniques are state-of-the-art methodologies to monitor changes in body composition, total energy expenditure and human milk intake in breastfed infants and thus provide tools to monitor the effects of altered diet and physical activity as well as interventions specifically targeted to improve nutrition.

2.5 Effect of HIV on nutritional status of lactating women

Women who are Human Immuno-deficiency Virus (HIV) infected in developing countries are particularly vulnerable to nutrient deficiencies because of likely inadequate dietary intake and potentially increased nutrient requirements associated with HIV and other infections and the nutritional demands of lactation (FANTA, 2004). HIV infection in adults has been associated with weight loss, progressive loss of fat-free mass (FFM) and fat mass (FM), and wasting, all associated with increased morbidity and mortality risks (Macallan,1999_a).

Grinspoon *et al*. (1998) found that resting energy expenditure in 33 HIV-positive women was higher (119% ± 23%) than in 26 HIV-negative weight matched control women. In a study of non-pregnant women in Zaire, Thea *et al* .,(1996) found that 51 HIV positive asymptomatic women had significantly more subcutaneous fat as measured by skin fold thicknesses and more lean body mass as measured by bioimpedance than 48 women with AIDS but less subcutaneous fat and lean body mass than 11 HIV negative women. Several studies in HIV positive women showed that at least initially, weight loss is primarily in the form of FM (Grinspoon et al,1997; Kotler et al,1985).

Lindan *et al., (1992)* conducted a prospective study of predictors of mortality in 460 HIV positive Rwandan women of childbearing age (mean age 28 years). The 2-year mortality for all women was 7% (95% CI 5%, 10%); for the 40 women with AIDS at

entry, mortality was 21% (95% CI 8%, 34%). HIV accounted for 90% of deaths during the 2 years of follow-up; independent predictors of mortality included a BMI less than 21 at enrolment (relative hazard 2.3; CI 1.1, 4.8) and low income (relative hazard 2.3; CI 1.1, 4.5).

A complex bidirectional relationship exists among HIV infection, nutrition and immune Function (Macallan, 1999_b). Several studies in industrialized countries found an association between low blood levels of vitamins (vitamin A, vitamin E, riboflavin, pyridoxine and cobalamin) and minerals (copper and zinc) and HIV disease progression (Beach et al,1992; Baum et al,1995; Bogden et al,2000; Skurnick et al,1996; Tang et al,1997).

However, little is known about macronutrient intake and status and lactation in developing countries nor about the macronutrient profile of women infected with HIV. In HIV negative adults, inadequate macronutrient intake is associated with altered lactation output. Hence there is concern that similar inadequate diets may compound the susceptibility to lack of milk during lactation with a further reverting to mixed breastfeeding in HIV positive individuals (IOM:U.S, 2002).

2.6 Nutritional needs during lactation

Requirements for many nutrients such as energy, vitamins A, C, B12 and E, riboflavin, and minerals iodine, selenium and zinc are considerably higher during

lactation than during pregnancy (IOM:U.S,1998 & 2000) and are proportional to the intensity and duration of breastfeeding. Ideally, some of the nutrients stored during pregnancy, including energy stored as fat, will be available during lactation. To support the mother and infant during the first 6 months of lactation, breastfeeding mothers need to consume approximately 500 kcal/day in addition to usual energy intake before pregnancy. This assumes that approximately 170 kcal/day will be used from stores accumulated during pregnancy (IOM: U.S, 2002).

If the mother has not gained adequate weight during pregnancy, additional energy will need to be consumed during lactation to make up for the lack of additional stores. As with pregnancy, several metabolic adaptations meet the metabolic needs of lactation. These include increased appetite and food intake, mobilization of tissue stores and reduced physical activity (IOM: U.S, 2002).

2.7 Weight change postpartum

In their Rwandan study of HIV positive and negative women, Ladner *et al(1998)* reported weight changes through 5 months postpartum. Between 10 days and 5 months postpartum, both HIV positive and negative mothers gained weight, but the HIV negative mothers gained more (HIV positive mothers: 0.7 kg, SD 3.8; HIV negative mothers:1.9 kg, SD 4.7; P= 0.03). BMI increased in both groups (HIV positive mothers: 0.3, SD 1.5; HIV-negative mothers: 0.80, SD 1.9; P= 0.03).

Ladner et al (1998) do not report whether women were breastfeeding, exclusively or otherwise. In a smaller sample at 9 months postpartum, weight change was not different between the two groups (HIV positive mothers: 1.1 kg, SD 4.6; HIV-negative mothers: 1.8 kg, SD 4.8; *P*= 0.28). Though reports on lactating women of unreported HIV status worldwide indicate that most lose some weight postpartum and with breastfeeding, the authors suggest that HIV infection impairs weight gain postpartum (Villamor et al,2003).

In a randomized clinical trial of formula feeding versus breastfeeding in Kenyan HIV positive mothers, weight measured between 0.5 and 3 months postpartum was compared with weight measured between 5 and 9 months postpartum (Nduati et al, 2001). Formula-feeding mothers lost no weight whereas breastfeeding mothers lost 0.17 kg/month (*P*= 0.03). The wide time span for each measurement makes interpretation of this finding difficult. A limitation of both these studies is that the first weight measurement, if taken before 6 weeks postpartum, may still include the increased fluid from pregnancy and thus may not represent a true baseline weight (Papathakis et al, 2004).

In a longitudinal study of 73 HIV positive and 47 HIV negative South African breastfeeding women, the HIV positive mothers lost more weight between 8 and 24 weeks postpartum than did the HIV negative mothers (–1.41 kg, SD 3.1, versus +0.27 kg, SD 3.33; *P*= 0.006, respectively), or approximately 0.35 kg/month loss in

HIV positive and 0.08 kg/month gain in HIV negative mothers (Brewer et al, 1989). As measured by bioimpedance spectrometry, 92% of the weight loss was fat loss.

2.8 Nutrition assessment of body composition during lactation

When women face food shortages during lactation, they mobilize fat or lose body weight to support milk production at the expense of nutritional status (Huston et al, 2000 and Dewey, 1998). Whether HIV poses a similar burden in marginally nourished women is not known.

In his review of weight and fat store changes during lactation, Dorea (1997) suggested that changes in maternal body weight and FM postpartum vary considerably depending on the mother's nutritional status, reproductive history and stage of lactation. Most of the maternal physiological and body composition changes occur during the first 3 months postpartum, with weight loss being the norm internationally regardless of socioeconomic status.

Changes in fat are highly variable. In developing countries, mothers lose subcutaneous fat from all sites, and triceps skin fold thickness appears to be a sensitive measure in marginally nourished mothers. Affluent mothers, however, show more variable results: triceps skin fold measurements generally increase but the sum of skin fold thicknesses measured at several sites either increases or decreases. Therefore, it is recommended that the triceps skin fold measurement be used as an indication of fat mobilization but not necessarily of total body fat.

Similarly, Butte and Hopkinson (1998) suggested that triceps skin fold thickness measurements may reflect fat stores or redistribution during the first 4 months of lactation and should not be used alone to reliably predict total body fat. They found that the biceps and triceps skin fold measurements did not change significantly but that the suprailiac and subscapular skin fold measurements did.

In the previously mentioned body composition study in South Africa, all skin fold thickness measurements (triceps, biceps, subscapular and suprailiac), BMI and MUAC were strongly correlated with FM in HIV positive and negative breastfeeding mothers and were useful in measuring changes in FM.(Papathakis et al,2005) For HIV positive breastfeeding mothers, however, none of the anthropometric measurements was correlated with FFM, suggesting that other measurements such as stable Isotope technique should be used to accurately assess FFM. This finding is similar to that of Grinspoon et al. (1998), who found that BMI was not correlated with FFM in non-breastfeeding HIV-positive women.

Reference data for assessing nutritional status and identifying individuals at risk during lactation are not available, but it has been suggested that a cutoff value for BMI of 20.3 at 1 month postpartum should be used rather than the usual 18.5; by 6 months postpartum, however, the cutoff of 18.5 can be used to identify women at risk (WHO, 1995). This compares with a study conducted in the Gambia, where

BMI<18 in HIV-positive men and women was a predictor of mortality (Van Der Sande et al, 2004).

As with HIV positive pregnant women, when determining the best measurement to assess nutritional status during lactation, it is important to consider the purpose of the indicator. Body Mass Index, Mid-Upper Arm Circumference or change in weight can be used to identify women who are malnourished and in need of intervention (WHO, 1995). Cut-off values for anthropometric indexes related to improved maternal outcome or delay of disease progression during lactation in HIV positive women have not yet been developed.

2.9 Feeding patterns

The concern around increased morbidity and mortality associated with the use of formula feeding underlies the World Health Organization (WHO)/United Nations Children's Fund (UNICEF)/United Nations Programme on HIV/AIDS (UNAIDS) recommendation that exclusive breast-feeding (EBF) should be practiced by HIV infected women who cannot practice safe formula feeding (Bland et al., 2002).

The possibility that EBF might also be associated with a lower transmission risk than MBF has prompted several clinical studies in which the feasibility of EBF at a population level is examined and to encourage women to avoid mixed breastfeeding (MBF). Notable studies (Leroy et al, 2002 and Bland et al, 2002) have tested this

hypothesis and the risks associated with stopping breast-feeding earlier or more abruptly than usual. A study conducted in Zambia (Kuhn et al., 2002) established that, among infants born to HIV positive mothers, infant mortality was more than 3-fold greater ($P < 0.01$) among infants who were subjected to mixed breast feeding by 3 months of age compared with those who were exclusively breast-fed to at least 3 months of age.

According to Latham et al, (2000), EBF is an acceptable and feasible feeding option for many HIV positive women, especially where practical support is available. Early introduction of water is common in many communities and achieving EBF beyond the first months can be difficult if only limited support is available. Avoiding the introduction of additional formula feeding rather than just other fluids such as, water, is more acceptable and easier to achieve.

Moderate facility-based or community-based support both antenatally and around delivery can significantly increase EBF rates. Obvious determinants of practice such as physical resources at home are not always the principal basis of choice or practice. Women's status in many societies often prevents them from making and exercising choices. Counseling approaches need to effectively guide women to informed choices with support available to make these choices viable and sustainable. Further investigation is needed of methods to increase community acceptability of feeding interventions to reduce HIV transmission and increase child survival.

The scientific data regarding HIV positive women who become pregnant and choose to breast feed their infants do not exist in a vacuum but in the context of communities, health systems, government policies and international recommendations. The latter, however, are constrained because of lack of empirical data on which to base clear and explicit guidelines. Common perceptions are that HIV positive women rapidly deteriorate when pregnant or while breastfeeding (Papathakis et al, 2007). Although these perceptions likely reflect the course of women with more advanced disease, they prejudice the attitudes of health care workers in their daily clinical practice.

In most studies investigating mother-to -child transmission, 12–15% of HIV positive pregnant women have CD4+ counts of less than 200 x 10^6 cells/L (Baum et al., 2003). It is not clear whether the effect of pregnancy and lactation is the same for women with advanced disease as for those who are still immunologically competent. This information would affect some or all aspects of antenatal, delivery and postnatal care.

Clinic and hospital staff reflect their own views and those of their communities on what is the best way to feed young infants irrespective of the HIV status of women. Breastfeeding is more than just providing nutrition to a young infant; in many communities it also represents an important symbol of care. Hence a mother may

feel obliged to breastfeed even when she feels unwell or when food is not readily available for herself.

Maternal survival is of obvious importance to the mother but also for the survival of her children. Preliminary evidence, though scanty, suggests that an HIV-positive mother who is well nourished in both macro- and micronutrients is likely to have adequate health and immune function as determined by CD4+ cell count and viral load (Baum et al., 2003). Therefore, determining the best way to optimize the nutritional status of HIV-positive women is essential.

Operational research is needed on the delivery of comprehensive nutrition and health services to HIV positive women to support maintenance and improvement of body composition and micronutrient status. Body composition assessment methods need to be investigated and validated in HIV positive lactating women.

CHAPTER 3: MATERIALS AND METHODS

3.1 Study design

The study was a randomized double controlled intervention trial in which both participants and field staff were blinded to treatment allocation. The study targeted HIV infected mothers attending PMTCT, who had been counseled on available infant feeding options and had made an informed choice to exclusively breastfeed their infants for six months. One hundred and sixty nine HIV infected mothers within their first six weeks of delivery were recruited and randomly assigned to either of the two study arms.

The women were randomly allocated to either of the two treatment groups receiving *Amaranthus cruentus L.* flour (experimental group) or whole-meal *Zea mays L.* /wheat composite flour preparation (control group). Since the colour of amaranth flour is golden brown and in order to control for colour of intervention flour as a confounder in the control group, 20% wheat was used to mask the colour of maize and make it close to the colour of amaranth flour. The proportions of maize and wheat needed to arrive at the desired colour were tested for in a series of trials and a proportion of 80%:20% gave the best results.

Women in the intervention groups received a controlled amount of Amaranthus *cruentus L.* flour or whole meal *Zea mays L.* / Wheat composite, which comprised 4

kg pack per participant every 2 weeks for 6 months (equivalent of about 300 g/day). 300 g/day translates to approximately 1100 k/cal per day in the intervention group and 1087 k/cal per day in the control group. The flour was carried home, which they used to prepare porridge according to prior instructions. For both groups, daily porridge rations were prepared using 300g of flour in three litres of water and were served in the morning, mid-morning and at four o'clock in the evening. Each serving provided 370 k/cal in the amaranth group and 362 k/cal in the maize composite group.

Compliance to feeding instructions was followed on monthly basis at individual level through home visits and follow up questionnaires *(Appendix 3)* were used to capture this information. Individual dietary diversity questionnaires *(Appendix 2)* were used to collect information on the participant's dietary habits .The amount of porridge to be consumed daily was expected to provide a substantial proportion of the daily recommended energy in kcal/kg body weight. Preliminary analysis of amaranth and maize/ wheat samples were done to determine the energy content of each treatment *(Appendix 4)*.

Table 3.1 shows nutrient composition of the amaranth and maize / wheat composite flour preparations.

Table 3.1: Energy content in amaranth and maize preparations

Parameter	100% Amaranth flour	Whole maize/wheat flour (80% maize, 20% wheat)
Protein	**44.1**	**30.3**
Carbohydrate	**198.6**	**216.7**
Calories	**1100**	**1087.2**

3.2 Sampling

The sample size estimate was based on existing data for interventions previously studied in HIV positive women (McKinley et al (1994) and Papathakis et al (2007). The proportion of women with normal fat free mass levels at baseline was set at 0.5 whereas the proportion of study population with normal fat free mass levels after intervention was set at 0.7.

Sixty two women per treatment arm were required to detect a 0.3 unit difference between amaranth and maize groups, with a 0.05 level of significance and 90% power. An attrition rate of 20 % was anticipated, with a target sample size of 85 per group.

3.3: Sample size calculation

The sample size was determined using formula described below:

$$n = \{\underline{Z_{1-\alpha/2}\sqrt{2P(1-P)} + Z_{1-\beta}\sqrt{P_1(1-P_1) + P_2(1-P_2)}\}^2}$$
$$(P_1-P_2)^2$$

(source: Kirkwood et al, 2003.Medical statistics)

Where:

$Z_{1-\alpha/2}$ - (level of statistical significance) 95% confidence level = 1.96

$Z_{1-\beta}$ - (value of the power desired) 90% power = 1.28

$P = (P_1+P_2)/ 2$

P_1 = proportion of study population with normal fat free mass levels at baseline.

P_2 = proportion of study population with normal fat free mass levels after intervention.

3.4 Randomization process

Participants were randomly assigned to receive either *Amaranthus cruentus L.* flour (300 g /day) or whole *Zea mays L./* wheat flour composite (300 g /day) for porridge preparation at home. The randomization scheme was generated by a computer program in blocks of four and six at the KEMRI- Centre for Public Health Research, by an individual not involved in study implementation.

Randomization codes were sealed in opaque envelopes in accordance with the allocation sequence. After being deemed eligible for enrollment, participants were assigned the next envelope in the sequence to determine treatment assignment. The randomization code was held at the coordinating center and was broken at the time

of data analysis. Adjacent treatment arm positions were grouped into blocks and then treatment was randomly assigned within each block as illustrated in the flow chart (Figure 3.1).

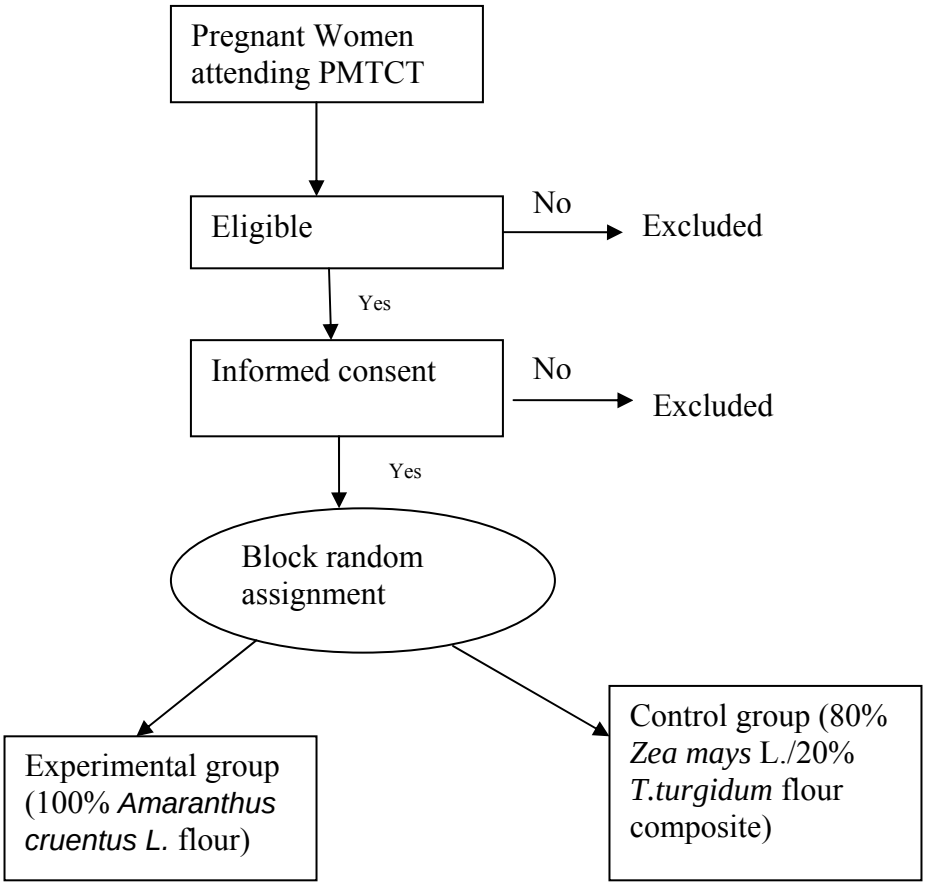

Figure 3.1. Randomization Process.

3.5 Study site

The study was conducted at the Nyambene District Hospital, about 320 km from Nairobi, an economically marginalized district classified among the Arid and Semi-

arid Lands (ASAL) climatic regions in Kenya. Since *Amaranthus cruentus L.* grows well in semi-arid climates, this region can support its cultivation. The climatic conditions of this district generally support cultivation of *Zea mays L.* which is the staple food in this locality.

The hospital implements the Prevention of Mother To Child Transmission (PMTCT) programme at the antenatal clinic (ANC), the maternity and the HIV comprehensive care clinic. The average monthly attendance in these PMTCT implementation sites during the study period was 2820 women and 187 of them were HIV positive.

3.6 Study population

3.6.1: Inclusion Criteria

Mothers enrolled in the food supplementation study were simultaneously participating in a larger study to investigate the pregnancy and lactation outcome of food supplementation using a control. All the mothers included in this study had been counseled on PMTCT and opted to exclusively breastfeed their babies for the first months of life until an option that was acceptable, feasible, affordable, sustainable, safe (AFASS) was available.

- All the recruited subjects included HIV positive women at the last month of gestation attending the antenatal clinic at Nyambene District Hospital and who planned to deliver at the hospital maternity.
- Women who had been counseled and tested for HIV at PMTCT clinic, had tested positive and had opted to exclusively breastfeed their infants for 6 months
- HIV positive mothers within six weeks after delivery
- Both pre –anti retroviral therapy (ART) mothers and those already on ART opting to exclusively breastfeed their infants were included in the study.
- Women whose CD4+ count was above 200 cell / mm^3
- Women without breast feeding problems (Abscess, mastitis and breast and nipple disease) or cracked nipples
- Women not mentally challenged

3.6.2 Exclusion Criteria

- Women whose CD4+ count was less than 200 cell / mm^3
- Women with breast feeding problems (Abscess, mastitis and breast and nipple disease) and/or cracked nipples
- Women mentally challenged, and/or bed-ridden
- Women who developed AIDS or whose CD4+ count fell below 200cell/mm^3 during the study

- Women who developed breast feeding problems (Abscess, mastitis and breast and nipple disease) and/or cracked nipples during the study

3.7 Data Collection

3.7.1 Body composition

Body composition was measured using the dose to the mother deuterium-oxide turnover technique (Coward et al, 1982; Orr-Ewing et al, 1986; Butt et al, 1988). Three clean, dry sample vials (4 ml screw cap cryogenic vials) were used for each subject (one baseline and two post- dose samples). Every participant was asked to empty bladder before her weight was taken in light clothing to 0.1 kg. Clean gloves were used for each participant.

Baseline saliva sample was then collected by giving the participants a cotton wool ball to chew. They were asked to move it round their mouth until sodden. They were asked to think about their favourite food to increase salivation. After removing the plunger from a new 20 ml disposable syringe, the participant was then asked to transfer the cotton wool to the front of their mouth and further transfer it directly from the mouth into the body syringe. The plunger was then replaced into the body of the syringe.

A sample storage vial was then labeled with participant's ID, date and time of collection. Names were not written on sample vials to preserve confidentiality. The

lid was then removed from the vial and using the syringe plunger, saliva was extracted from the cotton wool into the sample storage vial. At least 2 ml of saliva were collected to allow for repeat analysis. This sample was labeled T_0. The used syringe, cotton wool and gloves were discarded between participants and, there was strictly no reuse of sample vials or syringes.

The deuterium oxide dose bottle was inverted sufficiently to ensure the contents were fully mixed before administering to the participants. Using a clean straw, the participant was asked to drink the 30 g deuterium oxide. Some 50 ml drinking water was added into the same bottle and the participant was asked to consume. The participant was then asked to wait for 3 hours to allow the dose to equilibrate with the body water. After three hours, the post dose saliva sample was collected and labeled T_1. The saliva sample collection was repeated 3 months and six months post intervention for every participant.

Containers were firmly closed to prevent loss of water by evaporation and cross contamination between samples. Sample vials were stored in zip-lock bags to prevent cross- contamination. The baseline samples were placed in a different bag from the post-dose samples and together, these samples were placed in a third zip-lock bag and transported to KEMRI laboratories.

Samples were then analyzed using Fourier Transform Infra Red spectrophotometer to first determine the concentration of stable deuterium isotopes and these concentrations were then used to determine body composition in terms of Fat Mass and Fat Free Mass. The mother's body composition was estimated from her Total Body Water (TBW), which is measured by deuterium dilution. The calculations assume that the body is composed of fat and fat-free mass (FFM). Fat mass (FM) is the difference between body weight and FFM.

$TBW\ (kg) = V_D/1.041$

Where: V_D is the volume of distribution of deuterium also known as the pool space. It must be corrected for non-aqueous isotopic exchange which is assumed to be 4.1% of the pool space for deuterium.

FFM in adults is assumed to be 73.2% water. This is known as the hydration of FFM; therefore,

$FFM\ (kg) = TBW\ (kg)/\ 0.732$

Fat Mass (FM) is calculated as the difference between FFM and body weight,

$FM\ (kg) = body\ weight\ (kg) - FFM\ (kg)$

$FM\% = FM\ (kg)/body\ weight\ (kg) \times 100$

3.7.1.1: Cut off points for body composition parameters

The cut off points used for body composition in this study were adapted from similar studies that assessed fat free mass in study subjects (Khongsdier, 2005 and Fufa et

al, 2007). For the purposes of this study, Body Fat Free Mass Index of less than 15.1 was defined as malnourished, between 15.1 to 18.0 as normal and greater than 18.0 as overweight.

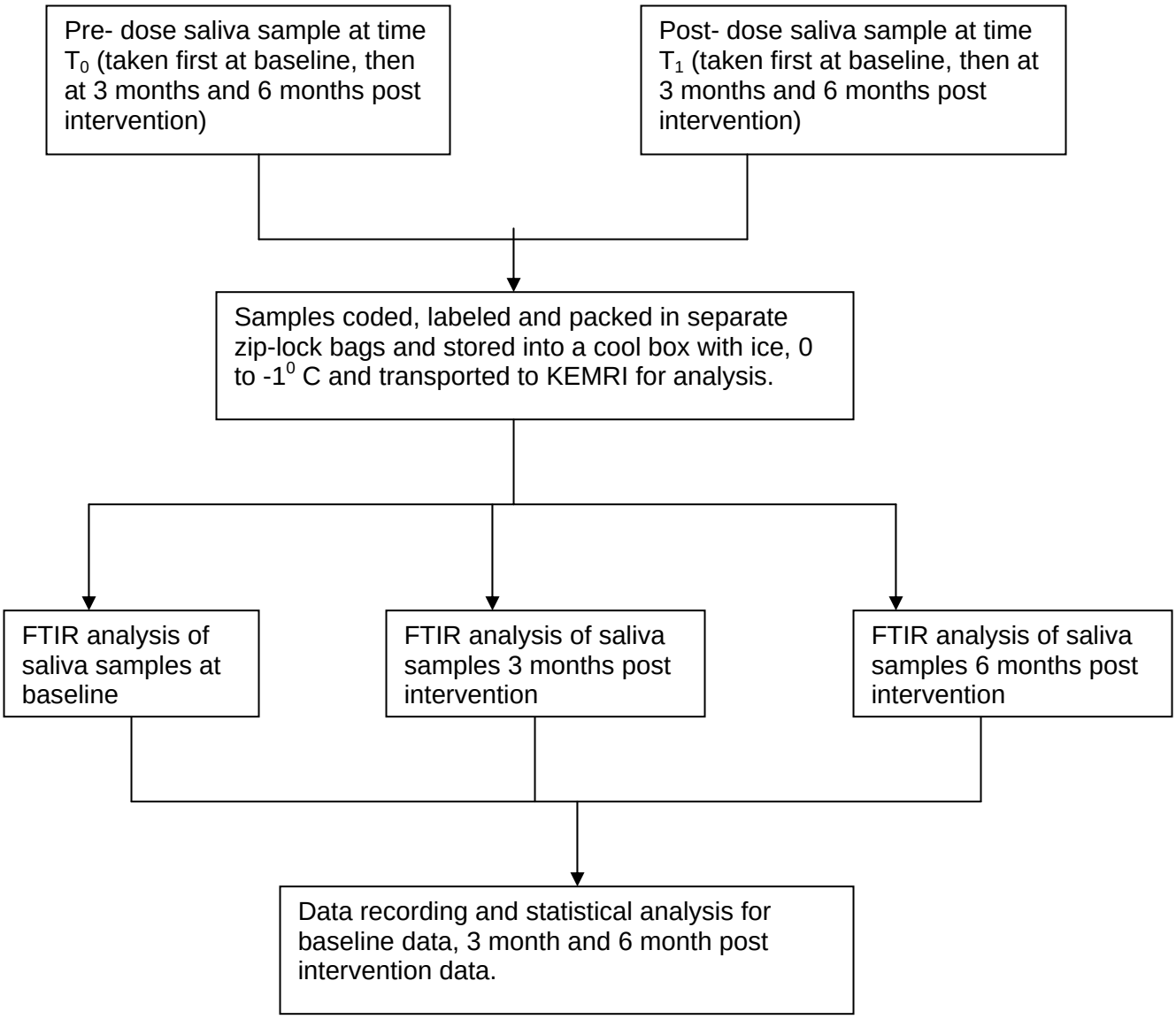

Figure 3.2: Flow chart of the saliva sample collection process

3.7.2 Anthropometric measurements

For Body Mass Index (BMI) measurements, weight was measured with the participant wearing no shoes and only light clothing. The weighing scale was placed on a flat surface for accurate readings. Weight was measured to the nearest kilogram (kg). Height was measured with the participant standing on a flat surface looking straight ahead to the nearest centimeter (cm). Measurements of both height and weight were used for the calculation of BMI. BMI was calculated by dividing weight in Kilograms by height in Meter squared. A repeat of weight measurements was done after 3 months and six months of intervention for every participant.

Mid Upper Arm Circumference (MUAC) measurements were taken with the participant being asked to keep their arm in a relaxed position by the side of their body, preferably while standing. The half way point of the upper arm was identified and using a MUAC tape, the measurement around the upper arm was taken at this point. The corresponding measurement on the tape was then recorded. MUAC measurements were repeated for every participant three months and six months post intervention.

3.7.2.1: Cut off points for anthropometric parameters

Body Mass Index (BMI) and Mid Upper Arm Circumference (MUAC) cut off points used in this study were derived from those defined by World Health Organization (WHO, 1995). BMI of less than or equal to 18.5 was defined as malnourished,

greater than 18.5 to 25.0 as normal and greater than 25.0 to 30.0 as overweight. The upper limit for BMI cut-offs was set at 30 since past BMI records at the comprehensive care clinic for HIV infectected individuals at the hospital did not show a higher value for lactating HIV infected mothers.

MUAC of less than or equal to 21.0 was defined as malnourished and greater than 21.0 as normal.

3.7.3 Blood sampling for CD4 count

Two milliliters of venous blood was drawn from the cubital vein by venepuncture and put in a non- citrated bottle. The samples were stored and transported for laboratory analysis in a cool box packed with ice packs. Clean gloves were used for every participant to ensure safety.

For the purposes of this study, CD4 counts for the participants was done together with those of other HIV patients attending the Comprehensive Care Clinic at the hospital. This was therefore done by a clinician trained in CD4 count blood sampling. In addition, CD4 counts for every participant were determined before enrollment into the study.

3.8 Statistical analysis

Data was analyzed using a computer aided statistical package (i.e. Statistical Package for Social Sciences (SPSS). For demographic and nutritional variables at each time point, differences between experimental and control groups were tested for significance with the use of a chi- square test for categorical variables (i.e. demographics) and Student's *t* test for continuous variables (i.e. Body Mass Index, Mid - Upper Arm Circumference, and Body Fat Free Mass Index).

Descriptive analysis on selected demographic and nutritional variables was performed using summary statistics i.e. Mean $\pm$ SD, Median, Minimum and Maximum for continuous variables and frequencies for categorical variables.

Distribution of continuous variables was tested for normality using Shapiro Wilk test. All were found to follow normal distribution; therefore Independent T-test was used to test for mean comparison between control and experimental groups.

To test for association between treatment groups and categorical variables e.g. demographic characteristics and categorized nutritional variables, χ^2-test or Fisher exact test was used.

Multivariate modeling for continuous outcome of BMI changes between baseline and end point was fitted using analysis of variance (ANOVA). Four variables were

fitted into the model; the treatment group was fitted as a fixed factor while age, occupation, and number of births were fitted as covariates.

Graphs and Summary tables were used for data presentation.

3.9: Ethical consideration

The study was approved by both the Ethical Review committee and the Scientific Steering committee of Kenya Medical Research Institute and an informed consent was signed by the participants. The consent form is attached as A*ppendix 1.*

Confidentiality of clients information was observed by allocating them coded numbers. The rights of the respondents were observed by informing them that they could withdraw or decline responding at any time they deem necessary.

CHAPTER 4: RESULTS

4.1. Respondents

A total of 169 lactating mothers between 15 and 47 years were enrolled into the study. About half of those (80) were randomized into experimental group and the rest (89) into control group. Respondents' retention in the study was at 98% as only two respondents dropped out of the study. Of these two dropouts, one relocated to an area far from the study site, while the other was excluded from the study following secession of breastfeeding due to frequent opportunistic infections which led to a CD_4 count of below 200.

4.2 Demographic characteristics

In order to assess for confounder effect on treatments, demographic characteristics were analyzed for both control and experimental groups.

The mean age of the mothers enrolled was 32.7 ± 9.1. Majority (64.5%) were aged between 25 to 35 years, while 25.5% were aged more than 35 years. A small proportion (10.1%) was aged less than 25 years.

Majority of the mothers (60.9%) were married, 23.1% were single, 14.2% were separated while a small proportion (1.8%) were widowed. The majority of the women (52.7%) practiced farming and 10.1% had some form of business. About one quarter (26.0%) were house wives while 10.7% were unemployed.

The mean number of children per household was four. The number ranged from a minimum of one child to a maximum of ten children. The level of education among the women was generally low. Most (88.8%) of the mothers had acquired education up to primary level. A small proportion (10.1%) had acquired secondary education while only 1.2% had acquired teacher -college level education

4.2.1 Relationship between demographic characteristics and treatment groups

Analysis of demographic characteristics by treatment groups revealed that there was no significant difference ($P>0.05$) in age, marital status, education, occupation, and number of children per household between the two treatment groups. None of the characteristics was significantly associated with the treatment groups ($P > 0.05$). Distribution of each of the characteristics was fairly similar in both treatment arms. The control and experimental groups were therefore reasonably comparable by the socio- demographic characteristics and no effect on randomization. **Table 4.2** shows distribution of specific demographic characteristics on each treatment group.

Table 4.2: Demographic characteristics by treatment groups

Variables/ categories		Total		Control		Experimental		P value
		N	%	N	%	N	%	
Age years								
	< 25	17	10.1	6	6.7	11	13.8	0.117
	26 to 30	44	26.0	20	22.5	24	30.0	
	31 to 35	65	38.5	37	41.6	28	35.0	
	36 to 40	17	10.1	13	14.6	4	5.0	
	More than 40	26	15.4	13	14.6	13	16.3	
Marital status								
	Married	103	60.9	55	61.8	48	60.0	0.362
	Separated	24	14.2	11	12.4	13	16.3	
	Single	39	23.1	20	22.5	19	23.8	
	Widow	3	1.8	3	3.4	0	0.0	
Education								
	Nil	17	10.1	9	10.1	8	10.0	0.960
	Primary	133	78.7	69	77.5	64	80.0	
	Secondary	17	10.1	10	11.2	7	8.8	
	College	2	1.2	1	1.1	1	1.3	
Occupation								
	Housewife	44	26.0	22.0	24.7	22	27.5	0.788
	Farmer	89	52.7	49	55.1	40	50.0	
	None	18	10.7	8.0	9.0	10	12.5	
	Business lady	17	10.1	9	10.1	8	10.0	
	Student	1	0.6	1	1.1	0	0.0	
Total number of children								
	1 to 2	35	20.7	19.0	21.3	16	20.0	0.649
	3 to 4	66	39.1	31	34.8	35	43.8	
	5 to 6	44	26.0	26.0	29.2	18	22.5	
	More than 6	24	14.2	13	14.6	11	13.8	

4.3: Nutritional assessment

4.3.1: Body Mass Index

The overall proportion of malnourished mothers at baseline among all the 169 mothers was 47 %. About half of the mothers were malnourished at baseline in both control group (43%) and experimental group (53%). The mean BMIs of the two groups were not significantly different (P>0.05). The number of malnourished mothers reduced steadily through out the study period in both groups. However, the reduction was notably higher in experimental group (31%) compared to control group (10 %) as demonstrated in **Table 4.3**.

Table 4.3: Percent of study mothers per BMI category at baseline, mid-point and endpoint.

	Baseline (0 months)		**Mid point (3 months)**		**End point (6 months)**	
	Control (n=89)	**Amaranth (n=80)**	**Control (n=88)**	**Amaranth (n=79)**	**Control (n=88)**	**Amaranth (n=79)**
BMI category in kg/m^2	**Mean BMI=20.7**	**Mean BMI =20.2**	**Mean BMI =21.1**	**Mean BMI =21.2**	**Mean BMI =21.6**	**Mean BMI =22.2**
	%	**%**	**%**	**%**	**%**	**%**
≤18.5 (malnourished)	43	53	38	34	33	22
>18.5 – 25.0 (normal)	46	43	50	60	56	68
>25.0 – 30.0 (overweight)	11	5	13	6	11	10

Changes in BMI were analyzed to see the effect of the treatments on nutritional status of the mothers. The changes in BMI for each participant between baseline,

midpoint and endpoint were calculated and these values were then used to calculate the mean changes.

As demonstrated in **Table 4.4**, mean changes for BMI were higher in experimental group compared to control group. The experimental group had a significantly higher increase in BMI compared to the control group ($P< 0.05$) at both mid point and end point.

Table 4.4 Mean BMI changes at mid point and endpoint

	Group	Mean	SD	
Mid point changes (3 months)	Control	0.4	1.1	
	Amaranth	1.1	1.3	
	P value < 0.001			
End point changes (6 months)	Control	0.9	1.1	
	Amaranth	2.1	1.4	
	P value < 0.001			

4.3.1.1: Multivariate analysis

Analysis of variance (ANOVA) was used to model BMI changes between baseline and end point. Four variables were fitted in to the model. The treatment group was fitted as a factor while age, occupation and number of births were fitted as covariates. The outcome of the model is shown in **Table 4.5.**

Table 4.5: Analysis of variance (ANOVA) in BMI changes between baseline and end point

Source of variation	Sum of Squares	df	Mean Square	F	P value
Treatment group	57.07	1	57.07	34.486	<0.001
Age	0.01	1	0.01	0.004	0.952
Occupation	2.72	1	2.72	1.642	0.202
Number of birth	0.55	1	0.55	0.334	0.564
Error term	266.46	161	1.65		
Total	326.81	166			

Adjusting for age, occupation and number of birth, the ANOVA model revealed significant treatment effect on BMI changes (P<0.001). Total variation in BMI changes attributable to the model was 18.5%. The treatment effects accounted for 17.5% of the variability in BMI changes while all the covariates had no significant effect on BMI changes (P>0.05) contributing only 1.0% of the total variation in BMI changes.

4.3.2: Mid- Upper – Arm – Circumference (MUAC)

Mid - Upper – Arm - Circumference results at baseline showed that 18% of the mothers in control group were malnourished while 28% were malnourished in experimental group. The mean MUACs of the two groups were not significantly different (P > 0.05) as demonstrated by a comparison of the mean MUACs of the two groups.

There was a gradual reduction in the proportion of malnourished mothers in both groups through out the study period. However, the reduction was markedly higher in experimental group (26%) compared to control group (10%) as demonstrated in **Table 4.6**.

Table 4.6: Percent of malnourished mothers at baseline, mid-point and endpoint using MUAC.

MUAC category in cm	Baseline (0 months)		Mid point (3 months)		End point (6 months)	
	Control (n=89)	Experimental (n=80)	Control (n=88)	Experimental (n=79)	Control (n=88)	Experimental (n=79)
	%	%	%	%	%	%
≤ 21 (malnourished)	18	29	15	17	8	23

Changes in MUAC were analyzed to compare the effect of the treatments on nutritional status of the mothers. The changes in MUAC for each participant between baseline, midpoint and endpoint were calculated and these values were then used to calculate the mean changes. As demonstrated in **Table 4.7,** mean changes for MUAC were higher in experimental group compared to control. The experimental group had a significantly higher increase in MUAC ($P \leq 0.05$) than the control group at both mid point and end point.

Table 4.7 Mean MUAC changes at mid point and endpoint

	Group	Mean
Mid point changes (3 months)	Control	0.3
	Amaranth	1.0
	P value <0.001	
End point changes (6 months)	Control	1.0
	Amaranth	2.4
	P value <0.001	

4.4: Body Composition

4.4.1 Body Fat Free Mass Index

The results for BFFMI revealed that more than half of the mothers were malnourished at baseline in both control group (57%) and experimental group (67%). There was continuous reduction in the proportion of malnourished mothers in both treatment groups. However, the reduction was proportionately higher in experimental group (42%) compared to control group (32%) through out the study period as demonstrated in **Table 4.8**.

Table 4.8: Percent of mothers as per BFFMI category at baseline, mid-point and endpoint

BFFMI category in kg/m^2	Baseline (0 months)		Midpoint (3 months)		End point (6 months)	
	Control (n=89)	Amaranth (n=80)	Control (n=88)	Amaranth (n=79)	Control (n=88)	Amaranth (n=79)
	%	%	%	%	%	%
<15.1 Malnourished)	57	67	48	47	25	25
15.1 – 18.0 (Normal)	38	29	50	53	68	71
> 18.0 (Overweight)	5	4	2	0	7	4

Changes in FFMI for each participant between baseline, mid point and endpoint were calculated to see the effect of supplementation on BFFMI. These values were then used to calculate the mean changes BFFMI. As demonstrated in **Table 4.9,** mean changes for FFMI at mid point were higher in experimental group compared to control.

The change in experimental group was (1.2) while in control group it was (0.9) though the mean change in FFM at midpoint was not statistically significant (P>0.05). Similarly, at end point, change in experimental group was higher (3.3) than in control group (2.6) but the mean change in FFM at end point was not statistically significant.

Table 4.9 Mean BFFMI changes at mid point and endpoint

	Group	Mean	SD
Mid point changes (3 months)	Control	0.9	3.6
	Amaranth	1.2	3.4
	P value =0.661		
End point changes (6 months)	Control	2.6	2.9
	Amaranth	3.3	3.1
	P value =0.131		

4.4.2: Relationship between anthropometric variables and body composition variables

The results on nutritional status assessment revealed baseline equivalence on each of the indicators for every treatment arm. A test of relationship between the indicators of nutritional status was done.

There was a significant positive association between fat free mass and BMI (Correlation coefficient, (r) =0.459, P<0.001), implying that fat free mass had significant contribution to BMI. Similarly there was a significant positive relationship between fat free mass and MUAC (Correlation coefficient (r) =0.479, P<0.001). The relationship was much stronger compared to BMI and fat free mass. **Figure 4.1** demonstrates the two relationships.

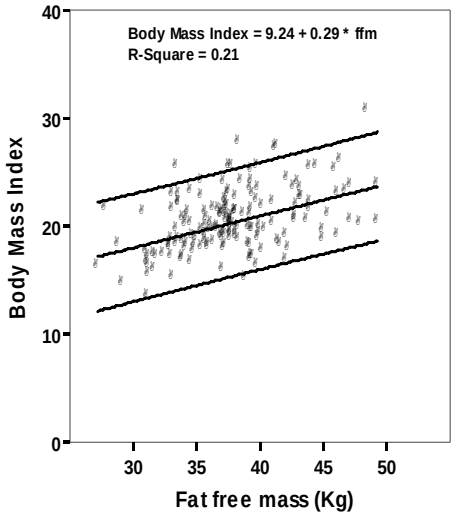

Figure 4.1(a): Relationship between fat free mass and BMI;

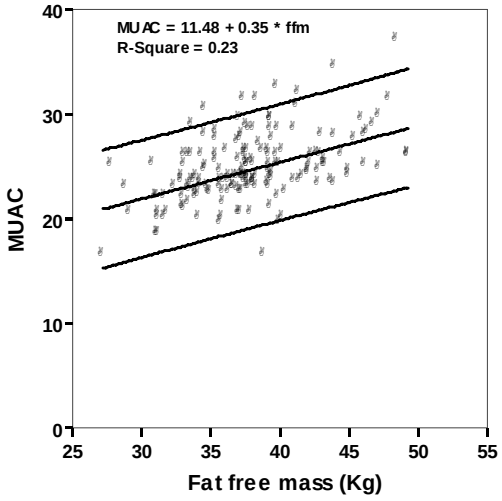

Figure 4.1(b): Relationship between fat free mass and MUAC

4.3.3: Relating weight changes to Fat Free Mass versus Fat Mass

Increase in weight throughout the study period was analyzed statistically to determine whether the increase in weight was in the Fat Free Mass or Fat Mass. There was a significant relationship between changes in weight and changes in FFM in experimental group ($P<0.047$) but not in control group ($P=0.270$). The increase in weight due to FFM changes was higher in experimental group (0.24 kg) compared to control group (0.13 kg). There was a significant change in FFM in the experimental group among those who improved in weight ($P< 0.001$). However, the change in FFM in the control group among those who improved in weight was not significant ($P > 0.05$).

In terms of relationship, there was a significant relationship between changes in weight and changes in FFM in experimental group ($P<0.047$) but not in control group ($P=0.270$). **(Figure 4.2).** Analysis of the relationship was performed using Pearson correlation coefficient (R). In control group 1% of the variability in change of fat mass between baseline and six months, is explained by variability in change of weight betweenbaseline and six months and vise versa. Similarly in experimental group 5% of the variability in change of fat mass between baseline and six months is explained by variability in change of weight between baseline and six months and vise versa.

(a) Control (b) Experimental

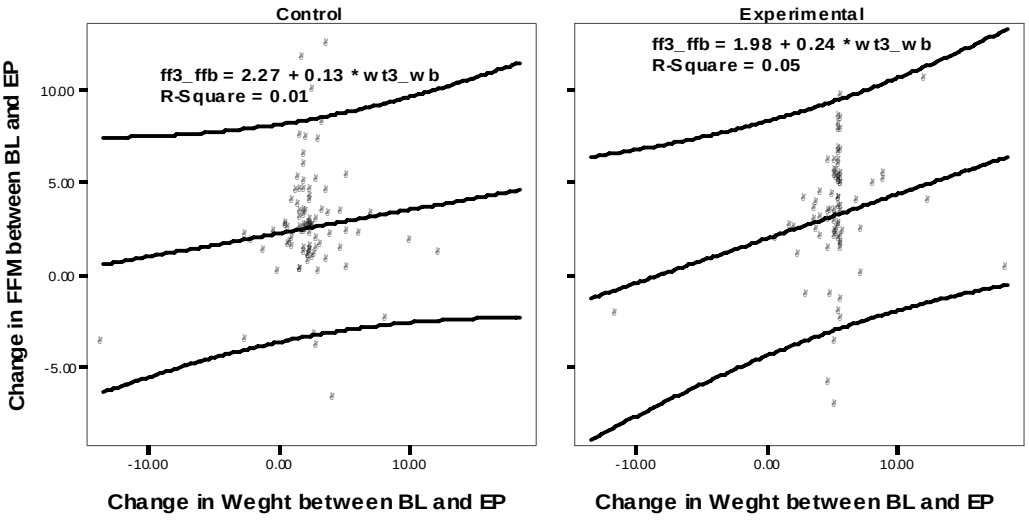

Figure 4.2: Relationship between weight changes and FFM changes by treatment groups: (a) control (b) Experimental.

On the other hand, the increase in weight among those who changed in fat mass was higher in control group (0.80) compared to experimental group (0.75). There was no significant change in fat mass in the experimental group among those who improved in weight ($P > 0.05$). However, the change in fat mass in the control group among those who improved in weight was significant ($P < 0.001$).

In terms of relationship, there was a significant relationship between changes in weight and changes in fat mass in both groups ($P<0.001$). **(Figure 4.3).** Analysis of

the relationship was performed using Pearson correlation coefficient (R). In control group 39% of the variability in change of fat mass between baseline and six months is explained by variability in change of weight between baseline and six months and vise versa. Similarly in experimental group 33% of the variability in change of fat mass between baseline and six months is explained by variability in change of weight between baseline and six months and vise versa.

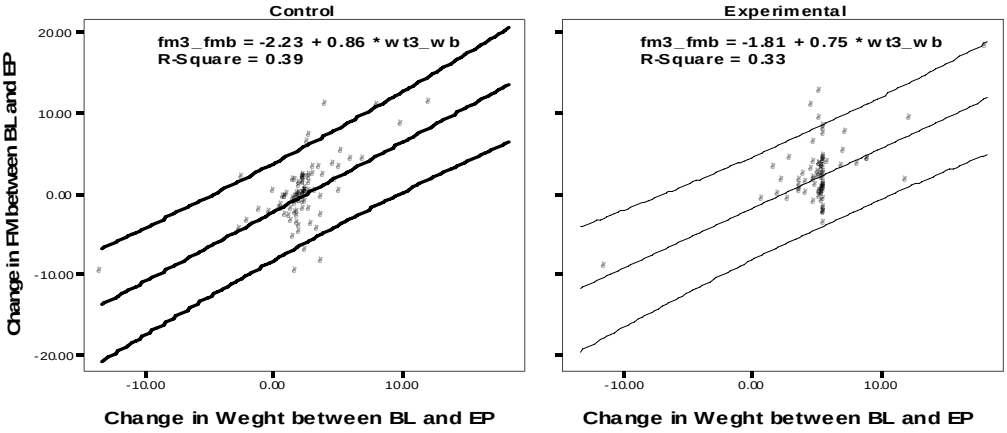

Figure 4.3: Relationship between weight changes and FM changes by treatment groups

4.5. Individual Dietary Diversity score

A total of 169 mothers were interviewed to establish their dietary diversity. The diet of the mothers in the study predominantly consisted of cereal grains, legumes, tuber crops and vegetables. There was very limited consumption of meat, eggs and fruits (**Table 4.10)**.

Table 4.10: Distribution of food diversity among study mothers

Food groups	**% (n=169)**
Cereals	95.8
Legume Grains	71.1
Tuber crops	57.2
Meats and eggs	12.4
Vegetables	81.6
Fruits	27.8

CHAPTER 5: DISCUSSION

5.1: Baseline Nutritional Status and dietary habits of participants

In this study, 47.3% of the mothers were malnourished at baseline. Several factors may explain this scenario among the study mothers. Key among these is the inadequacy and limited diversity of their diets as depicted by their dietary diversity results which showed that their diet was based mostly on cereals, tubers and vegetables. Such a diet is high in carbohydrates but is likely to be deficient in high quality proteins as well as some micronutrients. This is more likely so because consumption of fruits and meats was low. In addition, the vegetables are usually cooked for prolonged periods. This destroys the heat labile micronutrients.

The high level of malnutrition among study mothers at baseline can also be explained by the fact that the higher energy demands of lactation in HIV may increase weight loss which is also a risk factor for reduced HIV survival (Lindan et al., 1992). In addition, during lactation, energy, protein and micronutrient requirements increase to cater for milk production (FANTA, 2004). On the other hand, HIV causes nutrient loss and malabsorption further increasing nutritional needs (Bogden et al., 2000).

Another explanation for high malnutrition at baseline is the role HIV plays in complicating endemic malnutrition especially in Africa. For instance, studies of HIV positive mothers in Africa showed that nutrition status was a strong predictor of post

natal mortality (Nduati et. al., 2001). Moreover, it explains the fact that antiretroviral treatment alone in the absence of adequate diet, causes more harm than good.

There is strong evidence that malnourished people are less likely to benefit from antiretroviral treatment (Tang et al., 1997). A study in Malawi found that patients with mild malnutrition (a low body weight for their height) were twice as likely to die in the first three months of treatment. For those with severe malnutrition the risk was six times greater than those of healthy body weight (Zachariah et. al., 2006).

In addition, researchers in Singapore have reported similar findings (Paton et. Al.,2006). This information further qualifies nutrition interventions for lactating HIV infected mothers which strengthens their immune system, delays disease progression and enhances their quality of life as well as that of their infants.

5.2: Effect of Food Supplementation on study mothers

By the end of the study, BMI and MUAC results showed that there was about 30% reduction in malnutrition in the experimental group and about 10% reduction in malnutrition in the control group. The results also showed that mean changes for BMI and MUAC were higher in experimental group than control and that the experimental group had a significantly higher increase in BMI and MUAC ($P< 0.05$) than the control group.

These findings show that both treatments resulted in improved nutritional status. However, they also reveal that *Amaranthus cruentus L.* has a three fold ability to improve nutritional status of malnourished lactating mothers compared to maize. This was further verified by analyzing the degree of variance attributable to BMI changes between baseline and end point. The regression model for BMI changes showed only the intervention was a significant predictor of weight change and all other factors were not significant.

These findings agree with similar studies done on amaranth. In a study performed by Miguel (2007) in Mexico, one thousand children eating an equivalent of only twenty grams of amaranth grain daily for one year recovered at a rate of 61% percent while the control group only recovered at a rate of 15%. Such results show amaranth's potential in fighting malnutrition around the world.

The Fat Free Mass Index results also showed a continuous reduction in the proportion of malnourished mothers in both treatment groups. The reduction was slightly higher in experimental group (42%) compared to control group (32%) although the mean change in FFMI was not statistically significant ($P > 0.05$). This finding was important in explaining the location of change in weight gained during the intervention among the study mothers. It suggests that improvements in weight were located more in the fat free mass in experimental group than in control group.

After three months of intervention, BFFMI results in the percent of women who were overweight showed a decline to zero. This can perhaps be explained in terms of vulnerability of HIV infected mothers to opportunistic infections which leads to drastic decline in body weight. This decline can also be explained in terms of Isotope technique as a sensitive tool in detecting slight changes in body composition.

The results of this study showed significant association between weight and fat free mass among experimental mothers but not in the control group. Similarly, there was a significant association of weight and fat mass among the controls and not in the experimental group.

This suggests that the weight gained as a result of maize supplementation was located more in the fat mass while that which was gained due to grain amaranth supplementation was located more in fat free mass than in fat mass. Perhaps this can be attributed to the nutritional composition of these two intervention media. However, this observation requires further scientific investigation.

This might explain why weight gain in control group was located more in the fat mass compartment of body composition. However, there are several other metabolic interactions that come into play in the assimilation of food into either fat mass or fat free mass. From the findings of this study, there appears to be mandatory nutrient threshold levels that an intervention food in HIV patients must have for it to improve

FFM which then would translate specifically into improved nutritional status and into quality of life in general. Further studies need to be done to elucidate these arguments.

Moreover, studies have shown that Amaranth is a bio-chemically complete food and it contains 6 to 10% oil, which is found mostly within the germ (Betschart et al. 1981, Lorenz and Hwang 1985, Garcia et al. 1987). It is predominantly an unsaturated oil (76%) and is high in linoleic acid, which is necessary for human nutrition. In analyses conducted at the USDA Western Regional Research Center, amaranth oil was found to have 7% squalene, which is much higher than the amounts found in other common vegetable oils and as such can be used as a food – food fortificant.

The potential complimentary nature of amaranth protein has been studied by combining amaranth with wheat (Pant 1985), sorghum (Pedersen 1987) and maize (Tovar and Carpenter 1982; Sanchez Marroquin et al.,1985). Ordinary maize meal supplemented with as little as 12.7% (by weight) of toasted amaranth flour provides a nutritionally superior source of protein that can satisfy a good portion of the protein requirement of young children, and provide approximately 70% of diet energy (Morales et al. 1988). A combination of rice and amaranth in a 1:1 ratio has been reported to approach the FAO/WHO protein specifications (Singhal and Kulkarni 1988).

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

6.1: Conclusions

Based on findings of nutritional status at baseline, the majority of lactating HIV infected women were either malnourished or at a high risk of malnutrition.

In the absence of supplementation, the normal diet of the women was of limited diversity as shown by the predominance of cereals in their daily intakes.

Supplementation with an amaranthus enriched composite significantly improved the BMI , hence nutritional status of the women.

Nutritional interventions that target lactating HIV infected women should include provision of amaranthus enriched supplementations.

6.2: Recommendations

1. Larger studies need to be done on grain amaranth to confirm the findings of this study and therefore help advice policy on adoption of this crop at a national level as a staple food. This will help address the problem of household and national food insecurity. It will also be a practical tool for

eliminating malnutrition among all vulnerable groups. This is possible because amaranthus cruentus can safely be used as a weaning food as well.

2. Production and utilization of grain amaranth should be encouraged among Kenyan communities. This will help improve the nutritional status among vulnerable groups.
3. There is need for nutrition intervention programmes to incorporate amaranth supplementation for HIV positive women especially lactating mothers.
4. Further studies should be done to test the effect of grain amaranth supplementation on immune function of lactating HIV infected women.

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APPENDICES

APPENDIX 1: CONSENT FORM

The Effect of Amaranth (Amaranthus cruentus L.) supplementation on nutritional status of HIV infected lactating mothers attending Nyambene District Hospital in Eastern Kenya.

PART A. INFORMATION SHEET

The first part explains the reasons for the study and describes the study. It will be read aloud to participants. The second part will be read to the participants individually to obtain their consent.

Introduction: This is a study is being carried out by Dorothy K Murugu an MSC student with the Institute of Tropical Medicine and Infectious Diseases, JKUAT in collaboration with the Kenya medical Research Institute (KEMRI). The aim is to find out the effect of food supplementation on HIV infected mothers who are lactating and who attend Nyambene District Hospital.

Generally, the indigenous grains are believed to have a positive effect on health. The particular porridge flour being tested in this study is derived from one of locally grown grains and is believed to improve the health status of people living with HIV. However, there is inadequate documented scientific information to confirm this belief.

Objectives of the study:

The objective of this study is to assess the effect of supplementary food on the nutritional status of HIV infected lactating women. This will involve supplementing both ART and pre- ART clients with the supplementary food for a period of six months.

Participation in the study:

We are asking you to join this research study so that through your participation we will be able to address the objectives. The risks of taking part in this study are small. We wish to confirm that all the materials and instruments we shall use are new, sterile and clean. Our staff who will attend to you are all qualified to look after patients.

Alternatively, if you choose not to participate in the study, it will not affect your access to clinical care at this facility. You will be able to receive the same medical treatment and nutritional counseling as other clients at the facility.

What your participation will involve:

1. Upon enrollment in the study, you will be asked detailed questions regarding yourself and your health by our clinical staff attached to the project. You will be randomly allocated to either of two groups to receive either supplementary food 1 or supplementary food 2 for six months. The allocation will be entirely random, with a 50% chance of being allocated to either of the two food groups, 1 or 2. The supplementary food 1 is a powder that is derived

from a traditional grain crop that can be made into porridge and is believed to be highly nutritious. The supplementary food 2 is also a powder derived from whole maize meal and it is fortified with vitamin premix and iron. Both foods have sugar added to taste. The field staff who will issue you with the food do not know the difference between the two foods and as such cannot alter your random allocation.

2. You will be required to undergo standard medical examination at the comprehensive care clinic to confirm your eligibility for starting ART treatment. As part of your routine care in this clinic, you will be requested to provide blood at baseline and after six months for the CD4 test. In order to ensure a sufficient quantity of blood is available for this test, five milliliters of blood will be drawn each time. This information will be useful both for the research study as well as for your routine care at this clinic. For the purposes of the study, you will be requested to provide us with samples of your saliva at baseline, and after every 3 months. In order to collect the saliva sample, you will be provided with a clean and sterile piece of high quality cotton wool which you will be requested to put in your mouth and chew, without swallowing the saliva for a few minutes so as to soak it with saliva. We will then receive the saliva -soaked cotton wool into a clean and sterile 20ml syringe which we will use to squeeze the saliva into a sterile sample collection bottle. The saliva samples will be analyzed in KEMRI laboratories in Nairobi. This information will be recorded into standard forms and will

enable us provide you with good health care during the study period as well as assist in assessing changes resulting from food supplementation. On the other hand, a field assistant will ask you questions concerning your dietary habits, and measurements of your weight, height and mid upper arm circumference will be performed during the first visit. Periodically, our field assistant will visit you at home to enquire about your dietary intake. These tests will be repeated every three months for a period of six months to facilitate assessment of your progress during the intervention. The project will meet all the costs of these tests during the six months of follow-up.

3. You will receive simple instructions from our field staff on how to measure your daily portion of the porridge and how often to take it. You will not be required to pay for the food ration but we will expect you to pick it up from the designated point at the clinic once a month.
4. All information you provide us throughout the study will remain confidential and will only be used to provide you with nutritional care and medical assistance. Only the study team will have access to this information and it will not be relayed to any other persons without your permission.

Benefits for participating clients:

1. CD4 test at baseline and after six months
2. Nutritional assessments at baseline and every three months

3. Client monitoring and follow-up by the project staff for clinical and nutritional assessment performances.
4. Provision of food supplement for six months free of charge.

Risks for participating clients:

1. You may be allergic to ingredients in the food products in which case they may experience discomfort or adverse reactions to consumption of the food.
2. The flavour of the supplementary foods might require time to adjust or may be intolerable to some individuals.
3. You might suffer guilt for not sharing the food ration with their households.
4. The study involves responding to questions about dietary diversity, socio economic status and compliance to the food supplements which may make you feel uncomfortable or embarrassed.
5. You might be stigmatized if other individuals learn about your participation in the study by other means.

Withdrawal from the study:

You may withdraw from participating in this study at any time without giving the reason

and without jeopardizing your right to medical and nutritional care in this facility.

PART B: CONSENT FORM

Please read the information sheet (PART A) or have the information read to you carefully before completing and signing this consent. If there are any questions you have about the study, please feel free to ask them to the investigator prior to signing your consent form

If you have any questions about the study in the future or in between visits, or for any enquiries or issues related to the study, please contact the following staff:

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Consent form for the study:

FOR COMPLETION BY ALL PARTICIPANTS

I have read the information sheet concerning this study and I understand what will be required of me if I take part in the study.

Any questions I have concerning this study have been answered.

I understand that at any time that I may wish to withdraw from this study I can do so without giving any reason and without affecting my access to normal health care and management.

I agree to take part in this study.

Name ……..

Signed…….. date……..

APPENDIX 2: INDIVIDUAL DIETARY DIVERSITY QUESTIONNAIRE

Please describe the foods (all meals and snacks) you ate yesterday during the day and night. Start with the first food eaten in the morning.
Write down in the boxes provided, all food and drinks mentioned by the respondent. When a mixed dish is reported, ask about and write down all of the ingredients used in the dish.

Breakfast	Morning snack	Lunch	Afternoon snack	Dinner	Snack

Check the appropriate food groups consumed using the information recorded above. For any food groups not mentioned, clarify with the respondent whether or not a food.

Question number	Food group	Examples	YES=1 NO=0
1	CEREALS	Bread, noodles, biscuits, cookies or any other food foods made from millet, sorghum, maize, rice, wheat + insert local foods e.g. ugali, porridge,or pastes or other locally available grains.	
2	VITAMIN A RICH VEGETABLES AND TUBERS	Pumpkin, carrots, squash, or sweet potatoes that are orange inside + other locally available vitamin-A rich vegetables (e.g.sweet pepper)	
3	WHITE TUBERS AND ROOTS	White potatoes, white yams, cassava, or foods made from roots	
4	DARK GREEN LEAFY VEGETABLES	Dark green/ leafy vegetables, including wild ones + locally available vitamin A rich leaves such as cassava leaves etc.	
5	OTHER	Other vegetables including wild vegetables	

	VEGETABLES		
6	VITAMIN A RICH FRUITS	Ripe mangoes, papayas + other locally available vitamin A rich fruits	
7	OTHER FRUITS	Other fruits, including wild fruits	
8	ORGAN MEAT (IRON-RICH)	Liver, kidney, heart or other organ meats or blood based foods	
9	FLESH MEATS	Beef, pork, lamb, goat, rabbit, wild game, chicken, duck, or other birds	
10	EGGS		
11	FISH	Fresh or dried fish or shellfish	
12	LEGUMES, NUTS AND SEEDS	Beans, peas, lentils, nuts, seeds, or foods made from these.	
13	MILK AND MILK PRODUCTS	Milk, cheese, yorghurt or other milk products	
14	OILS AND FATS	Oil, fats or butter added to food or used for cooking	
15	SWEETS	Sugar, honey, sweetened soda or sugary foods such as chocolates, sweets or candies	
16	SPICES, CONDIMENTS, BEVERAGES	Spices (black pepper, salt), condiments (soy sauce, hot sauce), coffee, tea, alcoholic beverages or local examples.	
			YES=1 NO=0

Did you eat anything (meal or snack) OUTSIDE of the home yesterday?

APPENDIX 3:FOLLOW-UP: Socio-Demographic and Nutrition questionnaire

Instructions to Research Assistant: Circle and/or write the correct response.

1. BASIC INFORMATION

1.1 Date of interview (dd/mm/yy) ☐☐ ☐☐ ☐☐

1.2 Study Site ☐

1.3 Study Group [1] ART group [2] Pre-ART Group ☐

1.4 Month of follow-up (e.g. 01=Month 1 after baseline) _____ ☐☐

1.5 Name and ID number of Research Staff conducting the interview

Name of Research Staff _____ Staff ID # ☐☐

1.6 Name of Study Participant _____

2. SOCIO-DEMOGRAPHIC INFORMATION

2.4 What is/was your main occupation in the last month? ☐

[1] Student

[2] Employed, unskilled labor (assistants in carrying objects, building, plumbing, cleaners etc)

[3] Employed, skilled labor (technician or vocational skills such as electrical, chemical, or mechanical including car repair,

[7] Casual worker/part-time

[8] Farmer (large scale, subsistent farming, gardening)

[9] Housewife

carpentry etc)

[4] Agricultural/forestry worker
[5] Business (self employed)
[6] Professional (Teacher, doctor, nurse, manager, accountant etc)
[10] Unemployed
[11] Attendant in hotels, guest house, bars, or clubs
[12] Other (specify) _____
_____
_____

3. SOCIO-ECONOMIC INFORMATION

3.1 In the last month, what was the income of your household (choose from the categories below in Kenyan Shillings)?

[1] less than 1000
[2] 1,000-2,999
[3] 3,000-4,999
[4] 5,000-9,999
[5] 10,000-19,999
[6] 20,000-49,999
[7] 50,000+

3.2 Currently, who are you living with?

[1] Alone
[2] Parents
[3] Spouse
[4] Children
[5] Other relatives
[6] Friends

3.3 Excluding yourself, how many people live with you in your house?

Total # _____ of people

3.4 Excluding yourself, specify number of people who live with you by their sex and age:

[1]	Adults > 18 years	Females:			Males:	
[2]	Children 5 – 18 years	Females:			Males:	
[3]	Children <5 years	Females:			Males:	

3.5. Excluding yourself, on average how many people eat at your house in a day? _____

3.6 What is the main source of food for your household? (**Choose one response only**)

[1] Purchase (market/grocery store)
[2] Household farm/garden
[3] Relatives and friends
[4] Welfare/NGO support
[5] Other (specify) _____
[88] Don't Know

3.7. What percent of food currently consumed is from the _____% above mentioned source?

3.8 How much money in Kenyan Shillings do you usually spend on buying food for one day in your household _____ (write 88888 if Don't know)

4. BEHAVIORAL AND HEALTH HABITS

4.1 Do you perform physical activity or exercise at least 2 times a week?

[1] No
[2] Yes

5. SOCIAL SUPPORT AND CARE

5.1 In the past month, have you received any social support and/or care from any person listed below? (read responses to the client)

[1] Spouse
[2] Parent
[3] Brother/Sister
[4] Other relatives
[5] Friend
[6] Social worker
[7] Nurse
[8] Doctor
[9] Own child(ren)
[10] Other (specify) _____

5.2 In the past month, have you received any of the following support from an individual or organization?

[1] Food
[2] Money to but food
[6] Transportation
[7] Cloth

[3] Money for rent and other expenses
[4] Money to buy meds
[5] Shelter (place to live)
[8] Encouragement (socially)
[9] Medicines
[10] Other (specify) _____
_____

6. ADHERENCE (COMPLIANCE) TO FOOD SUPPLEMENTS

Instructions to the Research Staff: Please do not be judgmental when asking clients about their compliance in taking food supplements. In most situations patients are truthful about taking the supplements if asked in a nonjudgmental way and are given a specific time frame.

Instructions to the study participant: "Now I would ask questions on how you have been taking the food supplements in the past one-month. Please be aware that everyone misses doses in some of the time. Be assured that this information will neither change the way you receive the food supplements nor your opportunity to participate in this study.

6.1 Did you eat the food supplement yesterday?
[1] No
[2] Yes

6.2 If you did not eat the food supplement yesterday, when was the last time you ate the food?
past _____ days
[88] Don't know
[98] Did not reply (no response)

6.3 How many days did you eat the food supplements during the past 7 days (last week)?
_____# days [88] Don't know

6.4 How many days did you eat the food supplements last month?
_____# days [88] Don't know

6.5 ***If the client missed a visit or several visits ask***. How many days did you eat the food supplements since last time you received the food supply from the study clinic?
_____# days [88] Don't know [99] NA

6.6 How many packets of food supplement did you receive during last study visit?

_____# food packets [88] Don't know

6.7 How many packets of food supplement have remained as of today?

_____# food packets [88] Don't know

6.8 Date the food was supplied (issued) (dd/mm/yy)

6.9 Did you share the food supplement with anyone in your family or outside your home on any day since last visit?

[1] No [88] Don't Know
[2] Yes [98] Did not Reply (no response)

6.10 If you shared the food supplement who was the person?

[1] Spouse [4] Neighbor
[2] Own Child(ren) [5] Friend
[3] Other member of family (specify) _____ [6] Other (specify) _____

6.11 What was the amount per day of the food that you shared?. (provide mls or percent of food shared per day)

_____mls of food shared per day

_____% of food shared per day [888] Don't know

6.12 Everyone misses the medication or food doses some of the time, in the last one month, how many meals of food supplement did you miss? Note: A dose constitutes a measured portion or amount of food supplement taken at any one time during the day.

_____# food meals (doses) missed [888] Don't know

6.13 If the patient has missed any dose of food supplement dose, give reasons

[1] Forgot to take the food
[2] Fear of side effects/toxicity
[3] Shared with others
[4] Don't like the taste
[9] Stock was finished
[10] Lost/misplaced the food
[11] Unable to go get another stock
[12] Was away from home (traveled)

[5] Felt better
[6] Too ill
[7] Out of water/fluid to mix food
[8] Run out of cooking fuel
[13] Fear of stigma/disclosure
[14] Other (specify) _____
_____

6.14 In the past one month, did you have any family or community member who supported (remind, encourage, or helped) you to prepare or take your food supplement?

[1] No [2] Yes

6.15 If yes, who was the person who supported you? (check one response only)

[1] Spouse
[2] Immediate member of family (specify) _____
[3] Nurse
[4] Doctor
[5] Social worker
[6] Friend
[7] Other (specify) _____

6.16 Do you feel that preparing and taking the food supplements fits into your daily schedule of activities?

[1] No [2] Yes [88] DK

6.17 Did someone (a health professional) discussed with you the importance of taking the food supplements correctly and timely?

[1] No [2] Yes [88] DK

6.18 What do you think would happen if you do not take the food supplements daily?

[1] Will not gain weight
[2] My health may deteriorate
[3] Other (specify) _____
[88] Don't Know

7. NUTRITIONAL AND CLINICAL ASSESSMENTS

Perform the following examinations and fill the data where indicated during the correct visit/month of follow-up. (check the study follow-up chart)

7.1 MUAC (cm) _____ ☐☐.☐

7.2 Weight (Kg) _____ ☐☐☐.☐

7.3 Height (without shoes) (cm) _____ ☐☐☐.☐

Analysis of body composition (Isotope Technique)

7.4 baseline _____ ☐☐☐.☐

75.5 post dose _____ ☐☐.☐

Thank you for taking time to participate in this study and answer these questions. Now, I am going to ask you few more questions regarding your dietary habit over the past 24 hours. This information will assess your recall on the food diversity.

Before, we start on a 24-hour recall on food diversity, I would like to thank you for taking time to participate in this study and answer these questions. Now, I am going to give you the date of your next appointment.

8. Date of next appointment (dd/mm/yy) ☐☐ ☐☐ ☐☐

APPENDIX 4

Preliminary preparations

Two samples of amaranth and maize flour found in the market were subjected to proximate analysis to determine their nutritional value. This information was used to calculate energy content in the preparation of both the intervention and control media.

Proximate Analysis

Proximate analysis was done on several samples of Amaranth and maize as found in the market. The results were as shown in **table P1.** PA – pure Amaranth,PM –pure maize, MA – maize/amaranth

Table P: nutrient content of amaranth and maize samples from the market

	PA1	PA2	PM1	MA2	PM1	MA1
Moisture (% w/w)	11.2	6.3	11.3	9.3	12.6	10.3
Fats (% w/w)	7.1	7.1	4.0	7.4	6.8	8.1
Ash (% w/w)	2.5	3.1	1.0	1.3	2.4	2.0
Protein (% w/w)	12.7	13.2	12.4	10.0	7.0	7.5
Carbohydratyes (%w/w) (By difference)	66.5	70.3	71.3	72.0	71.2	72.1
Energy (Kilojoules/100gm)	1524	1592	1478	1577	1491	1563

Energy calculations were done using the Labelling of Food Regulations conversion factor which is given as 15.7 Kj/g for carbohydrate, 16.7Kj/g for protein and 37.7Kj/g for fat. These calculations are tabulated below **(table P2)**.

Table P2: Energy calculations of market samples

Energy per 100g sample	PA1	PA2	MA1	MA2	PM1	MA
FATS	267.5	267.5	150.7	278.8	256.2	305.2
PROTEIN	212.6	221.0	207.6	167.4	117.2	125.6
CARBOHYDRATES	1044.0	1103.7	1119.4	1130.4	1117.8	1132.0
TOTAL	1524.1	1592.2	1477.7	1576.6	1491.2	1562.8

Analysis Procedures used for proximate analysis::

1. **Fat**

5g dried ground sample was weighed into an extraction thimble and extracted with petroleum ether (BP 40^0-60^0C) in a continuous Soxhlet extractor for 5 hours. The solvent was then removed from the extractor by evaporation and the residue dried at 100^0 C for 30 minutes, and then it was cooled, weighed and reported as fat.

2. **Ash**

5g of ground sample was weighed into a pre-weighed porcelain dish, placed in a muffle furnace and ignited at 550^0C until free from carbon-residue appears grayish white after about 8 hours. The dish and its contents were then removed from the furnace, placed in a dessicator until cool and weighed. The increase in weight of the porcelain dish was reported as ash.

3. **Moisture:**

About 2g ground sample was accurately weighed into a pre- weighed moisture dish and dried in an air oven at 130±3^{0}C for 1 hour. The dish was then transferred to a dessicator, was left to cool to room temperature (for about 30 minutes) and weighed. The loss in weight was recorded as moisture.

4. Crude Protein:

The Kjehdal macro method was used to analyze for crude protein. 0.7- 2.2g of the ground sample was digested with sulphuric acid in the presence of mercuric oxide as catalyst to convert the proteins into ammonium Sulphate. The digest was made alkaline with sodium hydroxide and ammonia distilled into standard acid (0.1N HCL). The percentage nitrogen was calculated and the result converted to crude protein by a multiplication factor of 5.7.

5. Carbohydrates:

Carbohydrates were calculated by difference rather than available carbohydrates which excludes undigested forms.