LEUCINE SUPPLEMENTATION IN THE MANAGEMENT OF MODERATE WASTING IN CHILDREN AGED 6 – 24 MONTHS

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This PhD thesis is my original work and has not been presented to any other institution for any degree award

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DEDICATION

Being the first doctoral candidate in my extended family I dedicate this thesis to them including my late grandparents. I trust this will encourage my family especially the younger members to embrace education so they can reach and surpass my achievements.

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ACRONYMS AND ABBREVIATIONS

µg	Micrograms
ºC	Degrees Celsius
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
APHRC	African Population and Health Research Centre
BCAA	Branched-Chain Amino Acid
CFU/gram	Colony-Forming Units/gram
Cm	Centimetres
EAC	East African Community
FAO	Food and Agriculture Organization of the United Nations
G	Grams
GCP	Good Clinical Practices
IEC	Ion Exchange Chromatography
IFAD	International Fund for Agricultural Development
ISO	International Organization for Standardization
Kcal	Kilocalories
KDHS	Kenya Demographic and Health Survey
Kg	Kilograms

KNBS	Kenya National Bureau of Statistics
Mg	Milligrams
Mg KOH/100 g	Milligrams of potassium hydroxide per 100 grams food sample
MG/KG/DAY	MG/KG/DAY – Milligrams per Kilogram Body-weight per Day
Ml	Millilitres
Mm	Millimetres
MUAC	Mid Upper Arm Circumference
NHANES	National Health and Nutrition Examination Survey
NNAP	National Nutrition Action Plan
OPD	Outpatient Department
RDA	Recommended Daily Allowance
RE	Retinol Equivalents
TEE	Total Energy Expenditure
TVC	Total Viable Count
UNICEF	United Nations Children's Fund
WFP	World Food Programme
WHO	World Health Organization
WHZ	Weight-for-height Z-score

OPERATIONAL DEFINITIONS

Dietary intake	The daily eating patterns of children including specific foods eaten, the frequency of feeding and the quantities consumed.
Effective treatment	A treatment regimen using leucine supplementation that treats moderate wasting proven through a clinical trial.
Effectiveness	Capacity of leucine supplementation to produce the desired effect under 'real-world' conditions (outside of a controlled environment).
Efficacy	Capacity of the leucine supplement to produce the desired effect under controlled conditions.
Meal	The food eaten by moderately wasted children including snacks during the course of a day.
Moderate wasting	This study considers moderate wasting as a form of acute malnutrition in children in which their weight-for-height Z-score was below – 2 but greater than or equal to -3 standard deviations from the median of the reference population or a MUAC equal to or greater than 115 mm but less than 125 mm.
Nutraceutical (TheraPEM)	A food that is prepared for use in the treatment of moderate wasting.
Therapeutic food	Prepared foods designed to meet the special nutritional requirements of children with moderate wasting

GENERAL ABSTRACT

Wasting in children is a serious form of acute malnutrition that affects approximately 370,000 children under five years of age in Kenya. While guidelines for treatment of severe wasting are available, moderate wasting has no standardized treatment protocol. Studies have established that when leucine (an amino acid), is administered in a large dose in catabolic conditions in humans, it functions as a nutraceutical that accelerates muscle protein synthesis. The main objective of the study was thus to establish the effectiveness of leucine supplementation in treating moderate wasting in children and formulate a leucine-rich composite flour (TheraPEM) from locally available foods.

This study had two phases: Phase One was a double blind placebo controlled trial at Mbagathi Level IV Hospital that established the effectiveness of leucine supplementation in treating moderate wasting. Data on the risk factors and dietary intake of children with moderate wasting was also collected. Children, while under observation, were either supplemented with leucine capsules at a dose of 150 mg/kg bodyweight/day or given a placebo for 28 days. Phase Two involved formulation of a leucine-rich composite flour from locally available foods beans, millet and groundnuts) for use in management of moderate wasting. Ethical approval to conduct the study was obtained from the University of Nairobi – Kenyatta National Hospital Ethics and Research Committee (P 519/7/2016).

At the end of Phase One, it was observed that energy intake among 134 (92.7 %) of the study children was below 50 % of their daily requirement. In addition, their mean energy intake was 250.6 ± 59.3 kcal/day which represented 31.3 % of their daily energy requirement. Factors that were independently associated with moderate wasting were: daily frequency of meal consumption (r=0.7; p=0.00), female-headed households (r=-0.6; p=0.02), polygamous households (r=-0.4; p=0.02) and a high household dependency ratio (r=-0.4; p=0.03). On completion of the clinical trial, there was a significant difference (p = 0.00) in the proportion of children who recovered from moderate wasting between the study group receiving 150 mg/kg/day leucine capsules (93.1 %) and the placebo group (40.3 %). It was therefore concluded that

leucine supplementation is effective in treatment of moderate wasting and its usage should be scaled up in the treatment of moderate wasting. For this to occur, a policy brief should be formulated from this study and submitted to the Ministry of Health in support of adoption of leucine supplementation in the management of moderate wasting including the recovery phase after treatment of severe wasting. It was also concluded that the frequency of feeding in the majority of moderately wasted children is adequate however the meals are of a low energy density and therefore result in undernutrition and wasting in children. It is thus recommended that recurrent nutrition education to mothers on complementary feed preparation should be implemented during the growth monitoring clinics they attend. In addition, the study recommends adoption of nutrient-optimized combinations of affordable and locally available foods in the preparation of child complementary feeds.

In Phase Two, six leucine-rich composite flours branded TheraPEM were formulated. All six met the Codex Alimentarius food standards for minimum energy density (80 kcal/100g) and maximum fat content (27 %). All flours delivered above 1050 mg leucine per 100 grams of flour. Formulations 2 (Beans 300g +Groundnuts 200g + Millet 500g), 3 (Beans 400g +Groundnuts 200g+ Millet 400g) and 5 (Beans 300g +Groundnuts 150g+ Millet 550g) had the most preferred sensory attributes, hence was subjected to accelerated shelf-life evaluation. All three flours had a significant change in the Total Viable Count from the pre-storage counts by the third day of storage. The results led to the conclusion that all six formulations meet the minimum leucine requirement (1050 g/100g) in moderate wasting and that formulation 2, 3 and 5 are the most acceptable based on sensory attributes. It is recommended that these three flours be subjected to a feeding trial to further validate their effectiveness in treating moderate wasting.

CHAPTER ONE: GENERAL INTRODUCTION

1.1 Background Information

Wasting in children represents a depletion of the body's fat and lean tissue mass brought about by an acute failure to receive sufficient macronutrients (carbohydrates, proteins and fat) which results in a low body weight compared to the child's height (WHO, 2005). The deficit in both fat and lean tissue occurs when energy intake is inadequate and the body resorts to body fat breakdown for energy production through the process of gluconeogenesis. Where the protein intake is inadequate then the muscles are broken down into amino acids to provide energy hence the observed atrophy of muscles contributing to wasting in children (WHO, 2012). Since lean tissues account for the largest body compartment, their rate of loss is the most significant determinant of total body weight in most cases of wasting (Fink, 2011). The lean tissues represent the fat-free, metabolically active tissues in the body including; the skeletal muscles, viscera, cells of the blood and the immune system. Wasting is classified as either moderate or severe depending on the severity and this is measured as the deviation of the weight of the child from that of the reference value in healthy children of the same height (WHO, 2012).

Wasting in children has been shown to impair the immune system and therefore it increases the risk of death from infectious diseases. In addition, episodes of wasting undermine children's physical as well as mental growth resulting in impaired motor and cognitive development. This compromises a child's learning ability and ultimately their school performance (WHO, 2012). To improve child survival, it is then important to invest in interventions that prevent wasting in children and those that ensure timely recovery from wasting. Despite there being a standard treatment protocol for severe wasting there is none for moderate wasting resulting in a high variability of treatment practices and low predictability of outcomes of the treatment (WHO, 2012).

Leucine, a branched chain amino acid, in high amounts has been shown to accelerate protein synthesis and growth by directly activating a critical compound in muscles called mammalian target of rapamycin (Ham, 2014). Therefore, besides providing building blocks for protein synthesis, leucine plays a significant role in accelerating protein synthesis and reducing protein breakdown in the muscles Larger doses of leucine have been shown to be significantly more effective at stimulating muscle protein responses than doses equivalent to the average dietary intake of leucine (Glynn, 2010). Leucine levels must be adequately high to function in signalling and metabolic roles since structural roles must be satisfied a priori, and leucine's capacity to signal and function as an oxidative substrate is based on sufficient intracellular concentration (Churchward-Venne, 2012).

1.2 Statement of the problem

Current statistical estimates show that the prevalence of wasting in children under five years of age in developing countries has progressively fallen however the absolute numbers are rising. Moderate wasting is a precursor to severe wasting which causes suboptimal child growth and development. This has been shown to increase the susceptibility, severity and duration of infectious diseases such as diarrhoea and pneumonia (Pelletier, 2013). Wasting therefore confers a risk of death nine times higher than that of children who are of weight-for-height Z-score above -1 (WHO, 2005). There is currently no consensus on the most effective way to treat moderate wasting due to poor treatment outcomes of currently used methods (WHO, 2014). For children under five years of age, the World Health Organization recommends the package of 'essential nutrition actions' for the prevention and treatment of moderate wasting. The package involves promotion of optimal breastfeeding, nutrition counselling on complementary feeding and prescription of nutrition supplements (WHO, 2014).

Lipid-based nutrient supplements and blended foods are the nutrition supplements prescribed in the management of moderate wasting but they do not result in recovery for majority of children (Lazzerini, 2013). It is against this backdrop that there is a need to develop innovative methods that are more precise in the treatment of moderate wasting to reduce the burden of the disease.

Leucine, a branched chain amino acid, has recently received attention as a potential nutraceutical because of its role in increasing muscle protein synthesis in humans and rodents. A higher leucine concentration than that typical of high-quality protein food sources was found to produce transient decreases in muscle protein breakdown and enhance Mammalian Target of Rapamycin signalling, which may induce protein synthesis in muscle (Dreyer, 2008). This therefore creates a need to study the effectiveness of leucine supplementation in the management of moderate wasting.

1.3 Study Justification

It is estimated that globally, at any given point in time there are 34 million children with moderate wasting with a recovery rate of only 13 % (WHO, 2014). The low rate of recovery is attributed to lack of a specific treatment protocol due to limited consensus among stakeholders on the most effective approach to treating or preventing moderate wasting. This study will contribute towards prevention and timely treatment of moderate wasting in children and thus help improve their physical and mental growth and development. This by implication will contribute towards reducing the incidence of diseases in children associated with malnutrition and hence reduction in infant mortality rate. This addresses Kenya's Vision 2030 Social Strategy that aims at lowering infant mortality rates.

The study is in line with the Ministry of Agriculture's Food and Nutrition Security Policy (FSNP) which addresses associated issues of malnutrition. The Kenya Food and Nutrition Security Strategy (NNAP,

2011) which aims at contributing to the realization of optimal nutrition in Kenya highlights fourteen priority nutrition areas to be addressed towards achieving the bigger nutrition agenda in Kenya. Key among the fourteen are; emergency management and recovery and long-term management. Based on the Kenya Food and Nutrition Security Strategy, The Kenya Nutrition Action Plan (NNAP, 2011) highlights eleven strategic objectives that should be addressed so as to realize the goal of promoting and improving the nutrition status of all Kenyans. This project addresses two among the eleven objectives.

These are: (i) to improve the nutritional status of children under 5 years of age and (ii) to prevent deterioration of nutritional status and save lives of vulnerable groups in emergencies (NNAP, 2011). In addition, this study has the potential of indirectly addressing stunting (low height-for-age) which is listed as an emerging issue for food and nutrition security in the National Food and Security Policy (NNAP, 2011). Episodes of wasting can contribute to stunting depending on the severity, duration and recurrence especially if there is inadequate nourishment to support recovery (Fink, 2011).

1.4 Study Objectives

1.4.1 Main objective

To determine the effectiveness of leucine supplementation in treating moderate wasting in children aged 6 – 24 months.

1.4.2 Specific objectives

1. To establish the household socioeconomic factors and child dietary intake associated with moderate wasting in children living within an urban residence.
2. To determine the effect of leucine supplementation on moderate wasting.

3. To formulate a leucine-rich therapeutic food (TheraPEM) from locally available foods for use in the treatment of moderate wasting.

1.5 Hypotheses

1. Household socioeconomic factors have no association with the children's energy intake.
2. Leucine supplementation has no effect on moderate wasting.
3. TheraPEM formulated from locally available foods will not be effective in the treatment of moderate wasting
4. TheraPEM will not have the nutrient content and sensory acceptability of a therapeutic feed for children to effectively treat moderate wasting.

CHAPTER TWO: LITERATURE REVIEW

2.1 Wasting in children

Wasting refers to a loss of body weight in relation to height due to a loss in fat and lean tissue mass resulting in a low weight-for-height z-score of less than -2 standard deviation from the median of the reference population. Wasting occurs due to inadequate intake of energy-giving foods (macronutrients) that results in breakdown of fat stores and lean mass in the body to produce energy. The breakdown in body stores gives wasted children the distinct emaciated appearance (Briend, 2015). Wasting is classified as either moderate or severe depending on the weight-for-height z-score, mid upper arm circumference (MUAC) and the presence or absence of bilateral pitting oedema. A child with moderate wasting has a weight for height z-score of less than minus 2 but greater than or equal to minus 3 and/or a MUAC equal to or greater than 115 mm but less than 125 mm with an absence of pitting oedema. In severe wasting, a child has a z-score of less than minus 3, a MUAC of < 115 mm and the presence of bilateral pitting oedema (WHO, 2005).

2.2 Prevalence of wasting

Statistical estimates indicate that moderate wasting affects more than 33 million children globally (WHO, 2013). A report by the United Nations Children Fund (UNICEF) and The Food and Agriculture Organization of the United Nations (FAO) on September 15, 2017 stated that 370,000 children in Kenya suffer from wasting, 80.4% of whom have moderate wasting. This figure was an increase of 7,000 children from the February 2017 figure of 363,000 children (FAO, 2017).

Wasting levels are highest among children in the age group 6 - 11 months at seven percent. This is because at this age, children are being introduced to complementary feeds and are more susceptible to infectious diseases. Wasting in Kenya is highest in the counties towards including: Mandera, Marsabit, West Pokot,

Garissa, Wajir, Turkana and Samburu especially among households in the lowest wealth quintile. More than 11 % of children in these counties are wasted and as shown in Table 2.1, this is classified as a serious level of public health significance. Turkana County has the highest prevalence of wasting (23 %) and this is classified as critical (KDHS, 2014).

Table 2.1: Wasting cut-off values for public health significance

Prevalence of wasting cut-off value	**Public health significance**
< 5 %	Acceptable
5 – 9 %	Poor
10 – 14 %	Serious
≥ 15 %	Critical

Adapted from (WHO, 1995)

2.3 Causes and contributing factors of wasting

Wasting represents an acute failure to receive sufficient nutrition due to a rapid deterioration in food supplies or because of a recent illness that inhibits nutrient intake and utilization or that accelerates nutrient losses especially through diarrhoea and vomiting (Black, 2008).

When the body does not receive sufficient nourishment from dietary sources, it results to breaking down of fat tissues in order to form free glycerol which is transported to the liver and converted to glucose for energy production. Food deprivation for an even longer duration of time causes breakdown of lean mass into free amino acids which are then also used for energy production. Cumulatively, the breakdown of fat and lean mass causes wasting where the child appears thin and emaciated (Black, 2008).

Undernutrition (wasting) and illness are strongly related in a vicious cycle (Figure 2). Wasted children have reduced lean tissue and fat mass and are susceptible to contracting infectious diseases as a results of a compromised immune response system which heightens the child's risk of disease. Fat mass releases several hormones including leptin which has been shown to stimulate the immune system. The reduction in immune system response due to reduced fat stores ultimately contributes to increased child morbidity and mortality rates. Leptin has been shown to have an effect on linear growth as it stimulates bone growth. This gives a possible explanation to why children who are wasted have compromised linear growth for as long as their weight-for-height Z-score is below -2 (Briend, 2015).

Infectious disease increases energy and nutrient needs of the child to fight the infection while reducing the child's appetite and nutrient absorption ultimately reducing nutrient intake and utilization. This aggravates the already existent undernutrition and perpetuates the cycle increasing the risk of death and permanent growth and developmental malformations (Katona, 2008).

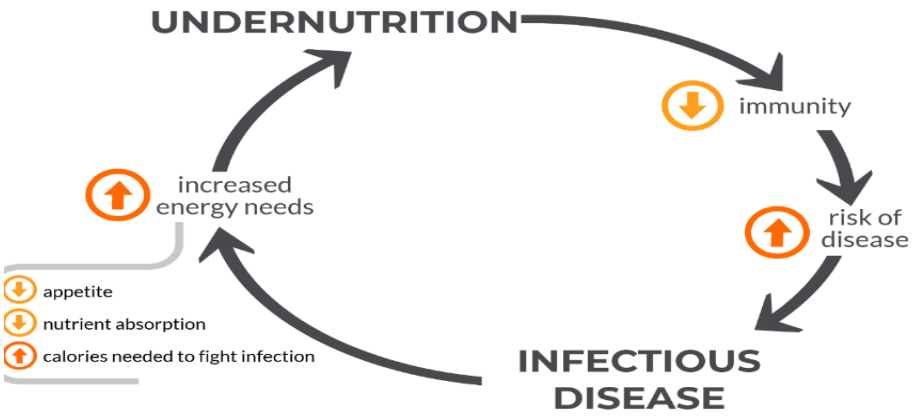


Figure 2.1: Vicious cycle linkage between disease and undernutrition

Adapted from (WHO, 2014)

Underlying causes of wasting include food insecurity where households lack adequate food quantities and dietary diversity resulting in monotonous diets of low energy and nutrient density. Other underlying causes include; insufficient child caring and feeding practices, limited access to effective, affordable and timely healthcare and residing in an unsanitary environment with inadequate access to clean water and hygiene services (WHO, 2014).

2.4 Treatment of wasting

Treatment of severe wasting requires the use of measures to correct fluid and electrolyte imbalances, and replenish calories, proteins and micronutrients (Baron, 2009). Treatment starts with modest amounts of calories and proteins based on a person's actual weight to avoid complications with simultaneous administration of minerals and vitamins. Administering water and sodium with carbohydrates can overload a heart that has already been weakened due to malnutrition resulting in congestive heart failure (Porth et al, 2011).

In the treatment of moderate wasting however, there is no definitive consensus on the most effective treatment protocol (Lazzerini, 2013). As such, different approaches are used to address moderate malnutrition with prepared foods that include: providing lipid-based nutrient supplements or blended foods, either a full daily dose or in a low dose as a complement to the usual diet. A study done to evaluate the safety and effectiveness of these specially formulated foods for children with moderate wasting concluded that both lipid-based nutrient supplements and blended foods have limited effectiveness in treating children with moderate acute malnutrition. In addition, lipid-based nutrient supplements do not reduce mortality, the risk of default or progression to severe acute malnutrition and they also induced more vomiting (Lazzerini, 2013). These poor outcomes that are attributed to the high variability and low

effectiveness of current treatment methods of moderate wasting justifies the need to explore other alternatives, including leucine, as an effective way to manage the condition.

2.5 Physical and chemical properties of leucine

Leucine is a branched-chain amino acid that occurs in the aliphatic R groups of amino acids which are nonpolar and hydrophobic (Nelson, 2005). It has aliphatic side-chains with a branch (a central carbon atom bound to three carbon atoms) and is stable under standard conditions of temperature (0° Celsius) and pressure (1 Atmosphere) (Nelson, 2005). As shown in Table 2.2, leucine occurs naturally in several food sources of both animal and plant origin. Foxtail millet grains have the highest amount of leucine (1764 mg/100g of food; 1044 mg/g total nitrogen) among the listed foods while custard apple, a fruit, had the lowest amount of leucine (106 mg/100g of food; 311 mg/g total nitrogen). Meat (beef, chicken and fish) and legumes (beans and groundnuts) are the richest sources of leucine.

Table 2.2: Locally available food sources of leucine

Food	**Leucine**	
	Mg/g total nitrogen	**mg/100g food**
Beef	507	1435
Chicken	460	1472
Fish (all types)	480	1445
Whole chicken egg	551	1091
Pasteurized cow milk	782	430
Bean (*Phaseoulus vulgaris*)	476	1685
Groundnut (*Voandzeia subterranea*)	489	1385
Foxtail millet (*Setaria italica*)	1044	1764
Custard apple (*Annona senegalensis*)	311	106
Alternanthera (*Alternanthera maritima*) leaves	500	375

Adapted from (FAO, 1981)

Leucine has a molecular formula of $C_6H_{13}NO_2$ (Figure 2) where the percentage composition of carbon is 54.94 %, hydrogen is 9.99 %, nitrogen is 10.68 % and oxygen is 24.39 %. It has a molecular weight of 131.18 g/mol while its density is 1.17 g/cm^3. It has a melting point of 293 – 295 degrees Celsius but has no boiling point. When isolated, leucine appears as a crystalline powder that is odourless and bitter in taste (Greenstein, 1961).

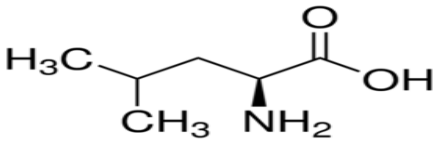


Figure 2.2: Chemical structure of leucine

2.6 Role of dietary leucine in the body

Leucine provides building blocks for muscle protein synthesis and also plays a significant role in accelerating the process and reducing protein breakdown in the muscles (Ham et al, 2014). Muscle protein synthesis is triggered by hydrolysis of all intracellular and extracellular proteins causing them to be broken-down to their constituent amino acids and replaced by new synthesis (Atherton, 2012). However, when the process of protein breakdown is accelerated due to several catabolic conditions including protein energy malnutrition it results in muscle mass loss (Atherton, 2012).

During states of insufficient caloric intake, the breakdown of cell proteins, particularly in skeletal muscle, increases to provide the body with amino acids essential for gluconeogenesis, energy production and new protein synthesis. This acceleration of protein breakdown resulting in muscle wasting. Leucine supplementation has been shown to be an effective strategy in increasing muscle protein synthesis in humans and rodents (Gallagher, 2007). Leucine supplementation activates messenger ribonucleic acid

translational machinery through mammalian target of rapamycin in an insulin dependent or independent process. The signalling of mammalian target of rapamycin is activated by insulin-like growth factor-I and insulin. These factors activate P13-k pathway and Akt resulting in mammalian target of rapamycin phosphorylation (Wang, 2006).

The phosphorylation of mammalian target of rapamycin, results in up-regulation of protein translation through the phosphorylation of the eukaryotic initiation factor 4E binding protein 1 (4E-BP1) and the ribosomal protein S6 kinase (S6K) leading to cell growth and proliferation (Gallagher, 2007; Shen, 2005; Hara, 2002). Unlike insulin and insulin-like growth factor-I, leucine has a direct effect at an intracellular locus modulating protein signalling pathways. Further, leucine does not appear to need the mediation of a cell membrane receptor (Beugnet, 2002), acting efficiently in the protein synthesis.

2.7 Use and safety of leucine supplementation

Branched-chain amino acid supplements and particularly leucine supplements are popular among athletes especially strength training athletes because of its implication as the key amino acid involved in stimulating muscle protein synthesis (Crozier et al, 2005; Nair, 2005).

It has been perceived that leucine improves athletic performance and endurance and increases lean body mass (Verhoeven, 2009). It is recommended that when chronic ingestion studies are designed, leucine intake levels should be in the range of 250–300 mg/kg bodyweight/day (Pencharz. 2012). An intake of greater than 500 mg/kg bodyweight/day could potentially increase the risk of adverse events and therefore is proposed as the upper limit for leucine (Cynober et al, 2012). A study done by Aschkenasy, et al (1979) on rats showed that a dietary leucine overload (3 % of the dry weight of the diet for 2 weeks and 7% for 6 weeks) induces a sharp decrease of the production of rosette and plaque forming cells in the spleen. In

rats that were maintained on diets that provided only minimally adequate amounts of tryptophan and niacin, an addition of 1.5 % leucine led to the depletion of tissue nicotinamide nucleotides causing pellagra. This therefore suggests that people living on a low level of protein nutrition and with only a marginally adequate intake of tryptophan and niacin may be at risk of developing pellagra if they have an excess intake of leucine (Barrett, 1985).

2.8 Gap in knowledge

Despite previous studies on leucine, none has investigated its effect on moderate wasting in children. In addition, studies have documented increases in muscle protein synthesis but they have not observed the change in body weight and nutrition status.

CHAPTER THREE: LEUCINE SUPPLEMENTATION IN THE MANAGEMENT OF MODERATE WASTING: A REVIEW ARTICLE

Abstract

Globally, wasting accounts for 4.7% of all deaths of children under 5 years of age globally. Currently there is no standard for treatment of moderate wasting in children resulting in high variability of treatment methods and low predictability of recovery outcomes hence the need to study treatment alternatives. Leucine, a branched chain amino acid, has recently received significant attention as a therapeutic agent for the treatment of numerous muscle wasting conditions. This is attributed to its ability to accelerate protein synthesis and reduction of protein breakdown in the muscles. The objective of this review was to establish the potential of leucine as a therapeutic agent in the treatment of moderate wasting. Based on defined key words a search was carried out on Pubmed that retrieved 47 articles on leucine supplementation and muscle protein synthesis. Only studies that met the search criteria were retrieved and the required data obtained. Eight unique studies that met the study criteria were included. The publications were analysed qualitatively to establish whether leucine supplementation had any effect on muscle protein synthesis and protein break down. Dosage levels used in the studies if available were also duly noted. The articles reviewed indicate that leucine supplementation either led to enhanced protein synthesis or reduced muscle mass loss in both healthy participants and participants with wasting conditions. Leucine supplementation is a safe and effective way to enhance muscle protein synthesis and reduce loss of lean mass in catabolic conditions. Given the low effectiveness of current therapeutic feeds used in the management of moderate wasting, leucine supplementation should be given significant consideration as a potential strategy for treating the condition.

3.1 Introduction

Wasting represents a depletion of the body's lean tissue caused by starvation or illness and if it is not adequately managed it can compromise growth and development and increase a child's risk of death from infectious diseases (Muller, 2005). When the weight for height Z-score of a child drops below minus two standard deviations from the median of the reference population, then that child is considered to be wasted. Wasting is classified as either: moderate, when the weight for height Z-score is greater than or equal to minus three but less than minus two, or severe where the Z-score is less than minus three (Muller, 2005). Globally, wasting accounts for 4.7 % of all deaths of children under five years of age (Kouanda, 2009). This is because wasted children are 11 times more likely to die than healthy children (Kouanda, 2009). It is estimated that at any given point in time in the world, 52 million children under the age of five years are wasted, with 17 million of those being severely wasted. These prevalence has not declined in the past 10 years and the world is therefore not on target to address Target 2.2 of the Sustainable Development Goals which is to reduce the prevalence of child wasting (UN, 2019). The World Health Assembly target is to reduce and maintain levels of childhood wasting to below five percent (WHO, 2014). In Kenya, four percent of children under the age of five years are moderately wasted and one percent are severely wasted. Wasting levels are highest among children in the age groups 6 – 8 months and 9 – 11 months (each seven percent). In this period, children are being introduced to complementary feeds and are more susceptible to illnesses. Wasting in children is highest among households in the lowest wealth quintile, as well as in the counties towards the north of the country including: Mandera, Marsabit, West Pokot, Garissa, Wajir, Turkana and Samburu. More than 11 percent of children in these counties are wasted with Turkana having the highest prevalence at 23 percent (KDHS, 2014).

Lipid-based nutrient supplements (LNS) such as Ready-to-Use Therapeutic food (RUTF) (Briend, 1999; Briend 2001) are the standard of care for severely wasted children and are also commonly used in moderate wasting. (Thakwalakwa, 2010) LNS refers to energy-dense pastes which characteristically contain carbohydrates, proteins and micronutrients embedded in a lipid base. (Briend, 1999) Sugar is often used at high concentrations in food supplements given to manage moderate wasting. This is a concern for both lipid-based nutritional supplements as well as blended flours. Foods with high sugar content have negative effects on dental health. They can also promote among young children a taste for sweet sugary foods that may negatively affect the acceptability of other local foods. (Thakwalakwa, 2010)

In the case of moderate wasting there is no definitive consensus on the most effective way to treat it and therefore different approaches are currently used to manage the condition (Lazzerini, 2013). Cereal and legume blends are recommended for supplementary feeding in moderately wasted children. (Dijkhuisen, 2000) Corn-soy blend (CSB), a blend of maize and soy flour, is also promoted as a supplementary feed for moderately wasted children as well as a complementary feed for primary prevention of undernutrition. (Thakwalakwa, 2010) These blended flours were designed more than three decades ago when emphasis was on the provision of large quantities of energy and protein. In a study done to determine the efficacy of CSB and LNS in the treatment of moderate wasting in children over a 12-week period, they did not lead to recovery for any of the study children at the end of the trial period. (Thakwalakwa, 2010) Lipid-based nutrient supplements also do not reduce mortality, the risk of progression to severe acute malnutrition and they also induce more vomiting (Lazzerini, 2013).

These poor treatment outcomes attributed to the high variability and low effectiveness of current management methods justifies the need to study other alternatives including leucine as an effective way to manage the condition. Leucine, a branched chain amino acid, has recently received significant attention as a therapeutic agent for the treatment of several muscle wasting conditions. Since lean tissues account

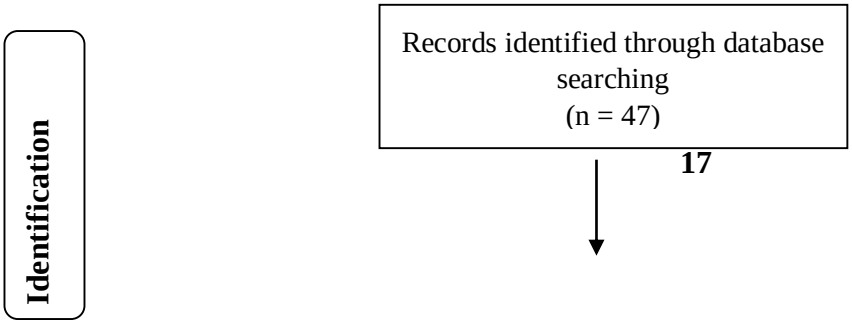
for the largest body compartment, their rate of loss is the most significant determinant of total body weight in most cases of protein energy malnutrition (Porth, 2011). The objective of this study was to therefore establish if leucine could be used as a therapeutic agent in the treatment of moderate wasting.

3.2 Materials and methods

Published material which covered leucine and protein synthesis were obtained on Pubmed from October 5th – 30, 2015. Articles were reviewed to establish whether there is any significant association between leucine supplementation and muscle protein synthesis. Literature search was done using the following keywords; 'leucine and protein synthesis', 'leucine and muscle wasting' and 'leucine supplementation' From the above search criteria 47 articles were retrieved and screened using the study selection criteria to ensure they were relevant to the study.

3.2.1 Inclusion criteria

Articles related to leucine usage in humans and rodents or their cultured tissues were included. Articles that did not discuss leucine in relation to muscle protein synthesis, those that were a review of other publications and where the statistical analysis used was not suitable were all excluded. As shown in Figure 3.1, out of the 47 articles retrieved 39 were excluded because they did not meet the aforementioned requirements leaving eight publications to be reviewed. The publications reported on eight studies from four countries. A form was developed to ensure that all relevant data from each of the studies was extracted. Data required included; the author of the study, the year the article was published, study group/study materials involved, study design, most effective leucine dosage used and the conclusion. This was followed by data synthesis where data from the eight studies was summarized narratively.



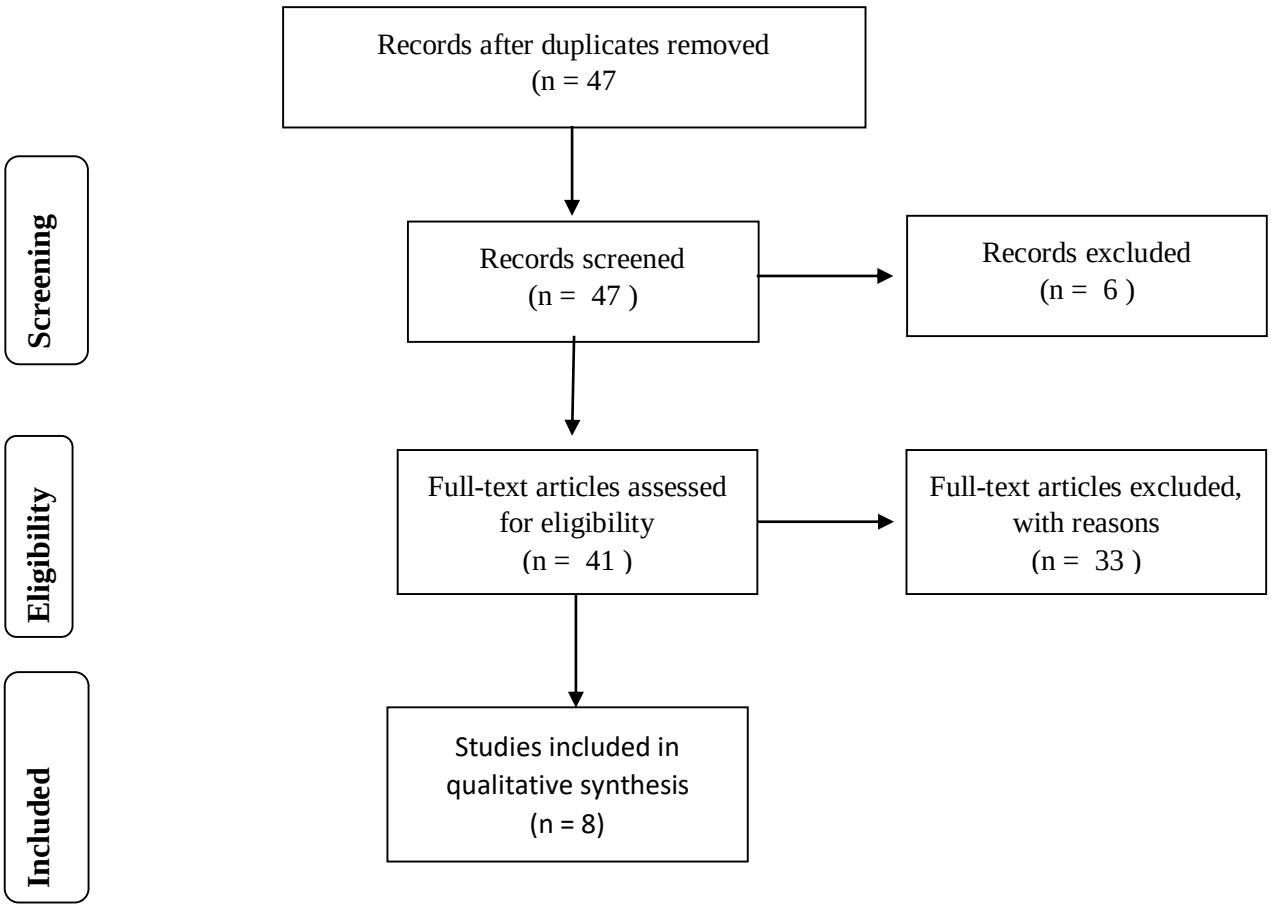


Figure 3.1: Sampling schema for selection of studies

3.3 Results

Table 3.1 shows key information obtained from the studies that were reviewed This included: the year and country of publication, the authors, the study group or materials used, the study design, leucine dosage used (where available) and the conclusion. From the studies reviewed, three were conducted on rats with varying characteristics, one was conducted on adults, one on premature infants, one on children with cystic fibrosis and the rest on cultured cells. Majority of the studies were conducted in both the USA and Netherlands (three each). There were no studies related to the research topic conducted in Africa and Asia. Majority of the articles reviewed were randomized controlled trials (six) with the rest being experimental

lab-based studies. In all the studies, it was noted that leucine supplementation either led to enhanced protein synthesis or reduced muscle mass loss.

The earliest study attempting to relate leucine and muscle protein was published by Kien L.C. et al in 1999. The study involved children with cystic fibrosis, a condition that causes poor weight gain and growth (synonymous with moderate wasting). Study results showed that leucine supplementation reduced protein breakdown in the children however the change in weight or other anthropometric measures was not documented. At a dosage of 2.4 g/kg/day, leucine supplementation resulted in increased protein synthesis in premature infants causing an anabolic state in the body. In a study published by Anthony J.C in 2000 involving rats that were food-deprived and hence wasted, leucine supplementation at a dosage of 270 mg stimulated protein synthesis in the muscles. This is consistent with what Crozier S. J observed in 2005 in a study performed on healthy lab rats using a leucine dosage of 1.35 g/kg/day. When studied on cancer cachectic mice by Peters S.J in 2011, leucine reduced the muscle wasting. At a dosage of five grams' leucine supplementation enhanced myofibrillar protein synthesis in the skeletal muscles of young male strength athletes, who were engaging in resistance training to enhance muscle strength and maximise muscle hypertrophy

Table 3.1: Studies done associating leucine supplementation and protein synthesis

Number	Year	Country	Author	Study group/study materials	Study design	Conclusion	Leucine dosage used
1	2014	Switzerland	Churchward-venne T.A et al	Young male strength athletes	Double-blind randomized trial	Leucine supplementation enhanced myofibrillar protein synthesis	5 gms
2	2012	Netherlands	Haegens A. et al	Cultured C2C12 skeletal muscle cells	Experimental lab-based	Leucine supplementation increased myofibrillar protein accretion	500 µM
3	2011	Netherlands	Peters S.J., et al	Cancer cachectic mice	Randomized control trial	Leucine supplementation reduces muscle	Not specified

						wasting in cancer cachectic mice	
4	2010	Brazil	Baptista I.L. et al	Immobilized hind limbs	Experimental lab-based	Leucine supplementation attenuated muscle mass loss caused by immobilization	Not specified
5	2006	Netherlands	Van der Akker C.H.P et al	Premature infants	Randomized control trial	Leucine supplementation resulted in anabolic state due to increased protein synthesis	2.4 g/kg/day
6	2005	USA	Crozier S.J. et al	Lab rats	Randomized control trial	Leucine supplementation stimulated muscle protein synthesis	1.35 g/kg/day
7	2000	USA	Anthony J.C	Food-deprived Lab rats	Randomized control trial	Leucine supplementation stimulates protein synthesis in the muscles of food-deprived rats	270mg
8	1999	USA	Kien L.C. et al	Children with cystic fibrosis	Randomized control trial	Leucine uptake resulted in reduced protein breakdown.	Not specified

3.4 Discussion

Muscle protein synthesis is triggered by continuous hydrolysis of all intracellular and extracellular proteins causing them to be broken-down to their constituent amino acids and replaced by new synthesis (Briend, 1999). However, when the process of protein breakdown is accelerated due to several catabolic conditions including wasting, it results in muscle mass loss (Briend, 1999). During states of insufficient caloric intake, the breakdown of cell proteins, particularly in skeletal muscle, increases to provide the body with amino acids essential for gluconeogenesis, energy production and new protein synthesis. This acceleration of protein breakdown resulting in muscle wasting mainly occurs due to activation of the ubiquitin (Ub) proteasome pathway (Briend, 1999).

Leucine supplementation has been shown to be an effective strategy in increasing muscle protein synthesis in humans and rodents (Datta, 1999) even in states of muscle wasting. Leucine supplementation activates

messenger ribonucleic acid translational machinery through mammalian target of rapamycin in an insulin dependent or independent process. The signalling of mammalian target of rapamycin is activated by insulin-like growth factor-I and insulin. These factors activate P13-k pathway and Akt resulting in mammalian target of rapamycin phosphorylation (Rommel, 2001). The phosphorylation of mammalian target of rapamycin, resulting in up-regulation of protein translation through the phosphorylation of the eukaryotic initiation factor 4E binding protein 1 (4E-BP1) and the ribosomal protein S6 kinase (S6K) leading to cell growth and proliferation (Datta, 1999; Gallagher, 2007; Hara, 2002). Unlike insulin and insulin-like growth factor-1, leucine has a direct effect at an intracellular locus modulating protein signalling pathways.

Further, leucine does not appear to need the mediation of a cell membrane receptor (Beugnet, 2002), due to its efficiency in protein synthesis. As seen in Figure 3.2, once in the body, leucine undergoes degradation mainly in the skeletal muscle where it is transaminated by branched chain amino acid transaminase. This yields α-ketoisocaproate which.is then decarboxylated and dehydrogenased by branched chain α-keto acid dehydrogenase. The resulting metabolite, isovaleryl-CoA undergoes a dehydrogenation reaction by isovaleryl-CoA dehydrogenase yielding isopentenyl-CoA. Biotin-dependent carboxylation yields methylglutaconyl-CoA. Addition of water by methylglutaconyl-CoA hydratase yields HMG-CoA. HMG-CoA lyase then splits HMG-CoA to acetyl-CoA and acetoacetate (Holmes, 1995).

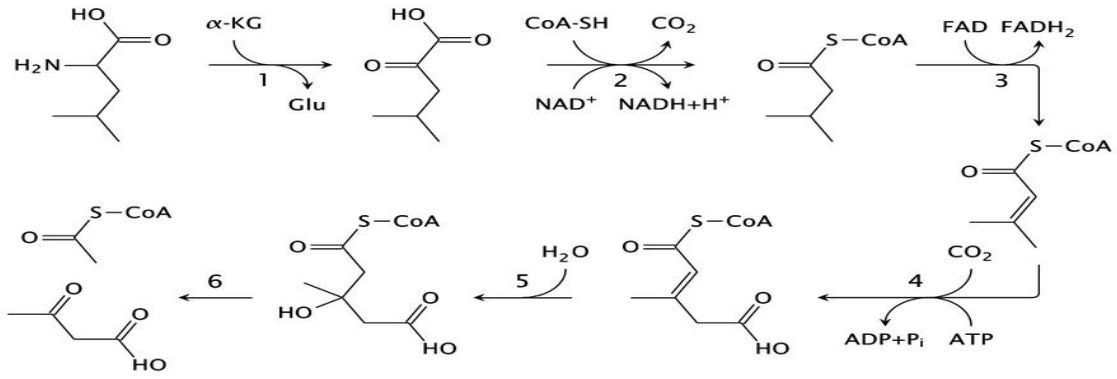


Figure 3.2: The pathway of Leucine degradation

When considering the safe intake level of leucine, 500 mg/kg/day is proposed as the upper limit (Cynober, 2012). This is because, with increasing intake amounts of leucine, a clear dose response in leucine oxidative capacity, measured as $F13CO_2$ from the oxidation of l-[1-13C] leucine, is observed. Significant increases in blood ammonia concentrations are observed at leucine intakes greater than 500 mg/kg^{-}/day.

At a leucine intake of 250 mg/kg^{-}/day, the mean fed-state ammonia concentrations are within normal limits, but at a leucine intake of 500 mg/kg^{-}/day, the mean ammonia concentration is beyond the normal limits (WHO, 2000). Therefore, when chronic ingestion studies are being developed, leucine intake levels should range between 250–300 mg/kg^{-}/day (Pencharz, 2008). In addition, one must also put into consideration the protein content of the diet when considering the safe intake level of leucine (Imamura, 2013). The relative amount of branched-chain amino acids in the diet is the main determinant of the adverse effects of excessive leucine (Imamura, 2013; Aschkenasy, 1979). Excess dietary leucine induces a secondary drop in the reserves of the structurally analogous antagonists. valine and isoleucine and the addition of one or preferably both of these branched-chain amino acids has been shown to reduce these

effects of leucine (Imamura, 2013). While there have been no adequate studies on the best ratio of Leucine to isoleucine to valine in leucine supplements most commercially available products have these branched-chain amino acids in a ratio of 2:1:1 per serving size.

3.5 Conclusion and recommendation

Leucine supplementation is an effective way to enhance muscle protein synthesis and reduce loss of lean mass in animals and humans. Given the low efficacy of current therapeutic feeds in the management of moderate wasting, leucine supplementation should be given significant consideration as a potential strategy for treating the condition.

CHAPTER FOUR: SOCIOECONOMIC AND DIETARY DETERMINANTS OF MODERATE WASTING IN CHILDREN LIVING IN URBAN RESIDENCE IN KENYA: A CASE STUDY OF NAIROBI COUNTY

Abstract

In Nairobi County, 60 % of the population live on under one US Dollar per day and occupy informal settlements which represent 6 % of the land area. Informal sector jobs are the urban poor's main source of income which often does not meet the household consumption needs. Millions of children living in these urban slums thus experience both chronic and acute malnutrition including wasting due to food insecurity. The study sought to generate information on the most significant factors associated with moderate wasting in children living within an urban residence. A cross sectional study was conducted at Mbagathi County Hospital in Nairobi between 8th May – 5th August 2017 on 144 moderately wasted children aged 6 – 24 months in outpatient care. The catchment area for study participants was Nairobi County. Household socioeconomic and child dietary intake data was collected from child caregivers. The mean number of household members was 4.64 while the mean dependency ratio was 1.5 (3:2) which was higher than that of Nairobi County (0.47) (1:2). The mean frequency of meal consumption among study children was 5.3 ± 1.9 meals/day however the mean energy intake was 250.6 ±59.3 kcal/day representing 31.3 % of their energy requirement. Factors that were correlated to energy intake were; the frequency of meal consumption (r=0.7; p=0.00), the household dependency ratio (r=-0.4; p=0.03), sex of the household head (r=-0.6; p=0.02) and the marital type (r=-0.4; p=0.02). The study thus concluded that polygamous and female-headed households as well as households with a high dependency ratio are likely to have a child with moderate wasting. In addition, the frequency of feeding for most children is adequate but the meals lack adequate energy density causing undernutrition which can lead to moderate wasting.

The study recommends for research to be done on precision interventions targeted at polygamous and female-headed households as well as those with a high dependency ratio to prevent moderate wasting occurring in children. The study also recommends adoption of nutrient-optimized combinations of affordable and locally available foods in the preparation of child complementary feeds.

4.1 Introduction

Wasting refers to a low weight-for-height Z-score indicative of a current severe weight loss commonly associated with acute starvation and/or illness. It is classified based on the level of severity as either; moderate, when the weight for height Z-score is greater than or equal to minus 3 but less than minus 2 (-3≤ Z-score<-2), or severe where the Z-score is less than minus 3 (Z-score < -3) (WHO, 2005). Children who have any form of wasting are at a risk of illness and/or death that is nine times higher than that of children with a weight-for-height Z-score above -1 (WHO, 2005). The prevalence of wasting in children typically peaks during the second year of life (WHO, 2009). In the year 2016, wasting affected globally the lives of nearly 52 million children (7.7 %) under the age of five years, 14 million of whom were living in Africa (UNICEF/WHO/World Bank Group, 2017). In Kenya, 280,000 (4 %) children are wasted with 210,000 (3 %) being moderately wasted (FAO, 2017). In Nairobi, the most populous County in Kenya, approximately 46,375 (2.5 %) of children are moderately wasted (KDHS, 2014) however prevalence is higher in the slums than in non-slum areas (Mohiddin, 2012). A prevalence of wasting higher than 5 % is considered an emergency due to an associated concurrent increase in child mortality (Toole, 1992).

The causes of malnutrition (including wasting) have been well articulated in the UNICEF conceptual framework for the causes of malnutrition and are divided into three levels; basic causes, underlying causes

and immediate causes (UNICEF, 1990). This framework covers a broad spectrum of physiological, environmental, cultural, sociological, economic and political causes of malnutrition. It however only provides a guide for what to look out for when identifying the causes of malnutrition within different contexts and therefore does not show exact relationships (UNICEF, 1990). The aim of the study was to contribute to enhanced management of undernutrition in children while the purpose was to generate information on the most significant factors associated with moderate wasting in children living within an urban residence.

The urban population in Kenya has been progressively growing over the years and it currently represents 26.7 % of the total population in the country. This is attributed to the economic growth Kenya, a developing country, is experiencing that draws youthful populations from rural areas to urban settlements in search of employment. Despite the benefits attributed to urbanization including higher incomes, better social services, economic and investment opportunities it also brings about socioeconomic and environmental challenges (Mohiddin, 2012). In Nairobi, 60 % of the population occupies only 6 % of the land area, most of this being in the city slums that include Kibera, Mathare and Mukuru (APHRC, 2012). In a study done by Olak B *et al*. (2011) in children aged between 6 – 59 months living in Nairobi slums, more than 47 % were reported to experience both chronic and acute malnutrition due to food insecurity. This is because the quantity and diversity of urban household diets is mainly dependent on the purchasing power of the household.

The urban poor's main livelihood is informal sector jobs which often do not meet the consumption needs leading to adoption of coping mechanisms including incurring debts to facilitate food purchases. Women in particular have irregular and insecure jobs, which offer limited social benefits (Mohiddin, 2012). This

directly affects child care practices including breastfeeding and infant and young child feeding (Mohiddin, 2012). Ultimately, the quantity and quality of the nutrient intake of children is compromised making them vulnerable to malnutrition and associated complications.

The main objective of this study was to therefore establish the household socioeconomic factors and child dietary intake associated with moderate wasting in children living within an urban residence. The information generated will assist in early detection of children at risk of developing moderate wasting. In addition, risk factors of significant importance identified can then be the focus of programme interventions whose expected outcomes include reducing the prevalence of moderate wasting among children living in urban residences. In Kenya, most interventions targeted at food insecurity and malnutrition focus on rural areas. This could however be misdirected since most poor people in the country live in urban areas (Mohiddin, 2012). In addition, the prevalence of wasting in urban areas is similar to that in rural areas (Ruel and Garrett, 2004).

4.2 Materials and Methods

4.2.1 Study Design and study setting

A cross sectional study was conducted at Mbagathi County Hospital (Figure 4.1), a level four hospital, in Nairobi County between 8th May, 2017 – 5th August, 2017 to collect household socioeconomic and dietary intake data among moderately wasted children.

The catchment area of study participants was Nairobi County which hosts the capital city of Kenya (Nairobi). The county, located 1.2921° south and 36.8219° east at an altitude of 1.78 km above sea level, sits on an area measured at 695 km². The County has a population size of 3,138,369 and a population

density of 4,515/km², the highest in Kenya. The county has a total of 17 parliamentary constituencies and 85 wards.

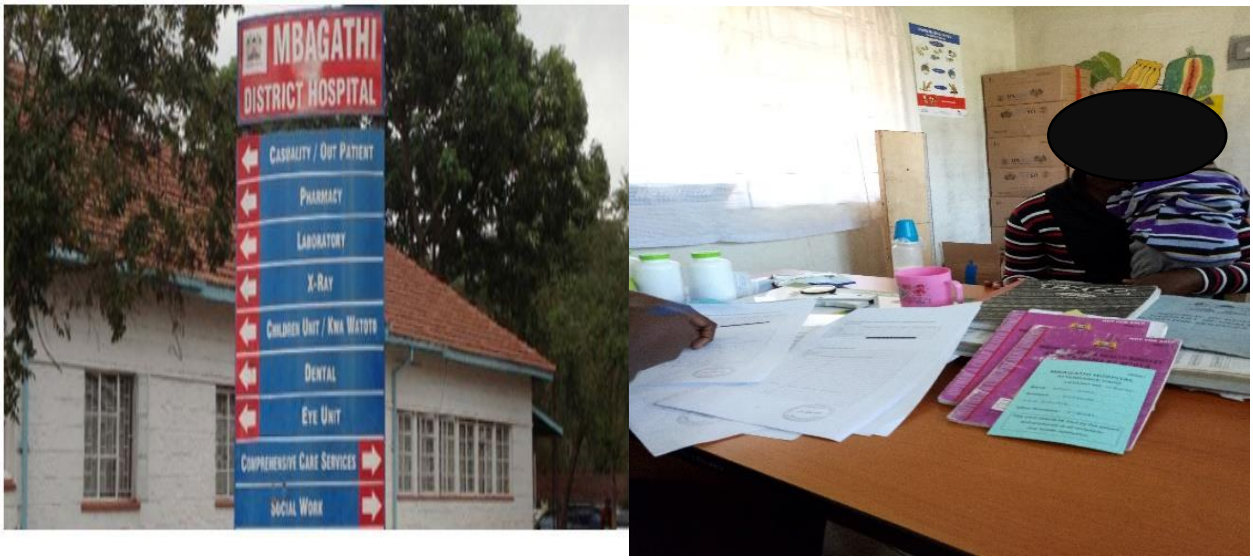


Figure 4.1: Mbagathi County Hospital (left) and on-going data collection at the Outpatient Therapeutic Program clinic (right)

Figure 4.2: Google map showing the position of Mbagathi County Hospital within Nairobi County

4.2.2 Study population

The study population comprised children aged 6 – 24 months with moderate wasting attending Mbagathi County Hospital and the study selection criteria formed the basis of recruitment. The inclusion criteria were children aged 6 – 24 months with a weight-for-height Z-score of less than -2 but greater than or equal to -3. Children with physical and mental disabilities as well as those with conditions that may affect nutrition status (diabetes, cerebral palsy, cancer, HIV/AIDS, epilepsy and congenital heart problems) were excluded. In addition, children who, for at least three months prior to the start of this study, had not been living in Nairobi and/or had participated in a study/pre-test were also excluded. Caregivers of the children had to give written consent prior to child enrolment in the study. Authority to review patient records was obtained from Mbagathi County Hospital (Appendix 8) to detect chronic childhood diseases that may lead to wasting. The preferred method of age verification was scrutiny of the child's – Mother Child Booklet obtained from the caregiver. Written consent was then obtained from the caregivers of the children prior to their enrolment into the study.

4.2.3 Sampling Procedure

Nairobi County was purposively selected to represent an urban residence in Kenya. Mbagathi Hospital was also purposively selected out of the three Nairobi County public hospitals because it specializes in the prevention and treatment of malnutrition in both children and adults through its outpatient therapeutic programme and supplementary feeding programme. A sample size of 144 children was computed using the formula by Chow (2008).

$$n_A = \kappa n_B \text{ and } n_B = \left(1 + \frac{1}{\kappa}\right)\left(\sigma \frac{z_{1-\alpha} + z_{1-\beta}}{\mu_A - \mu_B - \delta}\right)^2$$

Where:

K = n_A/n_B as the matching ratio = 1

σ is the standard deviation = 0.76

α is the Type 1 error = 0.05

β is type II error. = 0.1 Therefore power is 1 – 0.1 = 0.9

δ is the testing margin = 2

μ_A is the Group A mean = 0

μ_B is the Group B mean = -0.58

A referral system was established in the outpatient department (OPD) at Mbagathi Hospital where children found to be moderately wasted were referred to the study. As shown in Figure 4.3, exhaustive sampling was used where all children who met the selection criteria were enrolled in the study until the calculated sample size was met. Study child enrolment was conducted between 8AM and 5PM on all days apart from weekdays and holidays.

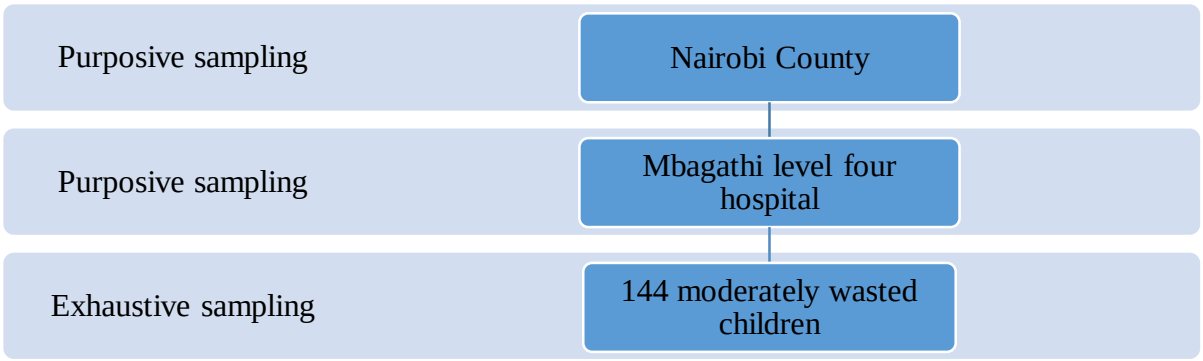


Figure 4.3: Sampling schema for study children recruitment

4.2.4 Ethical Considerations

Ethical clearance to conduct the study was obtained from the Kenyatta National Hospital – University of Nairobi Ethical Review Committee (P 519/7/2016) and the Mbagathi County Hospital Ethics Committee (17715/2017). Prior to enrolment, caregivers were informed on the purpose of the study, study objectives and possible benefits of the study after which they were given a chance to ask questions and have them answered to their satisfaction. Participation was purely voluntary and child caregivers had to therefore provide informed consent to be enrolled in the study. Caregivers were informed that there were no financial remunerations attached to participation in the study however they were compensated for their transport cost to and from the study site.

4.2.5 Data Collection

Data on household socioeconomic characteristics, child information and nutrient intake was collected on a pre-tested semi-structured questionnaire through face-to-face interviews with the child caregivers. Household socioeconomic data collected included; place of residence, household profile (monogamous or polygamous), sex of household head, number of household dependents as well as the age, education status and contribution to the household of the child's parents. Child information collected in the study comprised of; study children' age (in months), sex, presence of concurrent illness(es), frequency of feeding as well as breastfeeding practice. Average nutrient intake was estimated using a 24-hour dietary recall assessment administered through face-to-face interviews with the study child's caregiver. Caregivers of the children were required to list all foods and beverages consumed by the child in the preceding 24 hours. Details about the exact time and amount of food consumed as well as the cooking

method used were recorded. Estimated portion sizes of the foods and drinks consumed were converted to weight equivalents following the technique described by Gibson and Ferguson (1998).

4.2.6 Data Quality Management

Prior to data collection, two study enumerators were recruited and trained on how data collection forms were to be completed in order to standardize data collection procedures. They were also trained on communication skills and ethics in fieldwork. The tools and techniques were pre-tested in Ngumo area, Lang'ata Constituency on 10 children and adjusted where applicable in order to obtain the required data. Coded options were offered for some questions in order to standardize responses. During implementation of the 24-dietary hour recall, the interviewer probed for foods that may have been forgotten by the caregiver. Interviewers were required to be familiar with colloquial names and the appropriate common names of foods and beverages and to record both if the colloquial name was used by the respondent. Data collection forms were reviewed prior to termination of the interview and at the end of each day of data collection with errors being noted and duly addressed.

4.2.7 Statistical Analysis

Foods consumed and their weight equivalents were entered into Nutrisurvey® software to generate specific nutrient intake and to compare it with daily nutrient requirements of children aged 6 – 24 months. This data together with the household socioeconomic data was entered into Statistical Package for Social Sciences (SPSS®) Version 20. Statistical outliers were identified using the Grubbs' Test and expunged from the dataset. Descriptive statistics (means, medians, modes, percentages and standard deviations) for the data were computed. The significance of differences between socioeconomic characteristics of the study children's fathers and mothers were established using Chi-square, for categorical variables and

Analysis of Variance (ANOVA) for group means. The significance level was set at 0.05. Independent variables were correlated with energy intake in order to establish the strength and direction of correlation. The real household dependency ratio was calculated as the ratio of the economically inactive household members to the economically active household members. [*Children (0 – 17 years old) + Adults (≥ 18 years old) not contributing economically to the household: Adults (≥ 18 years old) contributing economically to household*].

4.3 Results

4.3.1 Residence of respondents

Study children were obtained from 11 out of 17 constituencies within Nairobi County therefore providing a 65 % representation of the county. As shown in Table 4.1, the highest number of respondents resided in Kawangware ward (26.2 %) while the joint lowest were from Embakasi, Nyayo Highrise and Lenana Wards (each 2.4 %). Kibera ward, where Mbagathi Hospital is located represented 19 % of the respondents.

Table 4.1: Place of residence for study respondents

Constituency	Ward	n=144 (%)
Kibra	Kibera	19.0
Dagoretti North	Kawangware	26.2
Makadara	Makongeni	4.8
Embakasi West	Kariobangi	4.8
Embakasi East	Embakasi	2.4
Embakasi Central	Kayole	11.9
Embakasi South	Pipeline	7.1
	South B	7.1
Mathare	Huruma	4.8
Kasarani	Ruai	2.4
Lang'ata	Lenana	2.4
	Nyayo Highrise	2.4
Westlands	Kangemi	4.8

4.3.2 Household profile (size and composition)

As shown in Table 4.2, 141 (97.6 %) of the sampled households were monogamous with 137 (95.2 %) having a male household head. The mean number of household members in the study was 4.64 (±1.36) and the mean household dependency ratio was 1.5 (3:2).

Table 4.2: Household characteristics of study children caregivers

Household characteristic		n=144 (%)
1. Marital type	Monogamous	97.6
	Polygamous	2.4
2. Household headship	Male	95.2
	Female	4.8
3. Household dependents' age group		**Mean**
	< 5 years	1.4 (±0.5)
	5 – 17 years	0.9 (±0.6)
	> 18 years	0.7 (±0.5)

4.3.3 Socioeconomic characteristics of study children parents/guardians

The mean age of the fathers to the study children was 33.8 ± 7.0 years while that of the mothers was 28.7 ± 6.5 years and this difference was statistically significant ($p < 0.01$). As shown in Table 4.3, there was also a statistically significant difference between the education status of the fathers and mothers with 51 (35.7 %) of fathers having completed secondary school education compared to 21 (14.3 %) of the mothers.

All fathers 144 (100 %) in the study contributed money to the household while only 65 (45.2 %) of the mothers contributed money. This difference was statistically significant but could not be computed since household heads contribution to the household was a constant 144 (100 %).

Table 4.3: Socioeconomic characteristics of study children parents/guardians

	Father (%) n=144	Mother (%) n=144	Significance of difference
1. Age (Years)			
<21	0	2.1	CBD
21 – 30	38.1	69.4	χ^2=28.3, df=1, p<0.01
31 – 40	50.0	26.4	χ^2=16.9, df=1, p<0.01
41 – 50	7.1	0	CBD
51 – 60	4.8	2.1	χ^2=1.6, df=1, p<0.01
2. Education status			
Tertiary	23.8	14.3	χ^2=4.2, df=1, p=0.04
Secondary complete	35.7	14.3	χ^2=17.5, df=1, p<0.01
Secondary incomplete	14.3	28.6	χ^2=8.7, df=1, p<0.01
Primary complete	19.0	28.6	χ^2=3.6, df=1, p=0.05
Primary incomplete	4.8	11.9	χ^2=4.7, df=1, p=0.03
No education	2.4	2.3	χ^2=0.00, df=1, p=0.96
3. Primary contribution to household			
Money	100	45.2	CBD
Labour	0	2.4	CBD
Child care	0	52.4	CBD

CBD – Cannot Be Determined

4.3.4 Study children characteristics

As shown in Table 4.4, 70 (48.6 %) of the study children were male and 74 (51.4 %) were female however this difference was not statistically significant different (p = 0.86). The mean age of study children was 12.7 ± 4.5 months with a majority 76 (52.4 %) aged between 6 – 10 months. The frequency of children consistently dropped with an increase in age where there were only 10 (7.1 %) children aged between 21-

24 months. The mean frequency of meal consumption by study children was 5.3 (±1.9) meals per day which is higher than the recommended range of 3 – 4 meals per day. There were only 18 (12.2 %) children who consumed less than three meals in a day.

It was however observed that all 144 (100%) study children were being breastfed. Majority of the study children 78 (54.7 %) had no concurrent illness to moderate wasting however 30 (21.4 %) had respiratory tract infections (common cold and pneumonia) and 20 (14.3 %) had rickets.

Table 4.4: Study children characteristics and frequency of feeding

Child characteristics	**n=144 (%)**
1. Sex of children	
Male	48.6
Female	51.4
2. Concurrent Illness	
Pneumonia	9.5
Common cold	11.9
Rickets	14.3
Anaemia	2.4
Diarrhoea	2.4
Convulsions	2.4
Cellulitis	2.4
No illness	54.7
3. Frequency of feeding (per day)	
< 3	12.2
3	2.4
> 3	85.4

4.3.5 Macronutrient and Energy Intake

Macronutrient and energy intake of study children were compared with the requirement needed to support optimal growth and development as well as to balance total energy expenditure (TEE) at a desirable level of physical activity with the goal of maintaining long-term health (Fomon and Nelson, 2002). As shown in Figure 4.4, most of study children 134 (92.7 %) had an energy intake of less than 50 % of their daily requirement (800 kcal/day) with none having an energy intake equal to or greater than 70 % of the requirement. Protein and carbohydrate intake was poor with only 14 (9.7 %) of study children having a protein intake ≥ 50 % of the requirement (13.5 g/day). All the study children consumed less than 50 % of their carbohydrate requirement (105 g/day).

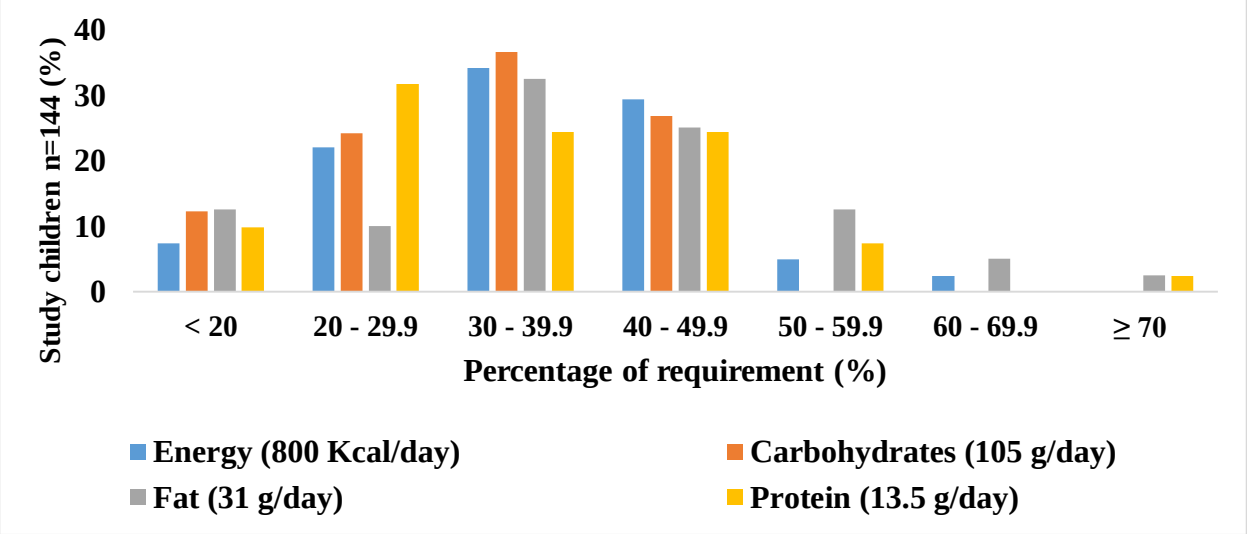
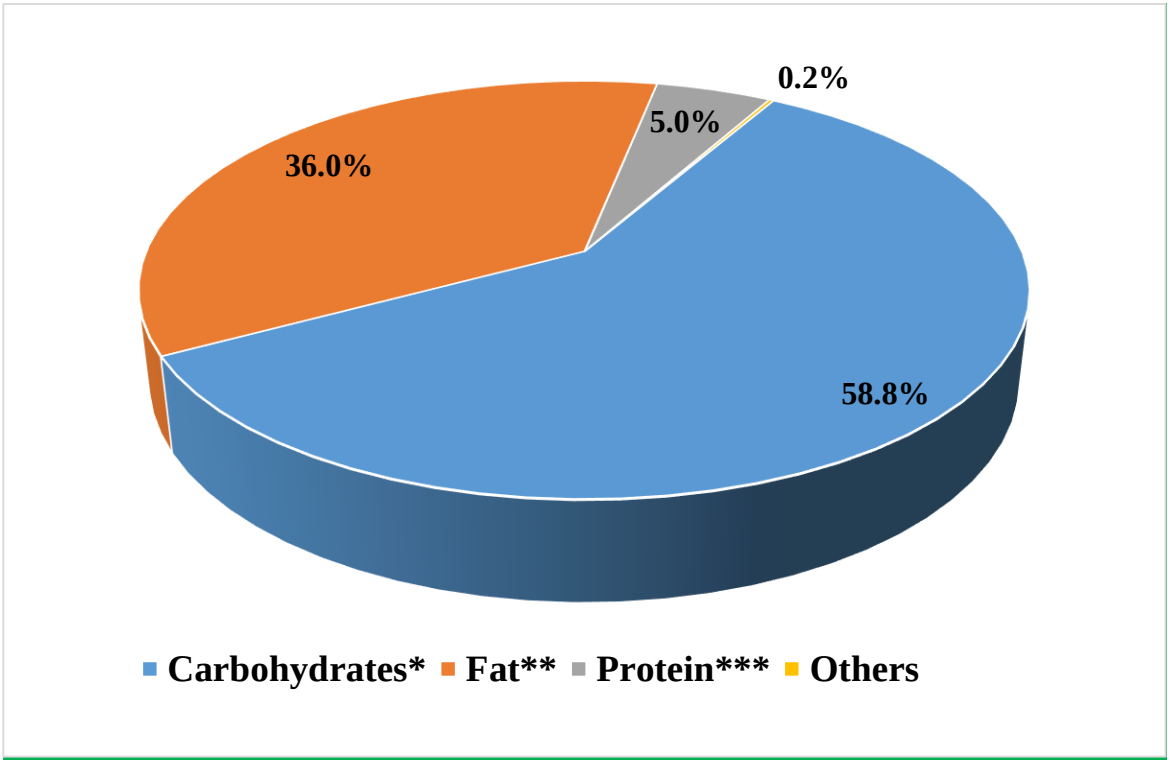


Figure 4.4: Distribution of study children by the percentage consumption of their daily energy and macronutrient requirements

4.3.6 Macronutrient contribution to the mean energy intake

The mean energy intake among study children was 250.6 ±59.3 Kcal/day which represented 31.3 % of the recommended amount of 800 kcal/day. As shown in Figure 4.5, the mean energy intake from carbohydrates (147.4 ±36.7 kcal/day) contributed the majority (58.8 %) of the mean energy intake whereas the mean energy intake from fats was 90.2 ±15.8 kcal/day and it contributed 36 % of the mean energy intake among study children. The mean energy intake from proteins was (12.5 ±2.4 kcal/day) and it constituted 5 % of the mean energy intake.



*Required to contribute 50% of daily energy intake
** Required to contribute 30 – 40 % of daily energy intake
***Required to contribute 6 – 9 % of daily energy intake

Figure 4.5: Macronutrient contribution to the mean daily energy intake of study children

4.3.7 Factors associated with energy intake

A multiple linear regression model helped in ranking independent variables observed to be correlated with the level of energy intake among study children were ranked in ascending order according to the strength of correlation. The number of meals consumed in a day had the strongest correlation with energy intake (r=0.7; p=0.00) where the higher the number of meals consumed in a day, the higher the energy intake (Figure 4.6).

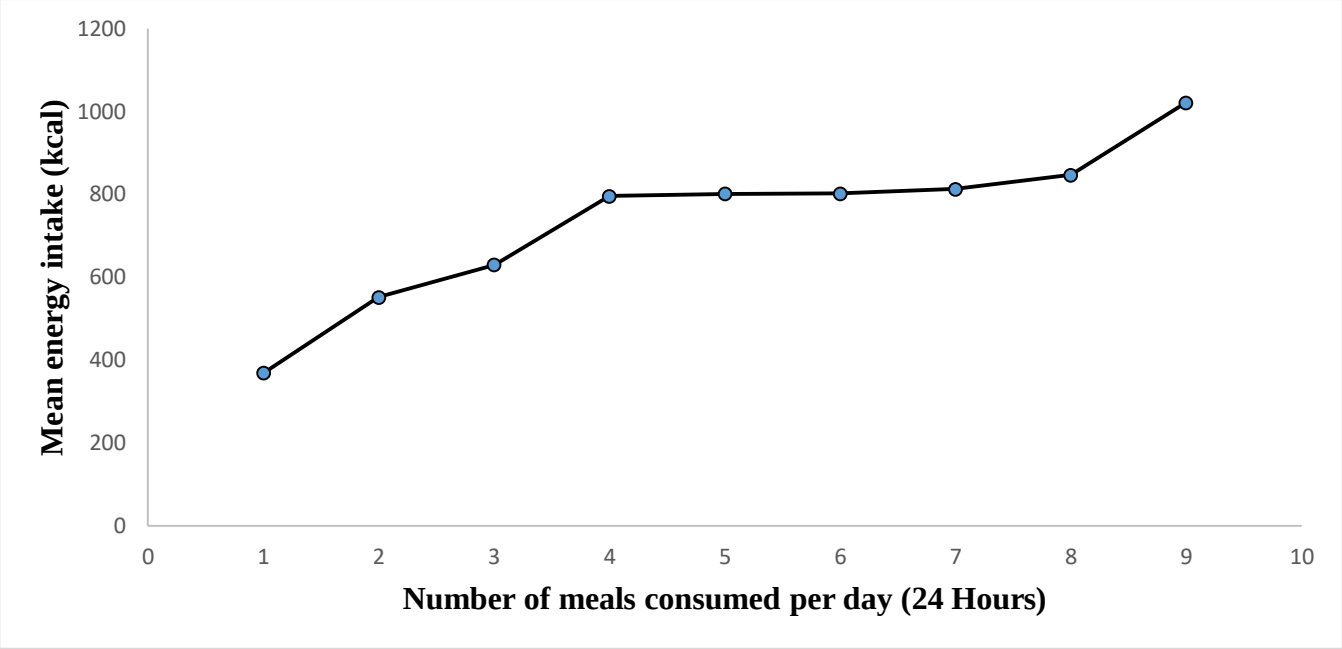


Figure 4.6: Association between daily meal frequency of study children and energy intake

Sex of the household head (r=-0.6; p=0.02), number of spouses in one household (r=-0.4; p=0.02) and the household dependency ratio (r=-0.4; p=0.03) were the non-dietary factors that significantly correlated to energy intake level. Therefore, households that were headed by a female and those that were polygamous were more likely to have a child with insufficient energy intake. Similarly, the higher the household

dependency ratio, the lower the energy intake among children. Table 4.5 summarizes the strength of correlation between select independent variables and the energy intake level.

Table 4.5: Correlation between percent energy intake by study children and selected factors

Characteristic	r - value	p-value
1. Dietary factors		
Daily frequency of meal consumption	0.7	0.00
2. Non-dietary factors		
Household head (male versus female)	-0.6	0.02
Household profile (monogamous vs. polygamous)	-0.4	0.02
Household dependency ratio	-0.4	0.03
Concurrent illness	-0.2	0.15
Caregiver (mother's) age	0.1	0.20
Caregiver (mother's) education	0.1	0.23

4.3.8 Vitamins intake

Majority of study children consumed above 100 % their recommended daily intake of vitamin C [75 (51.3 %)] and vitamin E [113 (78 %)]. A majority 102 (70.8 %) and 130 (90.3 %), also consumed foods that provided 80 % and above of their recommended daily intake of Vitamin B2 and B6 respectively. Vitamin A was consumed by a majority of study children 76 (53.6 %) who consumed ≥ 60 % of the daily requirement. Distribution of respondents according to their percentage intake of their vitamin requirements is summarized in Table 4.6.

Table 4.6: Distribution of study children by percentage intake of daily vitamin requirements

	n=144 (%)						
Percentage of requirement (%)	**Vitamin A (400 µ g RE/day)**	**Vitamin B1 (0.4 mg/day)**	**Vitamin B2 (0.5 mg/day)**	**Vitamin B6 (0.4 mg/day)**	**Folic acid (115 µg/day)**	**Vitamin C (30 mg/day)**	**Vitamin E (3 mg/day)**
<20	12.2	0.0	2.4	0.0	2.4	0.0	0.0
20 – 29.99	4.9	9.8	0.0	0.0	7.3	7.3	9.8
30 – 39.99	14.6	4.9	0.0	0.0	7.3	9.8	0.0
40 – 49.99	7.3	12.2	7.3	0.0	12.2	2.4	2.4
50 – 59.99	7.3	7.3	2.4	0.0	7.3	9.8	0.0
60 – 69.99	9.8	12.2	9.8	4.9	4.9	2.4	0.0
70 – 79.99	2.4	17.1	7.3	4.9	4.9	2.4	0.0
80 – 100	2.4	9.8	4.9	4.9	9.8	14.6	9.8
>100	39	26.8	65.9	85.4	43.9	51.3	78.0

4.3.9 Mineral intake

As shown in Table 4.7mineral intake was varied with most of the study children consuming above 100 % of their daily requirement for magnesium 120 (83.0 %). Majority of the study children 77 (53.7 %) consumed above 80 % of their daily requirement for calcium. In comparison, iron was not adequately consumed with 105 (73.2 %) of participants having an intake level that was below 80 % of the daily recommended intake. All of the study children consumed a diet that provided less than 20 % of their recommended sodium intake.

Table 4.7: Distribution of study children by their percentage intake of daily mineral requirements

	n=144 (%)						
Percentage of recommendation (%)	**Sodium (1000 mg/day)**	**Potassium (3000 mg/day)**	**Calcium (450 mg/day)**	**Magnesium (57 mg/day)**	**Phosphorous (327.5 mg/day)**	**Iron (9.3 mg/day)**	**Zinc (4.1 mg/day)**
<20	100	70.8	2.4	0.0	0.0	9.8	2.4
20 – 29.99	0.0	0.0	7.3	0.0	2.4	12.2	0.0
30 – 39.99	0.0	2.4	4.9	0.0	0.0	9.8	0.0
40 – 49.99	0.0	7.3	9.8	0.0	4.9	14.6	2.4
50 – 59.99	0.0	0.0	7.3	7.3	4.9	12.2	2.4
60 – 69.99	0.0	2.4	9.8	4.9	7.3	9.8	7.3
70 – 79.99	0.0	2.4	4.9	2.4	4.9	4.9	7.3
80 – 100	0.0	4.9	12.2	2.4	14.6	7.3	14.6
>100	0.0	9.8	41.5	83.0	61.0	19.5	63.6

4.4 Discussion

Less than five percent of household in the study were female headed which varies in comparison to the KDHS 2014 report that had female headed household in urban residence at 27.3 % (KDHS, 2014). The trend, however, in having a vast majority of urban households being headed by a male is consistent with the KDHS report of 2014 (KDHS, 2014). It was observed that sex of the household head was significantly correlated to energy intake of children. Female headed households have a high incidence of poverty and food insecurity because majority of the urban poor are women (Ngwenya, 2008). Most of the households in the study were monogamous and this did not vary with the overall Nairobi County figure of 94.5 % of households being monogamous. However, polygamous households were significantly correlated with low

energy intake among children. The mean number of household members (4.64) in the study was higher than the mean number of household members in an urban residence in Kenya (3.2) (SID, 2013). This coupled with a higher dependency ratio than that of Nairobi County (0.465) (SID, 2013) indicated a greater strain on household resources including food. It thus appears that a high dependency ratio compromised food and nutrient intake by the children that resulted in malnutrition in the form of moderate wasting. Majority of mothers in the study had a better education level than other women in Kenya (KDHS, 2014) with fewer having no education and dropping out of primary and secondary school. Additionally, more women in the study had attained tertiary level of education compared to other women in Kenya. Previous studies done have shown that the education status of a mother is a strong predictor of the nutrition status of their children (Abuya et al, 2012; Iftikhar et al, 2017). and it is strongly related to wasting than any other form of child malnutrition (Miller, 2009).

Majority of moderately wasted children in the study were aged between 6 – 10 months, consistent with the KDHS 2014 report. This is related to the introduction of complementary feeds that may vary in terms of quality and quantity. In addition, children at this age are more vulnerable to disease which can cause increased nutrient requirements, reduced nutrient intake and/or accelerated nutrient losses (WHO, 2012). This was evident whereby 45.3 % of study children had a concurrent illness mainly acute respiratory infections (pneumonia and common cold) and rickets. All of the study children were breastfed and majority consumed three or more meals in a day. Nevertheless, none of them met their daily requirement for carbohydrate, protein and fat and consequently none of them met their daily energy requirement. Failure to meet the energy requirement causes the body to hydrolyse the fats in adipose tissue to form free glycerol and breakdown muscle protein to form free amino acids both which are converted to glucose for

energy production. Overreliance on these body stores due to inadequate energy intake is what ultimately leads to moderate wasting in children (Briend, 2015). On-demand breastfeeding provides approximately half of all the child's energy requirements during the first12 months of life and one third of energy requirements of children aged 13 – 24 months (WHO, 2009). For infants with average breastmilk intake the energy requirements from complementary feeds are approximately 200 kcal/day at the age of 6 – 8 months, 300 kcal/day at 9 – 11 months and 550 kcal/day at 12 – 23 months of age (WHO, 2004).

The optimal frequency of feeding children aged 6 – 24 months who are breastfed on demand ranges between 3 - 4 times in a day to meet their daily energy requirements. This requirement assumes that the foods consumed have an energy density of between 0.8 – 1.0 kcal/g (WHO, 2009). The mean frequency of feeding in this study (5.3 times/day) was higher than this range. The frequency of feeding a child with complementary feeds should increase as the child gets older. The appropriate feeding frequency is dependent on the energy density of the foods prepared and the amounts consumed during each feeding. For children who are not wasted, complementary feeds should be provided 2 – 3 times per day at 6 – 8 months and 3 – 4 times per day for children between 9 – 24 months.

These frequencies of feeding require to increase in the case a child has moderate wasting. An additional nutritional snack should be provided 1 – 2 times per day or as desired by the child. A snack refers to foods eaten between meals that are easy to prepare and self-fed. If complementary feeds have low energy density and the child is no longer breastfeeding, a high frequency of feeding is required (WHO, 2004). For a child without any concurrent illness to thus be wasted despite having adequate meal frequency indicates that the foods consumed was of low energy density or in insufficient portion amounts.

4.5 Conclusion and Recommendations

Women headed households, polygamous households and households with a high dependency ratio are the most likely to have a child with moderate wasting. These together with a low energy and macronutrient intake are risk factors for moderate wasting in children living in an urban residence. Majority of moderately wasted children have an adequate meal frequency. The study recommends further research to be done on precision interventions targeted at women headed households, polygamous households and households with a high dependency ratio in urban residences to prevent moderate wasting occurring in children. The study also recommends that affordable complementary feeds for children with moderate wasting should be developed from local foods with high nutrient density. This together with on-demand breastfeeding should be promoted to provide adequate energy intake and prevent energy deficits that could result in breakdown of body stores leading to moderate wasting. This can be achieved through routine nutrition education of mothers during the mother-child health clinics. In addition to this, an investigation should be conducted to determine the appropriate number of meals that a child with moderate wasting should consume in a day to meet their daily energy requirement and enhance recovery.

CHAPTER FIVE: EFFECTIVENESS OF LEUCINE SUPPLEMENTATION IN THE MANAGEMENT OF MODERATE WASTING IN CHILDREN

Abstract

Wasting in children is a serious form of malnutrition where the affected child has a low weight-for-height. While there are published guidelines on the treatment of severe wasting, moderate wasting has no standardized treatment protocol and consequently, common feeds used to treat it have limited effectiveness. When leucine, an amino acid, is administered in a large dose in catabolic conditions it functions as a nutraceutical that accelerates muscle protein synthesis. The objective of this study was therefore to establish the effectiveness of leucine supplementation in treating moderate wasting in children. A double blind placebo controlled trial that involved 144 outpatient children aged 6 – 24 months with moderate wasting was conducted at Mbagathi Hospital (Kenya) between 6th March – 23 June, 2017. Study children were randomly assigned to the treatment group (n = 72) or the control group (n = 72). Children in the treatment group and control group were administered with the leucine supplement and placebo respectively every day for 28 days. Baseline weight and height measurements were taken then measured weekly and at the end of the study. Differences between study groups were analysed using analysis of variance and Chi-square. The significance level was set at $p < 0.05$.

A significant difference in the proportion of children who recovered from moderate wasting was observed between the treatment group (93.1 %) and the control group (40.3 %). This difference was more than two times and was statistically significant ($p < 0.01$). There was also a significant difference in the mean weight gained between the two study groups ($p = 0.02$). Occurrence of adverse reactions was not significantly different between the two study groups.

The study concludes that leucine supplementation in moderate wasting causes a significant weight gain that results in recovery and thus it is effective in the treatment of moderate wasting.

5.1 Introduction

Wasting in children is a form of malnutrition that manifests in the weight for height Z-score being below minus two standard deviations from the median of the reference population. It is classified as either; moderate wasting, when the weight for height Z-score is greater than or equal to minus 3 but less than minus 2 ($-3 \leq$ Z-score < -2), or severe wasting where the Z-score is less than minus 3 (Z-score < -3) (WHO, 2004). Statistical estimates indicate that moderate wasting affects more than 33 million children globally (WHO, 2013). According to a United Nations Children Fund (UNICEF) and The Food and Agriculture Organization of the United Nations (FAO) September 15, 2017 report, 370,000 children in Kenya suffer from wasting, 297,480 (80.4 %) of whom have moderate wasting. This figure reflects an increase of 37,000 children from the February 2017 figure of 363,000 children (FAO, 2017).

Wasting is brought about by an acute failure to receive sufficient nutrition which is related to inadequate nutrient intake or a recent illness. Illnesses inhibit nutrient intake and utilization or accelerate nutrient losses especially through diarrhoea and vomiting. Wasting in children has been shown to impair the immune system hence increased risk of death from infectious diseases. In addition, episodes of wasting undermine children's physical as well as mental growth resulting in impaired motor and cognitive development. This compromises a child's learning ability and ultimately their school performance (WHO, 2012).

To improve child survival, it is then important to invest in interventions that prevent child wasting and those that ensure timely treatment and recovery from wasting. Treatment guidelines for severe wasting have been defined and published by the World Health Organization (WHO, 2013). These comprise the use of measures to correct fluid and electrolyte imbalances and measures to replenish calories, proteins and micronutrients through the use of Ready-to-use therapeutic food (Baron, 2009). If a child responds positively to this treatment, they progress to moderate wasting for which there is no standardized treatment protocol despite ongoing research and consultation (Greiner, 2010; UNICEF/WHO/World Bank, 2012). In the absence of a standard protocol, practices in the treatment of moderate wasting as well as outcomes from these practices vary in different health care facilities (Greiner, 2010). The results of two separate studies on the effectiveness of the two commonly used foods in the treatment of moderate wasting - corn-soy blend and lipid-based nutrient supplements – show no significant difference in the outcomes between the treatment groups and the control groups (Thakwalakwa, 2010). In addition, lipid-based nutrient supplements do not reduce the risk of advancement to severe wasting and/or mortality as they also induce more vomiting (Lazzerini, 2013). The existing treatment methods of moderate wasting therefore have limited effectiveness and are less efficient, which compromises the quality of care in the treatment of wasting. Quality in healthcare can only exist when health services for individuals and populations increase the likelihood of attainment of the desired health outcomes and are based on current research and professional knowledge (Atkins, 2010). This therefore creates the need to conduct research on an effective treatment of moderate wasting to fill the existent gap in knowledge and management of moderate wasting.

A review article by Wamiti et al (2016) provided adequate evidence to suggest that leucine, a branched chain amino acid, has nutraceutical properties that accelerate muscle protein synthesis. Leucine was also

seen to maintain muscle mass in catabolic conditions associated with food restriction in rats(Anthony, 2000). A nutraceutical refers to a nutrient or nutritional component that occurs naturally and may have been purified and/or concentrated that is used to improve health through prevention and/or treatment of disease(s) (Lockwood, 2007). Leucine functions as a nutraceutical through activation of a compound in muscles called mammalian target of rapamycin which when activated, accelerates protein synthesis and growth. Larger doses of leucine – than those typically found in high-quality protein food sources – have been shown to be significantly more effective at stimulating muscle protein responses (Glynn, 2010). Leucine, primarily being an amino acid, must be sufficient in the body so as to first satisfy all structural roles before it can engage in signalling and metabolic roles. The capacity of leucine to function as a nutraceutical is based on sufficient intracellular concentration (Churchward-Venne, 2012). The main objective of this study was to therefore establish the effectiveness of leucine supplementation in correcting moderate wasting in children aged 6 – 24 months.

5.2 Materials and Methods

5.2.1 Study Design and study site

A randomized double blind placebo controlled trial was conducted at Mbagathi Hospital (Figure 3.1) in Nairobi County, Kenya between 8th May, 2017 – 5th August, 2017. This was done to demonstrate the effectiveness of leucine supplementation in the treatment of moderate wasting. As shown in Figure 5.1, a parallel trial design was used where study children were randomly assigned to either the treatment group in order to only receive the leucine supplement or the control group to only receive the placebo (Tinmouth, 2007). The hospital was purposively selected for the study because it has a large outpatient therapeutic programme and supplementary feeding programme. The two feeding programmes had a total of 807

children enrolled as of May 2016 based on the Outpatient Therapeutic Program Health Facility Record of 2016. The catchment area for children in this hospital is Nairobi County and this therefore sufficed as the catchment area for the study.

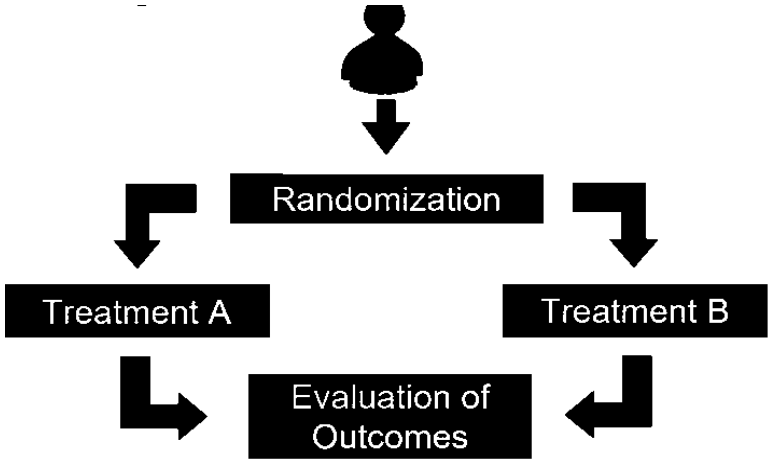


Figure 5.1: The parallel trial design used in this study

Adapted from (Tinmouth, 2007)

5.2.2 Sample size determination

A sample size of 72 children per group (144 in total) was computed based on the formula by Chow (2008) as described in subsection 4.2.3. This sample size was calculated based on the expected difference in the primary outcome, that is, change in weight among those provided with either leucine supplement or placebo. This expected difference was based on the assumed mean change in weight of + 0.5 ± 0.15 g among study children receiving leucine supplement and + 0.27 ± 0.15 g among those receiving the placebo. This gave the trial 80 % power and a type 1 error of no more than 5% to detect a difference of ≥ 0.23 g in the mean weight gain between the treatment group and control group.[8]

5.2.3 Sampling Procedure

Children aged between 6 – 24 months attending Mbagathi Hospital who had a weight-for-height Z-score of less than -2 SD but greater than or equal to -3 SD were referred to the study. After obtaining written consent from the child caregivers, the children were screened to determine their eligibility for enrolment to the study. Children who had chronic conditions and those who had participated in other research including pre-tests in the preceding three months were excluded from the study. This information was documented on the study case report form (Appendix Five) which thus formed the basis of inclusion or exclusion of study children. On enrolment, the study children were randomly assigned to either the treatment group or the control group. The study children, their caregivers and the research team were blinded on the assignment to ensure that neither knew what treatment each study child was receiving As shown in Figure 5.2, exhaustive sampling was used to recruit study children where all those that met the selection criteria and the caregiver gave informed consent were recruited until the computed sample size was attained.

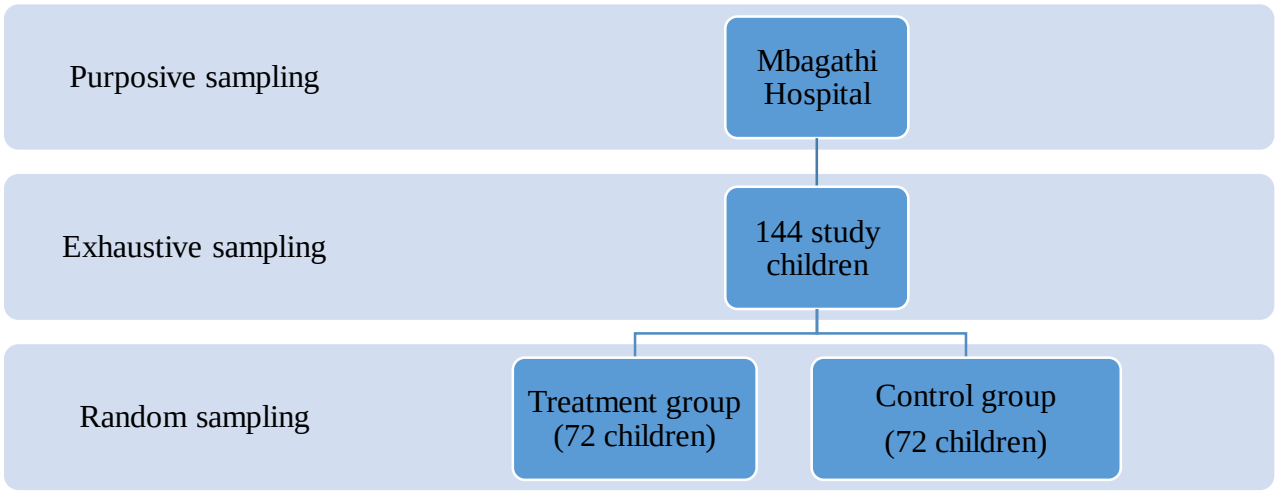


Figure 5.2: Sampling schema

5.2.4 Randomization Procedure

Simple random sampling was used where the randomization allocation sequence was computer generated by an independent statistician (who was not involved in the study) to ensure allocation concealment. Based on the sample size, 144 unique study codes were generated and fed into a computer program that randomly allocated 72 codes to the treatment group and 72 codes to the control group. Every n^{th} study participant recruited to the study received the next available study code and by default was allocated to the respective study group in which the code was assigned to.

5.2.5 Randomization allocation concealment and blinding

The randomization sequence was securely kept away from anyone who was blinded including the principal investigator, university supervisors and the study team until the end of the study. Only the independent statistician who generated the sequence and assigned study children to the study groups had access to it. A backup of the sequence was stored remotely in a computer away from the study team and password protected to ensure it was tamper-proof. Un-blinding of the study was done by the statistician in the presence of the principal investigator on completion of data analysis.

5.2.6 Ethical Considerations

Ethical consent was sought and obtained from the Kenyatta National Hospital Ethics Review Committee (P 519/7/2016) and the Mbagathi Hospital Research Committee (17715/2017). Mothers/caregivers were introduced to the study and informed on the objectives, possible benefits as well as risks of the study. Each mother/caregiver was given a chance to ask questions related to the study and have them answered satisfactorily. Afterwards they provided written consent prior to enrolment of the study child and this was captured on the consent form (Appendix Two). Participation in the study was purely voluntary and

mothers/caregivers were only financially compensated for their transport to and from the clinic. Upon completion of this study the control group was accorded similar interventions as the treatment group and therefore there were no discriminative ethical issues.

5.2.7 Data collection

Demographic characteristics for all the study children were collected through face-to-face interviews with the child's caregiver/mother using a semi-structured questionnaire then baseline anthropometric measurements were taken. This was done by measuring the weight (kg) using a Salter® Bathroom Scale for children who could stand on their own and Infant Weighing Scale for children who could not stand. Vertical height (cm) and supine length were measured using a Height Board for children who could stand and a Length Board for children who could not stand respectively.

Weight and height measurements were used to obtain the child's weight for height Z-score. This was used to determine the effectiveness of leucine supplementation in the treatment of moderate wasting by comparing the differences between the two study groups over the trial period. Mid Upper Arm Circumference (cm) (MUAC) was measured using a MUAC Tape. All anthropometric procedures used standard methods as described by NHANES, (2007).

5.2.8 Randomization implementation

Study children in the treatment group received leucine supplement capsules (150 mg/kg/day) while those in the control group received a placebo that was indistinguishable from the leucine capsule. Capsules given were sufficient for one week and therefore were to be administered orally at home by the child's caregiver as prescribed. Both capsules (active treatment and placebo) were packaged in identical, opaque

and securely sealed containers that were marked with only one of the unique study codes representing each study child. The capsules were either administered as a whole or they were opened and the content inside dissolved in a clean cup of 100 ml potable water at room temperature (20 - 25ºC) and the child ingests the entire content of the cup. A supplementation adherence form (Appendix Four) was given together with the capsules to enable weekly monitoring of adherence to the prescribed dosage. This supplementation adherence form was a written record by the caregiver of the times in a day and the number of capsules that were administered to the child. Capsules were administered every day for a period of four weeks (28 days). The children continued eating their regular diet and taking any prescribed medication and therapeutic feeds.

Clinic visits were scheduled after every seven days when the capsule refills were also done. During these visits: weight, height and MUAC were measured as well as monitoring for adverse reactions. Adherence to the previous week's prescribed dosage was observed before supplement refills were administered. Data collection forms were reviewed by the enumerators after each interview and at the end of the day to ensure completeness of data collected.

5.2.9 Intervention

Two types of capsules were administered to the study children: the active treatment and a placebo. The treatment capsules (Figure 5.3) administered contained all three Branched Chain Amino Acids (BCAAs): L-leucine (750 mg), L-Valine (500 mg) and L-Isoleucine (250 mg). This is because all the BCAAs compete for cell transport and metabolism and therefore ingestion of leucine alone may lead to depletion of plasma valine and isoleucine. All three amino acids therefore need to be consumed concurrently to

prevent plasma depletion of any one of the BCAAs. Leucine should however be the predominant nutrient because valine and isoleucine are ineffective in modulating nutrition status in stress situations (Cynober, 2012).

Figure 5.3: A sample of the leucine supplement capsules used in the study

Other constituents of the capsule were; gelatin, magnesium stearate and microcrystalline cellulose. All the children in the treatment group were required to consume 150 mg/kg/day of leucine and thus capsule prescription was based on the individual weight of each study child in the treatment group. The active treatment capsules were shipped from Milton Keynes, United Kingdom as they could not be sourced locally. The placebo in turn was a capsule similarly formulated with gelatin, magnesium stearate and microcrystalline cellulose but in place of the three BCAAs was glucose (7000 mg). This was locally obtained from a laboratory at the study site. The placebo was ensured to be indistinguishable in size, colour, shape as well as taste from the active treatment.

5.2.10 Monitoring of adherence to prescribed supplement dosage

Adherence by the study children to the prescribed dosage was monitored using the Pill Counts method together with the scrutiny of the supplement adherence forms. The pill counts method involved counting the number of pills (if any) in the container compared to the number given out on the previous visit. The difference between the two values was assumed to reflect the number of pills consumed. Supplement adherence forms (Appendix Four) were then inspected to compare the home-based capsule administration with the prescription given. Discrepancies between the two monitoring methods necessitated further probing of the caregiver to recall and describe how the capsules were administered.

5.2.11 Safety of study children

Under acute conditions, intake of leucine that exceed 500 mg mg/kg/day could potentially increase the risk of adverse events (Cynober, 2012) therefore, it was proposed as the upper limit for leucine. It is recommended that when chronic ingestion studies are designed, leucine intake levels should be in the range of 250–300 mg/kg/day (Pencharz, 2012). This study used a dosage of 150 mg/kg/day for a period of 28 days based on the 'Dose-toxicity approach' which uses the PKCOV statistical model to estimate the relationship between the probability of toxicity and the dose of leucine directly (Ursino, 2016). Notwithstanding, the study was keen to observe and document adverse reactions related to leucine supplementation in children that included: diarrhoea, vomiting, scaly pigmented rash on skin, swollen mouth and/or bright red tongue, depression, disorientation and apathy (Cynober, 2012).

Caregivers reported to the investigators any adverse reactions and other suspicious reactions if and when they occurred through a designated 24-hour hotline. All adverse reactions were recorded and the participation of the affected study children discontinued.

5.2.12 Contraindications

Leucine supplementation has no known contraindications.

5.2.13 Study dropouts and replacement strategy

The nine study children who developed adverse reactions and the 12 who defaulted more than one clinic visit were dropped from the study. Data collected from the study children who developed adverse reactions was however retained in the database but that collected from the defaulters was discarded. All the defaulters were immediately replaced with new study recruits who begun the study procedure afresh. Each of the 12 defaulters were notified on being dropped from the trial, their study codes discarded from the database and new ones were allocated to the replacement study children.

5.2.14 Data Quality Management

Two study enumerators were recruited: one a trained clinical officer and the other a registered nutritionist. The researcher/student as well as the enumerators were required to undertook a six-hour examinable online course on Good Clinical Practices (GCP). The course had 12 learning modules, key among them were Participant Safety and Adverse Events, Documentation and Record Keeping, Recruitment and Retention and Research Misconduct.

On successful completion of the course a certificate of completion was awarded (Appendix 8, 9, 10) and this was a prerequisite to participate in data collection. Afterwards the enumerators were trained on how data collection forms were to be completed in order to standardize data collection procedures. The study pre-tested tools and procedures on 10 children selected from households Ngumo area, Lang'ata Consituency. On completion, the study tools and procedures were adjusted where applicable to generate

the required data. Data collection procedures were standardized and all equipment were calibrated and recalibrated after every 10 measurements taken. All measurements were taken in triplicate at an accuracy of 0.01 before the mean between the three measurements was computed. Finally, before the mean value was entered on the tool provided all values were compared with established reference standards to enhance accuracy. Variation of the mean collected from the maximum allowable difference in the reference standards resulted in discarding of the values recorded and fresh ones being taken (Prem, 2016). Data collection forms were reviewed every day to determine completeness of data collected and to detect any errors.

5.2.15 Statistical analysis

Data was entered into the Statistical Package for Social Sciences (SPSS®) Version 22 then statistical outliers were identified using a Grubbs' test and expunged from the dataset. Descriptive statistics (means, medians, modes, percentages and standard deviations) were computed for the socio-demographic factors and anthropometric variables. Differences in the outcome variables (weight, height and MUAC) between the treatment group and control group were analysed using Analysis of Variance (ANOVA) and Chi-square. A p-value of <0.05 was considered significant in all the analyses.

The cohen's *d* (Lakens, 2013) was then computed to quantify the effect size in end-of-study differences between the two study groups. This was used to measure the magnitude of the effect of leucine (treatment effect) on the anthropometric measurements of the study children. The rate of change in anthropometric variables was calculated by dividing the overall change by the study period. Finally, a multiple linear

regression model was used to study the correlation between leucine supplementation and other independent variables.

5.3 Results

5.3.1 Study children flow through the parallel trial design

As shown in Figure 5.4, there were 157 children assessed for eligibility for enrolment into the study out of whom 10 did not meet the selection criteria and were thus excluded. Out of the remaining 147 children, the caregivers of three declined to participate and their children were also excluded. The study therefore had a sample size of 144 study children who were randomly allocated to either the treatment group or the control group. The data of all 144 study children was included in the data analysis including the nine who reported adverse reactions and their participation discontinued.

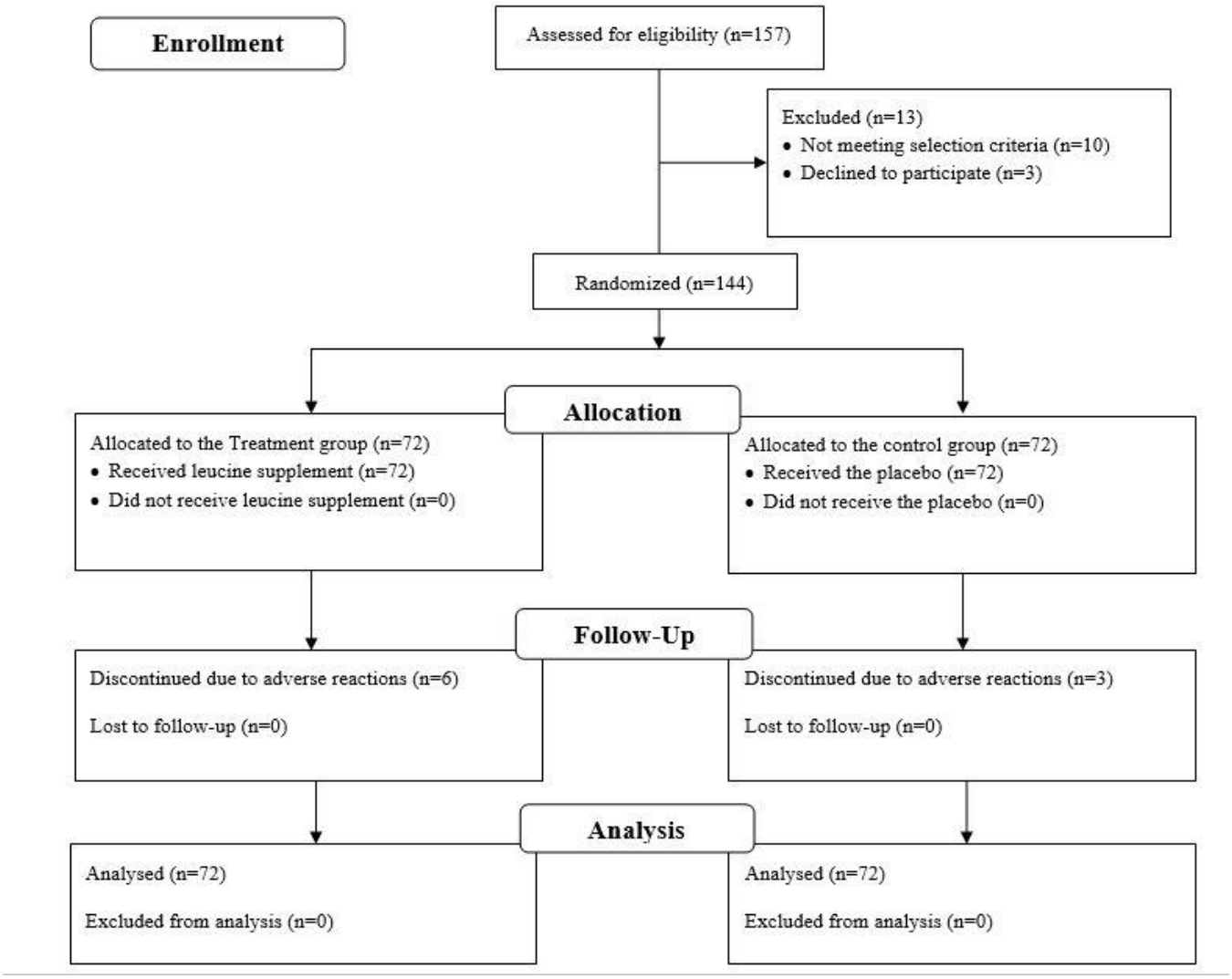


Figure 5.4: Flow diagram of the progress through the parallel randomized trial design

Adapted from (Moher D et al, 2010)

5.3.2 Demographic characteristics of child caregivers

As shown in Table 5.1, all of the child caregivers in both the treatment group and control group were female. The mean age of caregivers in the treatment group was 28.7 ± 6.5 years while in the control group was 28.9 ± 6.1 years. There was no significant difference in the age, education level and marital status of the respondents between the treatment group and control group ($p > 0.05$).

Table 5.1: Distribution of caregivers by demographic characteristics

		Treatment group n=72 (%)	Control group n=72 (%)	Significance of difference
Sex	Male	0.0	0.0	p=1.00
	Female	100.0	100.0	p=1.00
Mean age (years)		28.6	28.8	t = 0.19, df=142, p=0.85
Education level	Post-secondary	12.5	16.7	χ^2 =0.48, df=1, p=0.48
	Secondary	45.9	36.1	χ^2 =1.42, df=1, p=0.23
	Primary	38.9	45.8	χ^2 =0.70, df=1, p=0.40
	No education	2.8	1.4	χ^2 =0.34, df=1, p=0.56
Marital status	Married	87.5	88.9	χ^2 =0.07, df=1, p=0.80
	Separated	8.3	6.9	χ^2 =0.10, df=1, p=0.75
	Single	4.2	4.2	χ^2 =0.00, df=1, p=1.00

5.3.3 Baseline child characteristics and anthropometric measures

The study comprised of a sample of 144 children: 72 (52.8 % girls and 47.2 % boys) in the treatment group and 72 (51.4 % girls and 48.6 % boys) in the control group. The mean age of the study children was 12.1 ±4.5 months in the treatment group and 14.1 ±4.3 months in the control group. As shown in

Table 5.2, there was no significant difference ($p > 0.05$) in any of the baseline child characteristics between the two study groups.

Table 5.2: Distribution of the baseline study children characteristics and anthropometric measurements

		Treatment group n=72	Control group n=72	Significance of difference
Mean age (months)		12.0	14.0	t=2.72, df=142, p=0.59
Sex (%)	**Male**	47.2	48.6	χ^2 =0.03, df=1, p=0.87
	Female	52.9	51.4	χ^2 =0.03, df=1, p=0.87
Mean weight (Kg)		7.0	7.0	t=0.18, df=142, p=0.86
Mean height (cm)		70.8	72.1	t=1.35, df=142, p=0.18
Mean MUAC (cm)		12.4	12.5	t=0.07, df=142, p=0.94

5.3.4 Changes in anthropometric measurements in the treatment group

As displayed in Table 5.3, the mean weight of study children in the treatment group increased by 0.5 kg over the study period. There were also a 0.74 cm increase in the mean MUAC over the study period. The mean height of study children increased by only 0.2 cm.

Table 5.3: Changes in mean anthropometric measurements in the treatment group

	Baseline	**Day 7**	**Day 14**	**Day 21**	**Day 28**
Mean weight (Kg)	7.0	7.1	7.3	7.4	7.5
Mean height (cm)	70.8	70.9	70.9	71.0	71.0
Mean MUAC (cm)	12.4	12.6	12.7	13.0	13.2

5.3.5 Changes in anthropometric measurements in the control group

As shown in Table 5.4, the mean increase in weight of children in the control group from baseline to the end of the study was 0.3 kg. The mean height also changed over the study period from 72.14 cm to 72.74 cm. The mean increase in the MUAC through the duration of the study was 0.3 cm.

Table 5.4: Changes in mean anthropometric measurements in the control group.

	Baseline	**Day 7**	**Day 14**	**Day 21**	**Day 28**
Mean weight (Kg)	7.0	7.1	7.2	7.2	7.3
Mean height (cm)	72.1	72.3	72.7	72.7	72.7
Mean MUAC (cm)	12.5	12.5	12.6	12.6	12.7

5.3.6 Comparison of mean weight changes between the two study groups

The mean baseline weight of children in the treatment group (7.0 ±0.8 kg) and control group (7.0 ±1.2 kg) were not statistically different (t=0.18, df=142, p=0.86). However, as shown in Figure 5.5, the final mean weight of the treatment group (7.5 ±1.0 kg) was significantly different from that of the control group (7.3 ±1.2 kg) where (t=-1.02, df=142, p = 0.02). This represented a mean change in weight of +0.5 ±0.3 kg in the treatment group and +0.3 ±0.3 kg in the control group. This difference was also statistically significant (t=-4.34, df=142, p < 0.00) with the two means differing by 0.7 standard deviation representing a medium size difference (medium effect size).

The mean rate of weight gain among children in the treatment group (2.5 g/kg/day) was significantly different (p < 0.00) from that of the control group (1.5 g/kg/day). The largest mean weight increment in the treatment group occurred between day 7 - 14 (+ 0.2 kg) while in the control group was between day 14 – 21 (+ 0.1 kg).

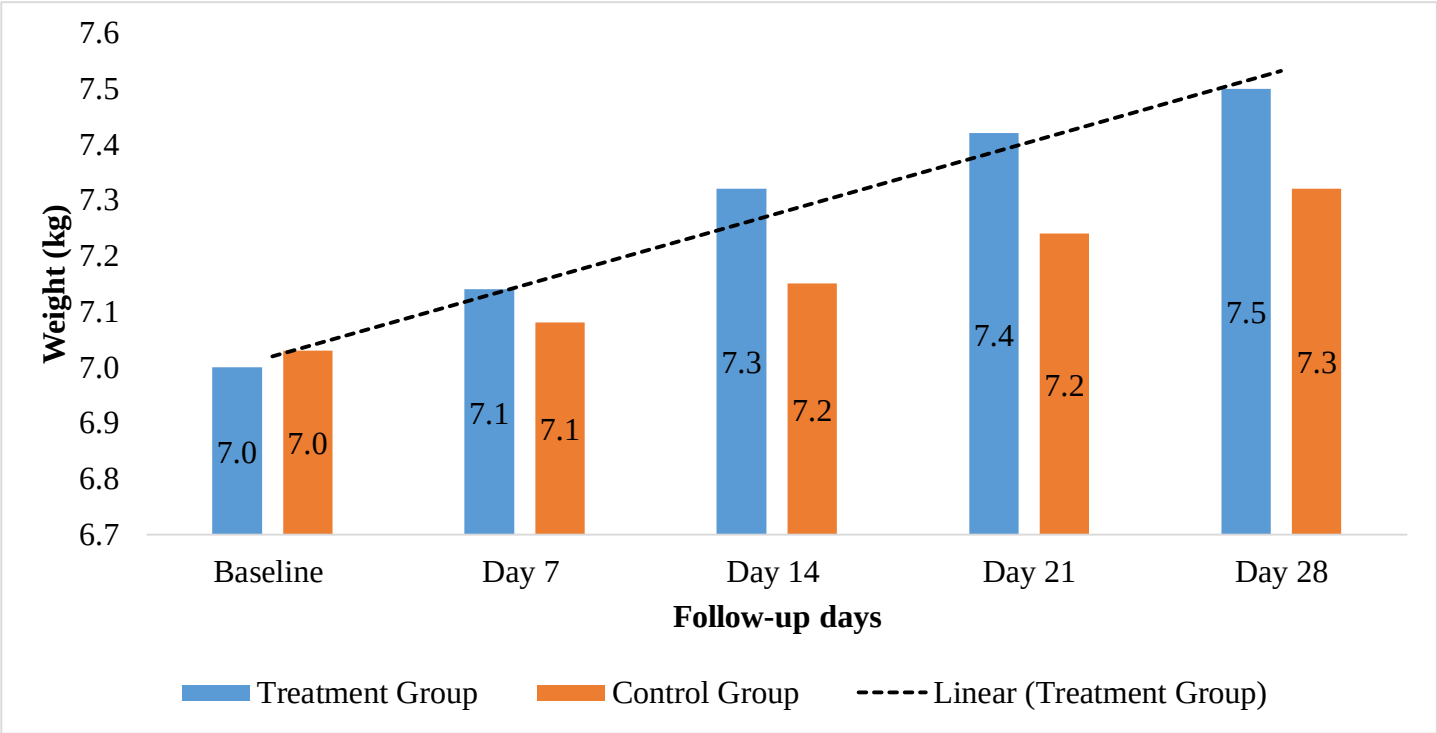


Figure 5.5: Comparison of the changes in mean weight of study children between the two study groups

5.3.7 Comparison of mean height changes between the two study groups

As shown in Table 5.5, mean baseline heights of study children in the treatment group (70.8 ± 4.9 cm) and in the control group (72.1 ± 6.8 cm) were not statistically significantly different (t=1.35, df=142, p=0.18).

The final mean height of the treatment group was 71.0 ±5.20 cm while that of the control group was 72.7 ±6.5 cm and this difference was not statistically significant (t=1.36, df=142, p=0.17). This represented an overall change in mean height of study children of + 0.2 cm in the treatment group and + 0.6 cm in the control group however, this difference was not significant (p = 0.46).

Table 5.5: Changes in mean height of the study children over the trial period

	Mean height (cm) ± SD	
Day of study	**Treatment group**	**Control group**
Baseline	70.8±4.9	72.1±6.8
Day 7	70.9±5.4	72.3±4.6
Day 14	70.9±4.9	72.7± 6.4
Day 21	71.0±5.34)	72.7± 5.8
Day 28	71.0 (±5.2)	72.74±6.8

5.3.8 Comparison of weight for height Z-scores changes between the two study groups

At the end of the study, 67 (93.1 %) of the children in the treatment group recovered from moderate wasting (Z-score equal to or greater than -2) compared to 29 (40.3 %) in the control group and this difference was statistically significant, (χ^2=44.80, df=1, $p < 0.00$). In the control group, only 4 (5.6 %) study children had a weight-for-height Z-score equal to or greater than -1 at the end of the study with a majority still having a weight-for-height Z-score equal to or less than -2 (Table 5.6). In comparison, 23 (31.9 %) of the study children in the treatment group had a weight-for-height Z-score greater than -1.

Table 5.6: Distribution of the end of study weight-for-height Z-scores of children

Wasting category	Z-score	Treatment group n=72 (%)	Control group n=72 (%)	Significance of difference
Moderate	<-2 but ≥ -3	7.0	59.7	χ^2=44.80, df=1, p < 0.00
At Risk	<-1 but ≥ -2	61.1	34.7	χ^2=9.98, df=1, p = 0.00
Normal	≥ -1	31.9	5.6	χ^2=16.33, df=1, p = 0.00
	Total	100.0	100.0	

5.3.9 Comparison of Mean Mid Upper Arm Circumference (MUAC) changes between the two study groups

As shown in Figure 5.6, baseline MUAC readings for both treatment group and control group were almost similar (12.4 ± 0.8 cm; 12.5 ± 0.8 cm respectively). The final MUAC reading in the treatment group was 13.2 ±0.8 cm while in the control group it was 12.7 ±0.8 cm however this difference was not statistically significant (p = 0.11).

At the end of the study, 58 (80.0 %) of study children in the treatment group had a MUAC equal to or greater than 12.5 cm (at risk of acute malnutrition) compared to 41 (57.1 %) in the control group. This difference was statistically significant (χ^2=8.70, df=1, p=0.00). The overall mean rate of MUAC gain was 0.03 cm/day for the treatment group and 0.01 cm/day for the control group and this difference was statistically significant (p = 0.03).

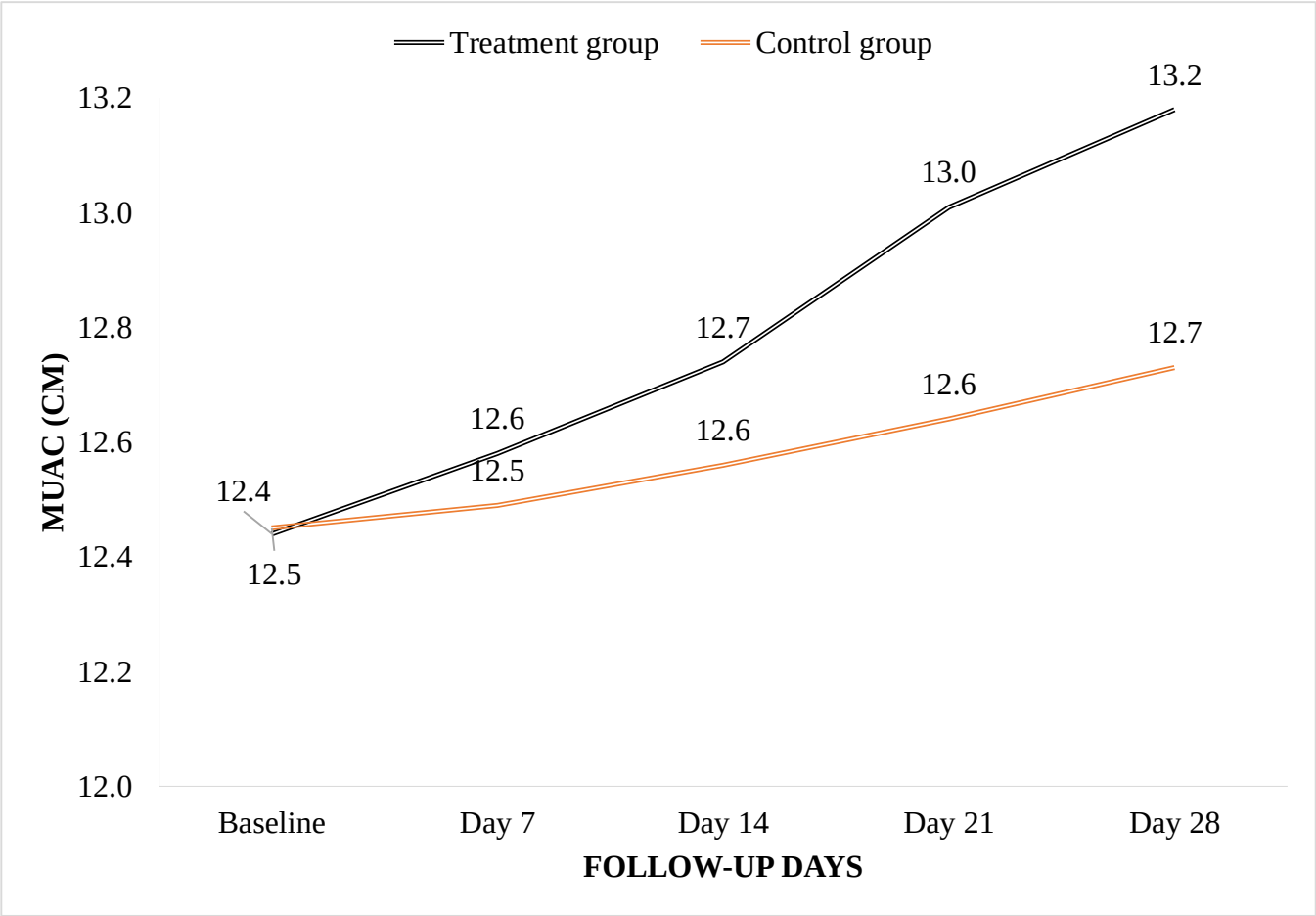


Figure 5.6: Comparison of the changes in mean MUAC of study children between the two study groups

5.3.10 Correlation between changes in anthropometric measurements and child characteristics in the treatment group

Multivariate regression models were run to investigate the correlation between change in anthropometric measurements and child characteristics in the treatment group. As shown in Table 5.7, there was a positive correlation observed between the age of the study children and their weight gain ($r = 0.41$, $p = 0.02$).

There was however no correlation between the sex of the child and their weight gain during the study period in the treatment group ($r = -0.11$, $p = 0.55$). Change in MUAC of the study children in the treatment group was positively correlated with their age ($r = 0.41$, $p = 0.01$).

Table 5.7: Correlation between changes in anthropometric measurements and child characteristics in the treatment group

Anthropometric measurement	**Age**		**Sex**	
	R-value	P-value	R-value	P-value
Mean weight (Kg)	**0.41**	**0.02**	-0.11	0.28
Mean height (cm)	0.11	0.55	0.27	0.63
Mean MUAC (cm)	**0.41**	**0.01**	-0.26	0.08

Values in bold are significantly correlated

5.3.11 Adverse reactions observed in the treatment group

As shown in Table 5.8, in total, nine (12.50 %) children from both study groups developed clinician-documented adverse reactions during the study period with six (66.67 %) and three (33.33 %) in the treatment group and control group respectively. When the significance of this difference was tested using the n-1 Chi-squared test as recommended by Campbell (2007) and Richardson (2011), it was not statistically significant ($\chi^2=1.89$, df=1, $p = 0.17$). Among the children in the treatment group, two (33.33 %) experienced diarrhoea while four (66.67 %) experienced vomiting. In the control group one child (33.33 %) experienced diarrhoea while two (66.67 %) had both diarrhoea and vomiting (Table 5.8). There was no correlation between development of adverse reactions and the age of the study child ($r = -0.08$, p

= 0.63) nor their sex (r = 0.15, p = 0.36). There was however a negative correlation between baseline weight and the development of adverse reactions (r = -0.03, p = 0.84). Children who weighed 6.50 kg and above at baseline were 0.5 times more likely to develop adverse reactions than those who weighed less than 6.50 kg at baseline.

Table 5.8: Occurrence of adverse reactions among study children

Adverse Reaction	**Treatment group n=6 (%)**	**Control group n=3 (%)**
Diarrhoea alone	33.3	33.3
Vomiting alone	66.7	0
Diarrhoea and vomiting	0	66.7

5.4 Discussion

There was no significant difference between the treatment and control groups in any of the baseline characteristics indicating that the randomization procedure was effective and the two groups were similar. Any observed change in outcome variables was therefore not attributed to the baseline characteristics. At the end of the study however most of the children in the treatment group had recovered from moderate wasting while majority of those in the control group were still moderately wasted. This was attributed to the rate of weight gain in the treatment group which was significantly higher than that in the control group as well as that attributed to lipid based nutrient supplements and corn soy blend, both of which are currently used in the treatment of moderate wasting (Thakwalakwa, 2010). These differences in outcomes can therefore be directly related to supplementation with leucine. It was also noted that, the weight of children in the treatment group continued to increase throughout the 28-day study period, but the increase was varied. This signals that weight increase could continue if the supplementation is carried out over a

longer period of time. Wasting represents a depletion of the body's fat and lean tissue mass but since lean tissues account for the largest body compartment, its rate of loss is the most significant determinant of total body weight in most cases of wasting (Porth, 2011). Treatment of moderate wasting should thus be built on a mechanism that accelerates muscle protein synthesis to make up for the significant losses incurred during the accelerated breakdown triggered by malnutrition. Current treatment methods fail to correct moderate wasting because of their emphasis on heavy calorie loading to enable gain in body fat which is wrongly assumed to contribute to overall gain in body weight (Porth, 2011). The results of the study have demonstrated that treatment of moderate wasting can be achieved with a large but safe level dose of leucine in which increased muscle protein synthesis directly contributes to weight gain in the affected children.

There was a trend of increase in the MUAC from baseline to end-of-study in both study groups. However, the rate of increase was significantly higher in the treatment group than in the control group. At the end of study, the number of children in the treatment group who had a MUAC reading above the cut-off point for acute malnutrition (12.5 cm) was significantly higher than that of the control group. During leucine supplementation, increase in body weight is recognized to be a result of an accelerated gain in lean body mass (muscle protein).

The difference in end of study MUAC between the two study groups can therefore be attributed to a greater increase in muscle protein synthesis in the treatment group related to leucine supplementation. Gain in body weight and MUAC in the treatment group were correlated to the age of the children where the older

the child the higher the rate of body weight and MUAC increase. This is consistent with the reported oscillating and increasing growth rate of children with increase in age [Karlberg, 1990].

There was no significant difference in changes in height over the study period between the two study groups. Leucine supplementation thus had no immediate impact on linear growth. However, it could possibly have an impact over a longer duration of time. This is similar to studies that have shown that linear growth follows weight increase with a lag period of approximately three months (Thakwalakwa, 2010). In addition, episodes of wasting may contribute to stunting (low height-for-age) depending on the severity, duration and recurrence especially if there is inadequate nourishment to support recovery [Fink, 2011]. By reducing the episodes and duration of wasting it would be possible to prevent the occurrence of chronic malnutrition and hence prevent stunting.

Diarrhoea and vomiting were the two adverse reactions noted to have occurred in study children. The occurrence of these adverse reactions was however not significantly different between the two study groups. Nevertheless, there were twice as many study children in the treatment group who developed adverse reactions than in the control group. The study could therefore not ascertain whether the occurrence of these adverse reactions was related to leucine supplementation.

5.5 Conclusion and recommendations

Leucine supplementation at a dosage of 150 mg/kg/day is a safe and effective way of treating moderate wasting in children aged 6 – 24 months. Leucine supplementation does not improve linear growth over 28 days. There is therefore a need to conduct a study to determine the impact of leucine supplementation on linear growth over a longer duration of time. Leucine supplements are not accessible to children with

moderate wasting thus the study recommends the formulation of a leucine-rich therapeutic food from locally available foods to enhance its accessibility and impact. The therapeutic food should deliver leucine at a dosage of 150 mg/kg/day and meet all minimum standards of complementary feeds for infants and young children.

5.6 Study limitations

This study was conducted during a time when nurses in public hospitals in Kenya had gone on strike. This limited the number of patients attending public hospitals including Mbagathi Hospital as patients sought alternative facilities for healthcare services. As such, attaining the pre-determined sample size took longer and consequently, the study ran for a longer period of time than first scheduled. However, this did not affect the credibility of the results of the study.

CHAPTER SIX: DEVELOPMENT OF A LEUCINE-RICH COMPOSITE FLOUR FOR TREATMENT OF MODERATE WASTING IN CHILDREN

Abstract

Wasting, categorized as either severe or moderate, is a form of child malnutrition that manifests with a low weight-for-height Z-score. There was previously no effective treatment for moderate wasting, which affects approximately 300,000 children in Kenya, because all prior treatments lacked a mechanism to replace the accelerated loss of lean tissue. Supplementation with leucine, has however proven to be a safe and effective method for treating moderate wasting. At a high dosage, leucine activates the mammalian target of rapamycin within the muscles which enhances gain of lean tissue. Leucine supplements are currently inaccessible to populations affected by moderate wasting in Kenya. The objective of this study was therefore to formulate a leucine-rich composite flour (TheraPEM) from locally available foods for treatment of moderate wasting. Six composite flours were prepared using unique combinations of beans (*Phaseoulus vulgaris*), groundnuts (*Voandzeia subterranea*), and foxtail millet (*Setaria italica*) selected for their high leucine content, high local availability and relatively low cost. Nutrient composition analysis and sensory evaluation were conducted on each of the six flours. The three preferred flours in terms of sensory attributes were subjected to accelerated shelf-life evaluation to determine changes in peroxide value, fat acidity, moisture content and Total Viable Count. Kraft paper, gunny bags and plastic containers were the packaging materials used.All six flours met the Codex Alimentarius food standards for minimum energy density (80 kcal/100g) and maximum fat content (27 %) in processed cereal-based foods used for complementary feeding of infants and young children. They all also met the required > 1050 mg leucine per 100 grams of flour.

Formulations 2, 3 and 5 had the most preferred sensory attributes and were thus subjected to accelerated shelf-life evaluation. At the fifth month, fat acidity was least in the flours packaged in plastic containers.

There was no peroxide formation in any of the three samples during the storage period. The study generated six formulations that meet the minimum requirement for leucine in treatment of moderate wasting, of which three (formulation 2, 3 and 5) are the most preferred based on sensory attributes. It is recommended that these three be subjected to a study to further validate their effectiveness in the treatment of moderate wasting prior to releasing them for up-scaled use.

6.1 Introduction

Wasting is a form of malnutrition that is diagnosed in children when their weight for height z-score is below minus two standard deviations from the median of the reference population. It is classified as either: moderate, when the weight for height z-score is greater than or equal to minus 3 but less than minus 2 ($-3 \leq$ z-score < -2), or severe where the z-score is less than minus 3 (z-score < -3) (WHO, 2004). Wasting represents a depletion of two body compartments: the body's lean tissue and fat mass. Lean tissue is the largest body compartment and thus its rate of loss is the most significant determinant of total body weight in most cases of wasting (Porth, 2011). There is no standardized treatment for moderate wasting which affects more than 33 million children globally and approximately 300,000 children in Kenya (FAO, 2017).

Current treatment methods have limited effectiveness. Lipid-based supplements, which are current treatment methods used, do not reduce the risk of advancement to severe wasting or mortality (Lazzerini, 2013). Corn soy blend, which is also used in the treatment of moderate wasting, has no significant effect on the weight-for-height Z-score of moderately wasted children (Thakwalakwa et al, 2010).

Treatment of moderate wasting should be built on a mechanism that accelerates muscle protein synthesis to make up for the significant losses incurred during the accelerated breakdown triggered by malnutrition. Current treatment methods fail to correct moderate wasting because of their emphasis on heavy calorie

loading to enable gain in body fat which is wrongfully assumed to contribute to overall gain in body weight (Wamiti et al, 2018).

In a clinical trial conducted on the effectiveness of leucine supplementation in the treatment of moderate wasting in children, it was shown that when administered at a dosage of 150mg/kg bodyweight/day it resulted in recovery (z-score ≥ -2) for majority (93 %) of the study children in the treatment group. Majority of those in the control group (60 %) remained wasted and leucine supplementation was thus proven to be effective in the treatment of moderate wasting (Wamiti et al, 2018). When administered at a dosage of 150mg/kg bodyweight/day, leucine functions as a nutraceutical by activating the mammalian target of rapamycin in muscles which triggers protein translation resulting in accelerated protein synthesis and growth (Glynn et al, 2010). This is what ultimately contributes to a significant gain in lean body mass and total body weight.

A nutraceutical is defined as a nutrient or nutritional component that occurs naturally and may have been purified and/or concentrated and that is used to improve health through prevention and/or treatment of disease(s) (Lockwood, 2007). For leucine to function as a nutraceutical by accelerating muscle protein responses, it is a prerequisite that its dosage is higher than that typically found even in high-quality protein food sources (Glynn et al, 2010). This is because primarily being an amino acid, it must be sufficient in the body to first satisfy all structural roles before it can then engage in signalling and metabolic roles.

As a result, the capacity of leucine to function as a nutraceutical is based on sufficient intracellular concentration (Churchward-Venne et al, 2012). Leucine supplements are currently used by strength-athletes to promote muscle growth and retention after strength training. They are made in either powder or capsule form both of which are expensive and inaccessible to the populations in Kenya that are most

affected by wasting. As a result, it is necessary to locally develop a leucine-rich therapeutic food from foods that are rich in the amino acid yet locally available and accessible for use in treatment of moderate wasting. The objective of this study was therefore to formulate a leucine-rich composite flour (TheraPEM) from locally available foods for use in the treatment of moderate wasting. The therapeutic food was required to deliver ≥ 1050 mg leucine/kg bodyweight/day to the users in order to confer its nutraceutical properties.

6.2 Materials and methods

Product development was carried out in four distinct but interrelated steps that included; nutrient optimization and formulation of composite flours, nutrient composition analysis, sensory evaluation and shelf life evaluation.

6.2.1 Food ingredients

The food ingredients that were selected for use in formulation of the leucine-rich composite flour included: bean (*Phaseoulus vulgaris*), groundnut (*Voandzeia subterranea*), and foxtail millet (*Setaria italica*). These ingredients were selected because of their high availability and accessibility in Kenya food markets as they are produced locally by farmers. In addition to this, compared with other locally produced food crops, they have the highest leucine content and relatively lower cost as shown in Table 6.1.

The food ingredients were procured at Kangemi Market, Nairobi and stored at the Chemistry laboratory at the Department of Food Science, Nutrition and Technology, University of Nairobi.

Table 6.1: Leucine content and market price of selected foods locally available in Kenya

	Ingredients	**Leucine content* (g/kg)**	**Price (KES/kg)**
1	foxtail millet (*Setaria italica*)	17.64	90

2	Bean (*Phaseoulus vulgaris*)	16.85	90
3	Groundnut (*Voandzeia subterranea*) whole	13.85	110
4	Maize (*Zea mays*) grain or whole meal	11.90	49
5	Millet (*Pennisetum spp.*) grain	9.27	90
6	Wheat (*Triticum spp.)* whole grain	8.71	90
7	Rice (*Oryza spp.*) brown or husked	6.48	158
8	Potato (*Solanum tuberosum*)	1.21	40
9	Sweet potato (*Ipomoea batatas*)	0.71	38
10	Cassava (*Manihot esculenta*) meal	0.64	26

*Dry matter basis

Adapted from (FAO, 1981)

6.2.2 Formulation and optimization

As shown in Figure 6.1, the beans were first sorted to remove foreign matter then washed under running water and soaked overnight to reduce the phytochemical content which has known antinutritional effects (Martino, 2012). Afterwards, the beans were boiled in water for two hours then dried in an air oven at 100ºC overnight after which they were to a fine powder ground using a laboratory hammer mill. The powder was sieved to remove large particles then stored in a clean dry container. Afterwards, the millet was sorted and sieved to remove all foreign matter after which it was washed under running water.

The millet grains were then placed in a tray lined with a moist muslin cloth and covered with another then stored for 28 hours so that they could germinate. Prior to removal from the muslin cloth, the sprouts of the grains were observed, to ensure they had reached a length approximately that of the grain. The grains were then placed in air oven at 100ºC overnight to stop the germination process and dry after which they were ground to a fine powder using a laboratory hammer mill. Germination of millet was done to increase

the free amino acids within the grain and reduce antinutrients (Ahmed, 2013). The powder was finally sieved then stored in a clean dry container.

On completion of this, the groundnuts were sorted to remove all foreign matter then washed under running water. Afterwards, the groundnuts were dried overnight in an air oven at 100ºC then roasted to enhance colour, flavour and aroma. The roasted groundnuts were again sorted to remove the burnt groundnuts after which they were ground together using a laboratory hammer mill with the other two ingredients (millet and beans) to a fine powder. This was done to prevent the groundnuts producing peanut butter which occurs when they are ground alone. The flours were stored in dry aseptic plastic containers to avoid contamination prior to mixing in the defined ratios.

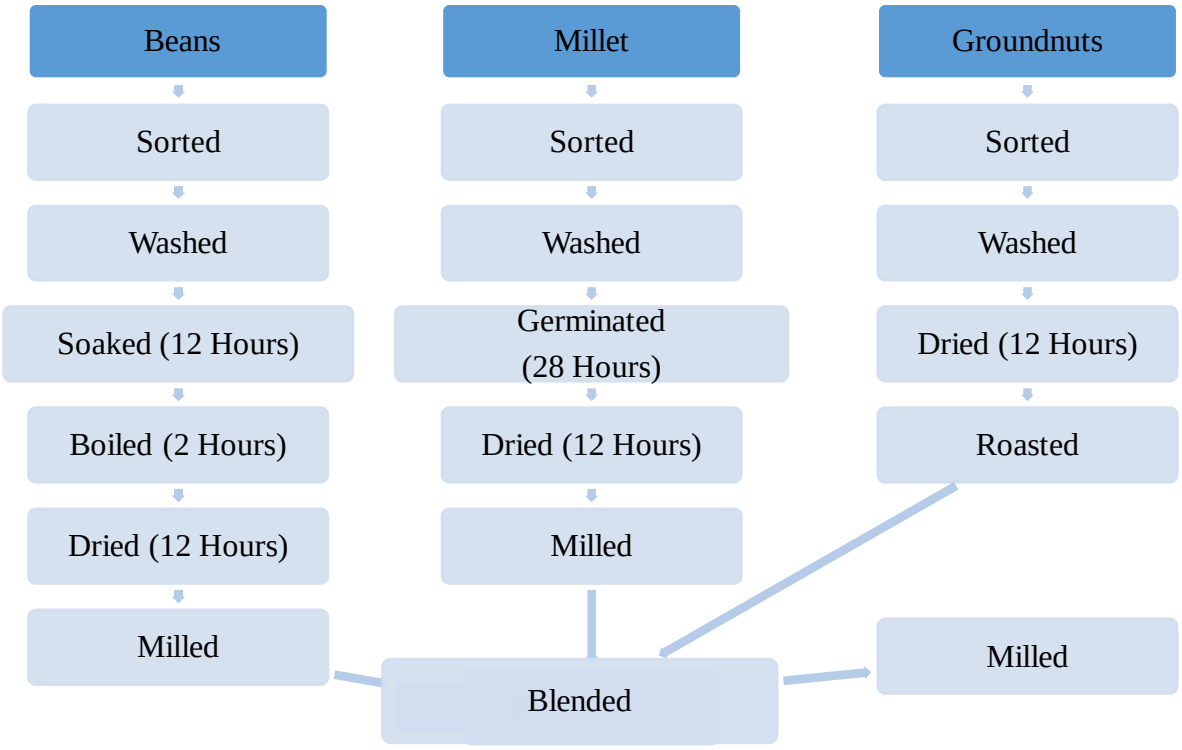


Figure 6.1: Process flow diagram for the development of the leucine-rich composite flours

A diet optimization model that uses linear programming was used to find unique combination of the three food ingredients (decision variables) that maximized the leucine content of the therapeutic food. The model provided six combinations (Table 6.2) that met this objective while fulfilling the constraint on the individual leucine content of the food ingredients. Three of these unique combinations were selected to be the leucine-rich therapeutic foods through sensory analysis.

Table 6.2: Ratios of beans, groundnuts and millet in each formulation.

Formulation	Beans (g)	Groundnuts (g)	Millet (g)	Total (g)
1	500	200	300	1000
2	300	200	500	1000
3	400	200	400	1000
4	550	150	300	1000
5	300	150	550	1000
6	300	100	600	1000

6.2.3 Nutrient composition analysis

Proximate analysis was conducted on each of the six formulations using standard AOAC procedures. Moisture content was determined through drying of the flour samples in an air oven at 105ºC whereas crude fat was determined through extraction in a Soxhlet extractor followed by evaporation in a rotary evaporator then drying in an air-oven for one hour at 105ºC. Crude protein was determined through boiling a mixture of the food sample and sulphuric acid in a Kjeldahl flask then distillation and back-titration with a sodium hydroxide solution. Total ash was determined by measuring the residue on ignition at 550ºC and crude fibre through digesting the food sample in sulphuric acid and sodium hydroxide solutions then ignition of the residue at 550ºC (AOAC, 2005). The results obtained were used to calculate the carbohydrates and energy in the formulations using the formula below.

% Carbohydrates = 100 – [% moisture content + % fibre + % ash + % protein + % fat]

Energy (Kcal/100g) = [fat (g/100g) × 9] + [protein (g/100g) × 4] + [carbohydrates (g/100g) × 4]

The branched-chain amino acid profiles of the formulations were determined using acid hydrolysis of the composite flour to release amino acids from the protein. This was followed by pre-column derivatization

with omicron-phthaldialdehyde (OPA) for ease of analysis. omicron-Phthaldialdehyde in the presence of mercaptan reacted rapidly with primary amino acids to form intensely fluorescent derivatives which were then separated on a reverse-phase Ultra Performance Liquid Chromatography (UPLC) with fluorescent detection hydrolysis (FAO, 2003).

6.2.4 Sensory evaluation

Sensory attributes of the composite flours were evaluated by 10 trained panellists for: taste, flavour, colour, mouth feel, odour and general acceptability of a porridge made from each of the six flours. For each of the composite flours, 150 mg was mixed in 250 ml of cold water. The mixture was then poured into 1000 ml of boiling water in a cooking pot and cooked for 15 – 20 minutes while constantly stirring then finally emptied into a storage jar. Different cooking utensils and storage jars were used to prepare and store each of the six porridges to avoid contamination.

Scoring of each of the porridges was then carried out on a scoring sheet using a seven-point hedonic rating scale where 7 = like very much and 1 = dislike very much for the evaluation of the six sensory attributes. The composite flours were then ranked based on their sensory attributes and the top three flours subjected to shelf-life evaluation.

6.2.5 Accelerated shelf life evaluation

Accelerated shelf life evaluation was conducted on the composite flours to determine the length of time the product would retain specific desired qualities including: acceptable microbial count, taste, appearance and odour. The formulations were stored in an air oven set at 55°C in three packages: Kraft paper bags, gunny bags and plastic containers for five days each day representing a month in storage. The foods were

analysed pre-storage and monitored every day in storage for changes in: fat acidity, peroxide value, moisture content and growth of yeasts and moulds.

Fat acidity was measured as the milligrams of potassium hydroxide required to neutralize the free acid in a one-gram sample of the flour. Peroxide value was determined by mixing a gram of the flour sample in a solution of potassium iodide and acetic acid followed by titration with a solution of sodium thiosulphate and starch. Moisture content was determined through drying in an air oven at 105ºC and weighing of the residue (AOAC, 2005). Yeasts and moulds were enumerated as the Total Viable Counts (TVCs) through the streak-plate technique. This involved serial dilutions of the food samples using a diluent (0.85% sodium chloride and distilled water) followed by streaking on potato dextrose agar in a petri dish (ISO, 2001).

6.2.6 Statistical analysis

Proximate analyses, branched-chain amino acid profiling and shelf-life evaluation were carried out in triplicate ($n = 3$). All values were entered in Microsoft Excel® and uploaded onto GenStat 15th Edition SP1 (32 bit) for analysis to obtain means and standard deviations. A two-way ANOVA was used to detect the significance of differences ($p < 0.05$) between the six formulations.

Sensory evaluation data ($n = 10$) was entered into Statistical Package for Social Sciences® (SPSS®) Version 20 for analysis where the means and standard deviations were computed for each sensory attribute. An overall mean score for all six of the sensory attributes was also computed.

6.3 Results and Discussion

6.3.1 Proximate composition

As shown in Table 6.3, there were significant differences ($p < 0.05$) in the fat, crude protein, crude fibre and energy of the six formulations. There were however no significant differences in the moisture content and total ash in all six formulations. The moisture content of all six formulations was below the maximum moisture content recommended for composite flours (13.5 %) (EAC, 2012). Formulation 1, however, had the highest moisture content (5.5 %) as well as the highest ash content (3.0 %) while formulation 4 had the lowest moisture content (4.6 %) but the highest crude fibre (6.6 %). The lower the moisture content of a flour, the higher its storage and microbial stability (Chukwu, 2015).

The Codex Alimentarius International food standards require that processed cereal-based foods for complementary feeding of infants and young children have an energy density of no less than 0.8 kcal/g (80 kcal/100g) and maximum fat content of 27 % (Joint FAO/WHO Codex Alimentarius Commission, 2017). All six formulations met this criterion. Formulation 2, which comprised of 20 % groundnuts, had the highest fat (13.0 %) and energy content (418Kcal/100g), while formulation 6 which had the lowest fat (5.0 %) and energy content (371.9 kcal/100g) consisted of the least amount of groundnuts (10 %).

This is because a 100 g serving of groundnuts contains 49.2 g of total fat which provides 165 % of the Recommended Daily Allowance (RDA) and 567 kcal of energy (29 % of RDA). Formulation 4, with the highest amount of beans (55 %), had the highest crude protein (17.95 g/100g), while formulation 6, with the lowest amount of beans (30 %) and groundnuts (10 %), had the lowest amount of crude protein (13.44 g/100 g).

Groundnuts contain 25.8 % crude protein (Arya, 2016) while the crude proteins in beans varies between 15 – 30 % both on dry matter basis (Martino, 2012). Millet has the lowest amount of protein (12.3 %) (Sharma, 2017) and therefore groundnuts and beans were ultimately the greatest contributors of crude protein in the formulations.

Table 6.3: Proximate composition of the formulations

Formulation	(g/100g sample)						Energy (Kcal/100g)
	Moisture content	Fat	Crude Protein	Total Ash	Crude Fibre	Carbohydrates	
1	5.5[a]	10.7[a]	17.4[a]	3[a]	3.9[a]	59.6[a]	404.1[a]
2	5.4[a]	13.0[b]	14.5[b]	2.8[a]	3.4[a]	60.9[a]	418.5[b]
3	5.4[a]	12.1[b]	16.7[a]	2.9[a]	4.5[a]	58.4[a]	409.2[a]
4	4.6[a]	9.0[a]	18.0[a]	3.0	6.6[b]	59.0[a]	388.4[c]
5	5.2[a]	10.8[b]	17.8[a]	2.7[a]	4.8[a]	58.7[a]	403.5[a]
6	5.1[a]	5.0[c]	13.4[b]	2.8[a]	5.3[a]	68.3[b]	371.8[c]

[a] Values in the same column with different lowercase superscript letters are significantly different ($p < 0.05$)

6.3.2 Branched-chain Amino Acid Profile

All six formulations contained above 1.1 g of leucine (the minimum requirement in moderate wasting) in 100 g of the product. As shown in Table 6.4, there was no significant difference ($p < 0.05$) in the leucine, isoleucine and valine content of the six formulations. Formulation 6, however, had the highest amount of leucine (1.4 g/100g) and the lowest amount of isoleucine (1.1 g/100g) and valine (0.8 g/100g). This was because it had the highest amount of millet (60 %) and millet was the ingredient used richest in leucine (1.8 g/100g). Formulation 4, which had only 30 % millet had the lowest amount of leucine (1.2 g/100g), but the highest amount of isoleucine (1.4 g/100g) and valine (0.9 g/100g).

It is a requirement that all three branched-chain amino acids (BCAAs) are consumed concurrently since all three compete for cell transport and metabolism. Ingesting leucine alone may lead to depletion of plasma valine and isoleucine nonetheless leucine should be the dominant BCAA (Cynober, 2012).

Table 6.4: Branched-chain amino acid profile of the formulations

Formulation	**g/100g product**		
	Leucine	**Isoleucine**	**Valine**
1	1.2[a]	1.4[a]	0.9[a]
2	1.3[a]	1.2[a]	0.9[a]
3	1.2[a]	1.3[a]	0.9[a]
4	1.2[a]	1.4[a]	0.9[a]
5	1.4[a]	1.2[a]	0.9[a]
6	1.4[a]	1.1[a]	0.8[a]

[a] Values in the same column with different lowercase superscript letters are significantly different ($p < 0.05$)

6.3.3 Sensory Evaluation

All six sensory attributes were ranked on a seven-point hedonic scale by 10 trained panellists after which a mean of each was computed to establish the overall score of the six porridges made from the six formulations. As shown in Table 6.5, there were significant differences ($p < 0.05$) in the perceived taste, flavour, colour, mouth feel and general acceptability of the six porridges made from each of the six formulations. The only exception was in the odour of the porridges, where there was no significant difference among all six. Formulation 3, which had a ratio of Beans: groundnuts: millet of 2:1:2, had the most preferred taste (5.2 ± 0.9), flavour (5.0 ± 0.9), colour (5.9 ± 0.7), mouth feel (4.6 ± 1.3) and general acceptability (5.4 ± 1.0). It also had the highest overall mean score among all six sensory attributes (5.3).

Formulation 5, which had the highest amount of millet (55 %), had the most preferred odour (5.6 ± 1.2). Formulation 4 had the highest amount of beans (55 %) compared to the other formulations and was the least preferred in taste (2.1 ± 0.9), flavour (3.0 ± 0.9), colour (4.5 ± 1.9), general acceptability (3.8 ± 1.5) and ultimately it had the lowest mean score (3.7 ± 1.0). Formulation 1, with the second highest amount of beans (50 %) had the least preferred mouth feel (2.5 ± 1.6) and odour (4.7 ± 1.6). A high amount of bean flour was therefore related to a low acceptability of the sensory attributes of the composite flour while a high amount of millet flour was associated with a desirable odour. A ratio of beans: millet of 1:1 however, gives the composite flour the most desirable sensory qualities. Formulations 2, 3 and 5 were the only ones subjected to shelf-life evaluation since they were the most preferred of the six.

Table 6.5: Sensory attributes of the formulations

Formulation	Sensory Attributes (1 – 7)						Mean score
	Taste	Flavour	Colour	Mouth feel	Odour	General acceptability	
1	3.1 ± 1.9[a]	3.4 ± 1.4[a]	5.3 ± 1.1[a]	2.5 ± 1.6[a]	4.7 ± 1.6[a]	4.2 ± 1.6[b]	3.9 ± 1.1[a]
2	4.3 ± 1.3[b]	4.7 ± 1.4[b]	5.3 ± 1.3[a]	4.1 ± 1.4[b]	5.2 ± 1.0[a]	5.1 ± 1.2[a]	4.8 ± 0.5[b]
3	5.2 ± 0.9[b]	5.0 ± 0.9[b]	5.9 ± 0.7[a]	4.6 ± 1.3[b]	5.4 ± 1.5[a]	5.4 ± 1.0[a]	5.3 ± 0.4[b]
4	2.1 ± 0.9[c]	3.0 ± 0.9[a]	4.5 ± 1.9[b]	3.8 ± 1.4[b]	5.0 ± 1.1[a]	3.8 ± 1.5[b]	3.7 ± 1.0[a]
5	4.2 ± 1.6[b]	4.3 ± 1.2[b]	5.5 ± 1.3[a]	4.5 ± 1.6[b]	5.6 ± 1.2[a]	5.1 ± 1.0[a]	4.9 ± 0.6[b]
6	3.6 ± 1.5[a]	4.2 ± 0.9[b]	5.2 ± 1.6[a]	3.3 ± 1.8[a]	4.7 ± 1.6[a]	4.5 ± 1.4[a]	4.3 ± 0.7[a]

[a] Values in the same column with different lowercase superscript letters are significantly different ($p < 0.05$)

6.3.4 Total Viable Count (TVC)

Growth of yeasts and moulds progressively increased with length of storage. As shown in Table 6.6, by the third day of storage, the TVCs of all six formulations were significantly different ($p < 0.05$) from their

pre-storage value. On the fifth day, formulation 2 packaged in a Kraft paper had the highest number of coliforms (3.2×10^8 CFU/gram). In comparison, formulation 2, 3 and 5 packaged in plastic containers had the lowest number of coliforms compared to the other two packaging materials on the fifth day (1.5×10^8, 7.6×10^7, and 2.8×10^7 CFU/gram respectively). The gradual but varied increase in the TVC in all three formulations indicated a high nutrient availability. In addition to this, the increase in TVC in storage also indicated favourable environmental conditions such as humidity as well as atmospheric gases including carbon dioxide and oxygen (Adejumo, 2012). The lower microbial proliferation in the samples stored in the plastic container implied a lower permeability to these environmental conditions compared to the other packaging materials.

Table 6.6: Total viable count of formulations 2, 3 and 5 during storage

		CFU/gram					
Sample	**Packaging**	**Day 0**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**
2	**Gunny bag**	1.4×10^{3a}	2.6×10^{3a}	5.7×10^{4a}	7.1×10^{5b}	9.2×10^{7c}	2.1×10^{8c}
	Plastic container	1.4×10^{3a}	2.3×10^{4a}	1.1×10^{4a}	7.9×10^{5b}	1.7×10^{7c}	1.5×10^{8c}
	Kraft paper	1.4×10^{3a}	2.6×10^{3a}	5.0×10^{4a}	1.1×10^{6b}	1.2×10^{7b}	3.2×10^{8c}
3	**Gunny bag**	1.8×10^{3a}	3.3×10^{3a}	1.1×10^{4a}	8.1×10^{5b}	1.2×10^{7c}	2.2×10^{8c}
	Plastic container	1.8×10^{3a}	6.9×10^{3a}	8.3×10^{3a}	7.2×10^{5b}	6.0×10^{6b}	7.6×10^{7c}
	Kraft paper	1.8×10^{3a}	6.9×10^{3a}	8.8×10^{3a}	4.2×10^{5b}	4.6×10^{6b}	8.9×10^{7c}
5	**Gunny bag**	2.5×10^{3a}	3.2×10^{3a}	3.9×10^{4a}	8.8×10^{6b}	1.2×10^{7b}	9.3×10^{7b}
	Plastic container	2.5×10^{3a}	2.5×10^{4a}	3.0×10^{4a}	1.2×10^{6b}	1.2×10^{6b}	2.8×10^{7b}
	Kraft paper	2.5×10^{3a}	1.2×10^{4a}	3.5×10^{4a}	3.9×10^{6b}	6.4×10^{6b}	5.2×10^{7b}

[a] Values in the same row with different lowercase superscript letters are significantly different from the Day 0 value ($p < 0.05$)

6.3.5 Fat acidity

Fat acidity (acid value) is a measure of the extent to which glycerides in the formulations have been decomposed by the action of the enzyme lipase. This is a process that is accelerated during storage due to the presence of heat and light and is an indicator of the condition and edibility of the food. It is measured

by the milligrams of potassium hydroxide required to neutralize the free acids in the food sample. (Eldin, 2010). As shown in Table 6.7, after one day of storage, there were significant changes ($p < 0.05$) in the acid value of formulation 2 packaged in a gunny bag and plastic container as well as formulations 3 and 5 packaged in the Kraft paper. Formulations 3 and 5, both packaged in gunny bags only, had significant changes in acid value after three days of storage. In addition, after three days of storage all three formulations except formulation 5 in the Kraft paper still met the minimum acceptable acid value (50 mg KOH/100g) as specified in the East African Community standards for composite flours (EAC, 2012).

On the fifth day of storage, all samples in all the three packaging materials had exceeded the minimum acceptable acid value. Formulation 5 had the highest increase in fat acidity with an increase of +61.4 mg/100g in the gunny bag, +60.9 mg/100g in the Kraft paper and +54.7 mg/100g in the plastic container. Formulation 3 had the lowest increase with an increase of +30.2 mg/100g in the plastic container, +31.7 mg/100g in the gunny bag and +33.1 mg/100g in the Kraft paper. Formulations 2, 3 and 5 stored in the plastic container had the lowest final fat acidity compared to those stored in the other packaging materials (74.6, 57.9 and 83.0 mg/100g respectively).

Table 6.7: Free acidity of sample 2, 3 and 5 during storage

Sample	Packaging	Fat acidity (mg KOH/100g)					
		Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
2	Gunny bag	27.5[a]	40.5[b] (+13.0)	43.5[b] (+16.0)	45.4[b] (+17.9)	47.8[b] (+20.3)	81.2[b] (+53.7)
	Plastic container	27.5[a]	35.9[b] (+8.4)	38.4[b] (+10.9)	42.0[b] (+14.5)	51.7[b] (+24.2)	74.6[b] (+47.1)
	Kraft paper	27.5[a]	31.9[a] (+4.4)	35.1[b] (+7.6)	39.8[b] (+12.2)	65.8[b] (+38.3)	76.4[b] (+48.9)
3	Gunny bag	27.7[a]	31.4[a] (+3.7)	33.9[a] (+6.1)	35.1[b] (+7.4)	52.6[b] (+24.9)	59.4[b] (+31.7)
	Plastic container	27.7[a]	33.7[a] (+6.0)	37.7[b] (+10.0)	45.4[b] (+17.6)	49.7[b] (+22.0)	57.9[b] (+30.2)
	Kraft paper	27.7[a]	38.4[b] (+10.7)	42.6[b] (+14.9)	44.3[b] (+16.5)	45.0[b] (+17.3)	60.8[b] (+33.1)
5	Gunny bag	28.3[a]	28.9[a] (+0.6)	33.4[a] (+5.1)	39.1[b] (+10.7)	71.6[b] (+43.2)	89.7[b] (+61.4)
	Plastic container	28.3[a]	34.1[a] (+5.8)	46.8[b] (+18.4)	48.3[b] (+20.0)	49.6[b] (+21.3)	83.0[b] (+54.7)
	Kraft paper	28.3[a]	36.6[b] (+8.3)	39.6[b] (+11.2)	50.6[b] (+22.3)	56.1[b] (+27.8)	89.2[b] (+60.9)

[a] Values in the same row with different lowercase superscript letters are significantly different from the Day 0 value ($p < 0.05$)

[*]Figures in brackets represent percent change from Day 0 values.

6.3.6 Peroxide value

The peroxide value was used to estimate the overall oxidation status of the fats in the formulations. It measures the hydroperoxides and lipid peroxides formed in the primary phase of oxidation (induction period) (Eldin, 2010). There was no peroxide formation in any of the three samples during the storage period. This was anticipated because peroxide formation during storage is slow at first during the incubation period which ranges from a few weeks to several months depending on the oils in the food as well as storage temperature. High peroxide value early in storage negatively impacts on the storage stability of the food (Decker, 2010).

6.3.7 Moisture content

Moisture content of the 3 formulations steadily declined in all packaging materials during storage as seen in Table 6.8. Formulation 2 and 3, stored in plastic containers, only had significant changes ($p < 0.05$) in moisture content from the pre-storage value on the fifth day. In comparison, those stored in the gunny bag and Kraft paper had a significant change in moisture by the fourth day. Formulation 5 stored in the plastic container had a significant change in moisture content on the fourth day while that in the gunny bag and Kraft paper significantly changed on the third day. This indicates that the gunny bags and Kraft paper permit a higher rate of moisture content loss in storage compared to the plastic containers. Formulation 5 had the lowest moisture content at day 5, with the samples stored in the gunny bag, plastic container and Kraft paper having 0.0 %, 0.1 % and 0.1 % respectively. It was also the formulation with the largest decrease in moisture content during storage, with the sample stored in the gunny bag having a change of -7.3 %, that in the plastic container, -7.2 % and that in the Kraft paper, -7.2 %.

Formulation 2 stored in the gunny bag had the smallest change in moisture content (-3.5 %) and consequently the highest moisture content on the fifth day (3.0 %). These changes in moisture content reflects the permeability of the packaging materials to environmental conditions in storage including temperature which cause evaporative loss of moisture from the flours. The rate of moisture loss would vary in different environmental conditions of storage reflective of the differing climatic conditions in separate regions.

Table 6.8: Moisture content of Formulation 2, 3 and 5 during storage

		Moisture Content (%)					
Formulations	**Packaging**	**Day 0**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**
2	Gunny bag	6.4[a]	5.0[a]	4.7[a]	3.5[a]	3.5[b]	3.0[b]
			(-1.4)	(-1.7)	(-2.9)	(-2.9)	(-3.4)
	Plastic container	6.4	5.2[a]	4.6[a]	4.5[a]	4.0[a]	1.0[b]
			(-1.2)	(-1.8)	(-1.9)	(-2.4)	(-5.4)
	Kraft paper	6.4	5.7[a]	5.2[a]	4.2[a]	2.7[b]	1.1[b]
			(-0.7)	(-1.2)	(-2.2)	(-3.7)	(-5.3)
3	Gunny bag	6.4	4.8[a]	4.7[a]	3.4[a]	1.5[b]	0.5[b]
			(-1.6)	(-1.7)	(-3.0)	(-4.9)	(-5.9)
	Plastic container	6.4	4.6[a]	4.5[a]	4.2[a]	4.1[a]	0.3[b]
			(-1.8)	(-1.9)	(-2.2)	(-2.3)	(-6.1)
	Kraft paper	6.4	4.5[a]	4.2[a]	3.1[b]	1.5[b]	0.8[b]
			(-1.9)	(-2.2)	(-3.3)	(-4.9)	(-5.6)
5	Gunny bag	7.3	5.8[a]	5.3[a]	2.6[b]	1.9[b]	0.0[b]
			(-1.5)	(-2.0)	(-4.7)	(-5.4)	(-7.3)
	Plastic container	7.3	6.1[a]	5.1[a]	4.8[a]	3.3[b]	0.1[b]
			(-1.2)	(-2.2)	(-2.5)	(-4.0)	(-7.2)
	Kraft paper	7.3	5.3[a]	4.5[a]	4.3[b]	2.5[b]	0.1[b]
			(-0.2)	(-2.8)	(-3.0)	(-4.8)	(-7.2)

[a] Values in the same row with different lowercase superscript letters are significantly different from the Day 0 value ($p < 0.05$)

[*]Figures in brackets represent percent change from Day 0 values.

6.4 Conclusion

All six formulations met the minimum leucine requirement (1050 g/100 g serving). Each contains sufficient amounts of isoleucine and valine to permit a ratio of the three branched-chain amino acids that would prevent the plasma depletion of either of the three. The formulations meet the minimum energy density and maximum fat content standards for processed cereal-based foods for infants and young children as prescribed by the Codex Alimentarius International Food Standards. Formulation 2, 3 and 5 were the most preferred based on sensory attributes.

6.5 Recommendations

It is recommended that further studies be conducted to determine the effectiveness of the formulations in the management of moderate wasting in children with priority given to the most preferred three. To slow down the decomposition of the fats by the action of lipase and to slow down microbial (yeasts and moulds) growth, plastic containers are recommended as the most effective packaging material for the composite flours. To improve the shelf-stability of the formulations a chemical preservation method should be considered to control growth of yeasts and moulds that is expected beyond the third month of storage.

6.6 Further research

The digestibility, bioavailability as well as antinutrient content of TheraPEM was not determined nor the possible reducing leucine content during storage, these should be investigated.

CHAPTER SEVEN: GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

7.1 General discussion

The main objective of this study was to determine the effectiveness of leucine supplementation in treating moderate wasting in children aged 6 – 24 months. The study also established the household socioeconomic factors and child dietary intake associated with moderate wasting in children living within an urban residence. Contrary to the null hypothesis that household socioeconomic factors had no association with the children's energy intake, women headed households, polygamous households and households with a high dependency ratio were the risk factors for moderate wasting in children. This caused poor intra-household food distribution ultimately leading to inadequate food intake by children. Majority of moderately wasted children however had an adequate frequency of feeding per day however the meals did not meet the children's daily requirement for carbohydrates, fat and proteins. These three are the main sources of total energy intake and therefore insufficient intake causes catabolism of fat and lean tissue to provide the body with energy resulting in child wasting (Katona, 2008).

Supplementation with leucine has the potential to treat moderate wasting in children through acceleration of muscle protein synthesis which results in increases in lean body mass and body weight. Leucine supplementation activates the messenger ribonucleic acid translational machinery through mammalian target of rapamycin resulting in acceleration of protein translation through the phosphorylation of the eukaryotic initiation factor 4E binding protein 1 (4E-BP1) and the ribosomal protein S6 kinase (S6K) leading to cell growth and proliferation (Datta, 1999; Gallagher, 2007; Hara, 2002). Contrary to the null hypothesis that leucine supplementation had no effect on moderate wasting, the clinical trial showed that leucine supplementation resulted in recovery from moderate wasting in over 90 % of study children. This was indicated by a significant increase in body weight and MUAC of children and improved nutrition status. Leucine supplementation did not have any significant effect on linear growth over four weeks.

Further studies on supplementation with leucine on moderately wasted children for longer than four weeks might reveal associations on linear growth. Some observed adverse reactions were diarrhoea, vomiting and diarrhoea and vomiting combined. There was no significant difference in the occurrence of these adverse reactions between the treatment group and the control group. Diarrhoea and vomiting are common in developing countries where hygiene and sanitation can be suboptimal and it causes rapid weight loss if not managed promptly (WHO, 2014). Given that leucine supplements are expensive and inaccessible to the vulnerable populations that can benefit from them, a leucine-rich therapeutic food was developed for use in the treatment of moderate wasting.

Six composite flours were formulated for use in the treatment of moderate wasting comprising different ratios of beans, millet and groundnuts, selected for their leucine content and local availability. All six formulations meet the minimum leucine requirement for treatment of moderate wasting (150 mg/kg bodyweight/day) and the Codex Alimentarius International food standards for minimum energy density and maximum fat content. A high amount of beans in the composite flour was related to a low acceptability of its sensory attributes while a high amount of millet flour was associated with a desirable odour. A ratio of beans: millet of 1:1 gave the composite flour the most desirable sensory qualities. During storage, there were significant increases in TVCs and acid value of the composite flours as well as significant losses in moisture content indicating spoilage. These changes were however significantly less in the formulations stored in a plastic container compared to a Kraft or gunny bag. The plastic container is the preferred storage material for the composite flour in order to ensure shelf-stability for up to three months. Plastic containers are less permeable to environmental conditions such as air, moisture and temperature, all which contribute to food spoilage (ref).

7.2 Conclusion

Households with a high-dependency ratio, those that are polygamous as well as those that are female-headed are the socioeconomic risk factors for moderate wasting in children living in an urban residence. Majority of children who are moderately wasted have adequate frequency of meal consumption but they do not meet their daily energy requirement. This is as a result of the meals consumed by the children having a low energy density resulting in muscle and fat catabolism to provide energy and therefore causing wasting. Leucine supplementation is a safe and effective way to enhance muscle protein synthesis and reduce loss of lean mass in catabolic conditions. At a dosage of 150 mg/kg/day, leucine leads to a significant gain in weight in moderately wasted children and therefore leading to recovery from the condition. There are no adverse reactions related to leucine supplementation making it safe for use however it has no effect on linear growth over a 28-day period.

TheraPEM, a composite flour developed from beans, groundnuts and miller for use in the management of moderate wasting meets the minimum leucine content required to treat the condition. It also meets the minimum energy density and maximum fat content of a complementary feed as described in the Codex Alimentarius International Food Standards. Plastic containers provide the best shelf-life for the composite flour to reduce moisture content loss, yeasts and moulds growth as well as preventing formation of free acids in the food all of which compromise the eating quality of the composite flours.

7.3 Recommendations

Future studies require to be done to determine the effect of leucine supplementation over a longer duration of time on the weight and height of moderately wasted children. This study was conducted over a four-week period and the weight of study children was still increasing even at the last day of measurement. The effect of leucine supplementation in other catabolic conditions including HIV/AIDS and tuberculosis needs to be investigated.

A study needs to be done to determine the digestibility, bioavailability and effectiveness of the leucine-rich therapeutic food on moderately wasted children. Formulation of the therapeutic food from other food sources rich in leucine including animal sources should be investigated. The therapeutic food also requires to be studied for reducing content of leucine during storage.

7.4 Public health significance

There is no standard protocol in the treatment of moderate wasting given the poor outcomes of currently used treatment methods. This study has proven the effectiveness of leucine supplementation in treatment of moderate wasting. Policy on how to handle moderately wasted children to prevent them deteriorating especially when aggravating factors (poor rain fall or poor harvest) are eminent and should include a management protocol using leucine supplementation.

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APPENDIX:

Appendix One: Plagiarism check

LEUCINE SUPPLEMENTATION IN THE MANAGEMENT OF MODERATE WASTING IN CHILDREN: A CASE STUDY OF KENYA

ORIGINALITY REPORT

13%	12%	15%	2%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

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Appendix Two: Consent Information Sheet and consent form

LEUCINE SUPPLEMENTATION IN THE MANAGEMENT OF MODERATE WASTING IN CHILDREN

You and your child are being invited to take part in a research study assessing the effectiveness of a new treatment in the management of moderate wasting in children.

Who can participate?

Persons who can participate in this study are children aged between 6 – 24 months with moderate wasting referred to the Outpatient Therapeutic Programme in Mbagathi County Hospital with written consent from their parents/caregivers. You are therefore being given this information because we would like your child to participate in the study. The study will take place over a period of 28 days in which you will be required to orally administer to your child the treatment in the prescribed dosage amounts.

What is involved in this study?

You will be required to answer some questions truthfully then afterwards, the weight, height, and mid upper arm circumference (MUAC) of your child will be taken. You will then be given some capsules to take home and administer them to your child every day in the prescribed amount. You will also be required to bring your child to the clinic after every seven days from your date of enrolment to the study for follow-up and capsule refills for up to a maximum of four visits.

Is there any compensation attached to this study?

You shall be compensated for your weekly travel costs to and fro the study site at a fixed rate of KES 200 per visit.

What are the benefits of this study?

The knowledge that is gained from this research will help improve the treatment outcomes of children with moderate wasting and contribute towards improving the physical and mental growth and development of children.

What are the risks?

During and after the study period, if you notice any of the following symptoms or any other suspicious symptoms please report to the following contact person immediately. [Jeff Wamiti – Principal Investigator. Tel: 0724409655 Email: jwamiti72@live.co.uk]. **Diarrhoea, vomiting, scaly pigmented rash on skin, swollen mouth and/or bright red tongue, depression, disorientation and apathy**

Participation is your choice.

Your child's participation in this research is completely voluntary so you will make the choice for him/her whether or not to participate. Nothing will happen if you choose for him/her not to take part. You may decide for your child to stop participating in the study at any time however we encourage you to remain in the study.

You have the right to demand that any data provided until that point be withdrawn/destroyed. Feel free to ask questions at any point. If you have any questions as a result of reading this information sheet, you should ask the investigator before the study begins.

How will we protect your child's information and confidentiality?

The data collected shall only be seen by people affiliated with the study, and shall not be linked to any identifying personal information such as name, address or other personal details that you supply.

What will happen with the results of the study?

Results of the study will be made available online and also in hard copy reports that will be surrendered to Mbagathi Hospital on completion of the study. The results shall also be presented in one session of the Comtinuous Medical Education meetings held weekly at the hospital. Results of this study shall also be used as part of the principal investigator's thesis work.

Do you have any questions at this time?

_____

_____

_____

_____

_____

_____

CONSENT FORM

LEUCINE SUPPLEMENTATION IN THE MANAGEMENT OF MODERATE WASTING IN CHILDREN

Instructions: Kindly tick in the box the statements you agree with

1. I confirm that I have read (or been read to) and understood the information sheet for the above study. I have had the opportunity to consider the information, ask questions and have had the questions answered satisfactorily ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my legal rights being affected. ☐
3. I understand that relevant sections of information and data collected during the study may be looked at by individuals affiliated to the study where it is relevant to my taking part in this research. I give permission for these individuals to have access to these records. ☐
4. I agree to take part in the above study without any demands ☐

Signature: _____ Date: _____

Name of interviewer: _____ Signature: _____

Cell: 0724409655

Appendix Three: Data Collection Tool

Questionnaire Serial Number: _____

LEUCINE SUPPLEMENTATION IN THE MANAGEMENT OF MODERATE WASTING IN CHILDREN

IDENTIFICATION

Sub-County_____ Estate _____ Household Number____

Name of interviewer_____ Date of interview _____/___/_____

Study Participant's Code_____ Sex _____

Household profile 1 = monogamous 2 = polygamous

1. SOCIO-DEMOGRAPHIC CHARACTERISTICS

S/No	Name	Relationship to HH head -codes-	Sex M=1 F=2	Age (Years)	Marital status -codes-	Education -codes-	Contribution to HH (occupation)
1.1							
1.2							
1.3							
1.4							
1.5							
1.6							
1. 7							
1.8							
1.9							
1.10							

RHHH	Marital Status	Education	Contribution to HH
1=HHH 2=Spouse 3=Son 4=Daughter 5=Grandson 6=Granddaughter 7=Relative 8=Parent 9=House girl 10=Gardener	1=Married 2=Separated 3=Widowed 4=Single 5=Divorced 6=N/A	1=College/University 2=Completed secondary 3=Completed primary 4=Dropped from primary 5=In primary 6=In secondary 7=Adult education 8= Illiterate 9 = N/A (Preschool)	1=Nothing 2=Money 3=Labour 4=Childcare 5=Savings

2. ANTHROPOMETRIC AND BIOCHEMICAL ASSESSMENTS OF THE INDEX CHILD

		Baseline	Day 7	Day 14	Day 21	Day 28
2.1	**Weight**					
2.2	**Height**					
2.3	**W/H Z-score**					
2.4	**MUAC**					

3. 24 HOUR DIETARY RECALL

Date		
Day of the week		
24 hour record		
Time	**Quantity eaten**	**Description of the food or drink**

Appendix Four: Supplement Adherence Form

Note: Kindly carry this form with you every time you visit the clinic.

Name of child			
Study code			
Name of the Guardian			
Date issued			
Date returned			
Day	**Time**	**Number of capsules (or amount) administered**	**Guardian's Signature**
Day 1			
Day 2			
Day 3			
Day 4			
Day 5			
Day 6			
Day 7			

Appendix Five: Study case report form

1. Identifying Data	
Study Participant ID _____	**Date** _____/_____/_____ *Date Month Year*
Study Participants Date of Birth (D.O.B) and age	**D.O.B:** _____/_____/_____ *Date Month Year* **Age *(in months):* _____**
Informed consent obtained	☐ Yes ☐ No (*Administer consent information sheet and consent form)*
2. Clinical History	
Have you participated in a clinical trial in the past three months	☐ Yes ☐ No
Current Illness	_____ *If none skip to section 3*
Current medication	Name: _____ Dose: _____ Frequency: _____ Duration: _____
3. Vital signs and physical measurements	
Date of physical exam	_____/_____/_____ *Date Month Year*
Physical Exam	☐ Normal ☐ Abnormal – Not clinically significant

☐

	Abnormal – Clinically significant *Specify abnormality in previous or current disease on page 2*
Height	_____ Cm
Weight	_____ Kg
Weight for Height Z – Score	_____
Adverse Events	
Did any adverse events occur after the last visit?	☐ Yes ☐ No
If *'yes'* please provide the information in the Suspected Adverse Drug Reaction Reporting Form (Appendix Six)	

Physical Exam Abnormalities

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

Appendix Six: Suspected Adverse Drug Reaction Reporting Form

PV 1
(rev.2.0)

IN CONFIDENCE

☐ Initial Report
☐ Follow-up Report

MINISTRY OF HEALTH
PHARMACY AND POISONS BOARD
P.O. Box 27663-00506 NAIROBI
Tel: (020)-3562107 Ext 114, 0720 608811, 0733 884411 Fax: (020) 2713431/2713409
Email: pv@pharmacyboardkenya.org

SUSPECTED ADVERSE DRUG REACTION REPORTING FORM

REPORT TITLE: .....

NAME OF INSTITUTION: ..... **INSTITUTION CODE:** .....

ADDRESS: ..... **CONTACT:** .....

COUNTY: .....

****

PATIENT NAME / INITIALS: ..... **IP/OP. NO.:** ..... **D.O.B / AGE:** .....

PATIENT ADDRESS: ..... **WARD /CLINIC:** ..... *(NAME / NUMBER)* **GENDER:** ☐ Male ☐ Female

ANY KNOWN ALLERGY:
☐ No
☐ Yes (specify).....

PREGNANCY STATUS
☐ Not Applicable
☐ Not Pregnant
☐ 1st Trimester ☐ 2nd Trimester ☐ 3rd Trimester

WEIGHT: ..... **Kg**
HEIGHT: ..... **cm**

DIAGNOSIS: (what was the patient treated for) .....

DATE OF ONSET OF REACTION: .....

BRIEF DESCRIPTION OF REACTION: .....

.....

LIST OF *ALL* DRUGS USED IN THE LAST 3 MONTHS PRIOR TO REACTION. IF PREGNANT, INDICATE DRUGS USED DURING THE 1st TRIMESTER (include OTC and herbals) {use rear side of this form for additional drugs}	BRAND NAME	DOSE	ROUTE AND FREQUENCY	DATE STARTED	DATE STOPPED	INDICATION	TICK (√) SUSPECTED DRUG(S)
1.							
2.							
3.							
4.							
5.							

SEVERITY OF THE REACTION: (Refer to scale overleaf)
☐ Mild
☐ Moderate
☐ Severe
☐ Fatal
☐ Unknown

ACTION TAKEN:
☐ Drug withdrawn
☐ Dose increased
☐ Dose reduced
☐ Dose not changed
☐ Unknown

OUTCOME:
☐ Recovering / resolving
☐ Recovered / resolved
☐ Requires or prolongs hospitalization
☐ Causes a congenital anomaly
☐ Requires intervention to prevent permanent damage
☐ Unknown

CAUSALITY OF REACTION (Refer to scale overleaf)
☐ Certain
☐ Probable / Likely
☐ Possible
☐ Unlikely
☐ Conditional / Unclassified
☐ Unassessable / Unclassifiable

ANY OTHER COMMENTS: .....

.....

NAME OF PERSON REPORTING: ..... **DATE:** .....

E-MAIL ADDRESS: ..... **PHONE NUMBER:** .....

DESIGNATION: ..... **SIGNATURE:** .....

You need not be certain… just be suspicious!

Your support towards the National Pharmacovigilance system is appreciated

Submission of a report does not constitute an admission that medical personnel or manufacturer or the product caused or contributed to the event.
Patient's identity is held in strict confidence and programme staff is not expected to and will not disclose reporter's identity in response to any public request.
Information supplied by you will contribute to the improvement of drug safety and therapy in Kenya. Once completed please send to:
The Pharmacy and Poisons Board on the above address

EXPLANATORY NOTES

CONFIDENTIALITY

All information collected in this form, identities of the reporter and patient, will remain confidential.

WHAT TO REPORT

An Adverse Drug Reaction (ADR) is defined as a reaction that is noxious and unintended, and occurs at doses normally used in man for prophylaxis, diagnosis or treatment of a disease, or for modification of physiological function.

Report all suspected adverse experiences with medications, especially those where the patient outcome is:

- Death
- Life-threatening (real risk of dying)
- Hospitalization (initial or prolonged)
- Disability (significant, persistent or permanent)
- Congenital anomaly
- Required intervention to prevent permanent impairment or damage

Report even if:

- You are not certain if the drug caused the reaction
- You do not have all the details

WHO CAN REPORT

All healthcare professionals (clinicians, dentists, nurses, pharmacists, physiotherapists, community health workers etc) are encouraged to report. Patients (or their next of kin) may also report

WHAT HAPPENS TO THE SUBMITTED INFORMATION

All information submitted is handled in strict confidence. The Pharmacy and Poisons Board will assess causality and statistical analysis on each form. Data will periodically be used for reviews and suggest any interventions that may be required to the Ministry of Health. Data will also be submitted periodically to the Uppsala Monitoring Center- the WHO Collaborating Centre for International Drug Monitoring in Sweden.

SUBMISSION OF FOLLOW-UP REPORTS

It is important to tick the appropriate box on the top right corner of the front page to indicate whether the report is an initial (original) report or is a follow-up (subsequent) report.
It is very important that follow-up reports are identified and linked to the original report.

WHERE TO REPORT

After completing this form, please forward the same to your Pharmacy Department for onward submission, or mail directly, to:

PHARMACY AND POISONS BOARD
Lenana Road
P.O. Box 27663-00506 NAIROBI
Tel: (020)-3562107 Ext 114, 0720 608811, 0733 884411
Fax: (020) 2713431/2713409
Email: pv@pharmacyboardkenya.org

Please use the space provided below for any further information. You may attach more pages to this form if required.

.....
.....
.....

LIST OF ALL DRUGS USED IN THE LAST 3 MONTHS PRIOR TO REACTION (include OTC and herbals)	BRAND NAME	DOSE	ROUTE AND FREQUENCY	DATE STARTED	DATE STOPPED	INDICATION	TICK (√) SUSPECTED DRUG(S)
6.							
7.							
8.							
9.							
10.							

Criteria for Assessment of Severity of an ADR

Mild	• The ADR requires no change in treatment with the suspected drug • The ADR requires that the suspected drug be withheld, discontinued or otherwise changed. No antidote or other treatment is required • No increase in length of stay.
Moderate	• The ADR requires that the suspected drug be withheld, discontinued or otherwise changed, and/or an antidote or other treatment is required. • Increases length of stay by at least one day • The ADR is the reason for admission
Severe	• The ADR requires intensive medicare care • The ADR causes permanent harm to the patient
Fatal	• The ADR either directly or indirectly leads to the death of the patient
Unkown	• When you have no information about the ADR

WHO-UMC Causality Assessment Scale

Causality Term	Assessment
Certain	• Event of laboratory test abnormality, with plausible time relationship to drug intake • Cannot be explained by disease or other drugs • Response to withdrawal plausible (pharmacologically, pathologically) • Event definitive pharmacologically or phenomenologically *(i.e. an objective and specific medical disorder or a recognized pharmacological phenomenon)* • Rechallenge satisfactory, if necessary
Probable / Likely	• Event or laboratory test abnormality, with reasonable time relationship to drug intake • Unlikely to be attributed to disease or other drugs • Response to withdrawal clinically reasonable • Rechallenge not required
Possible	• Event or laboratory test abnormality, with reasonable time relationship to drug intake • Could also be explained by disease or other drugs • Information on drug withdrawal lacking or unclear
Unlikely	• Event or laboratory test abnormality with a time to drug that makes a relationship improbable (but not impossible) • Diseases or other drugs provide plausible explanations
Conditional/ Unclassified	• Event or laboratory test abnormality • More data for proper assessment needed or • additional data under examination
Unassessable/ unclassifiable	• Report suggesting an adverse reaction • Cannot be judged because of insufficient or contradictory information • Data cannot be supplemented or verified

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Submission of a report does not constitute an admission that medical personnel or manufacturer or the product caused or contributed to the event.
Patient's identity is held in strict confidence and programme staff is not expected to and will not disclose reporter's identity in response to any public request.
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Appendix Seven: Sensory evaluation score sheet for TheraPEM

Date of Evaluation: _____

Please evaluate the porridge samples provided and indicate the degree of your liking for: taste, flavour, colour, mouth feel, odour and general acceptability.

Use the numerical scores from the 7-point hedonic scale provided. Enter your score under the sample code in the scoring sheet.

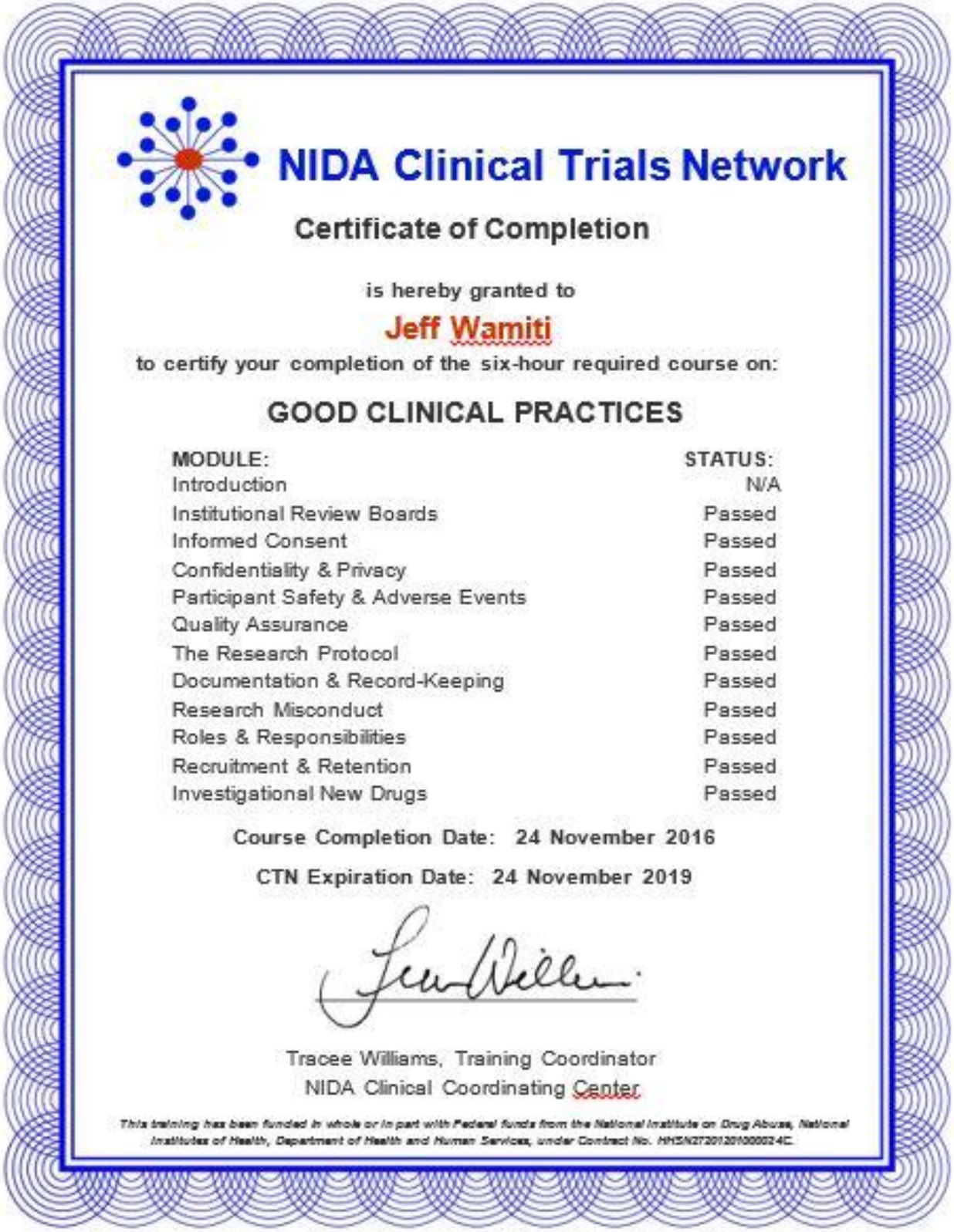
***7-point Hedonic Scale**

Quality	Score
Dislike very much	1
Dislike	2
Dislike slightly	3
Fair	4
Like slightly	5
Like	6
Like very much	7

Scoring Sheet

Sample	1	2	3	4	5	6
Taste						
Flavour						
Colour						
Mouth feel						
Odour						
General acceptability						

Appendix Eight: Jeff Wamiti GCP certificate



NIDA Clinical Trials Network

Certificate of Completion

is hereby granted to

Jeff Wamiti

to certify your completion of the six-hour required course on:

GOOD CLINICAL PRACTICES

MODULE:	STATUS:
Introduction	N/A
Institutional Review Boards	Passed
Informed Consent	Passed
Confidentiality & Privacy	Passed
Participant Safety & Adverse Events	Passed
Quality Assurance	Passed
The Research Protocol	Passed
Documentation & Record-Keeping	Passed
Research Misconduct	Passed
Roles & Responsibilities	Passed
Recruitment & Retention	Passed
Investigational New Drugs	Passed

Course Completion Date: 24 November 2016

CTN Expiration Date: 24 November 2019

Tracee Williams, Training Coordinator
NIDA Clinical Coordinating Center

This training has been funded in whole or in part with Federal funds from the National Institute on Drug Abuse, National Institutes of Health, Department of Health and Human Services, under Contract No. HHSN271201000024C.

Appendix Nine: Kariuki Lilian GCP certificate

NIDA Clinical Trials Network

Certificate of Completion

is hereby granted to

KARIUKI Lilian wachoro

to certify your completion of the six-hour required course on:

GOOD CLINICAL PRACTICES

MODULE:	STATUS:
Introduction	N/A
Institutional Review Boards	Passed
Informed Consent	Passed
Confidentiality & Privacy	Passed
Participant Safety & Adverse Events	Passed
Quality Assurance	Passed
The Research Protocol	Passed
Documentation & Record-Keeping	Passed
Research Misconduct	Passed
Roles & Responsibilities	Passed
Recruitment & Retention	Passed
Investigational New Drugs	Passed

Course Completion Date: 29 November 2016

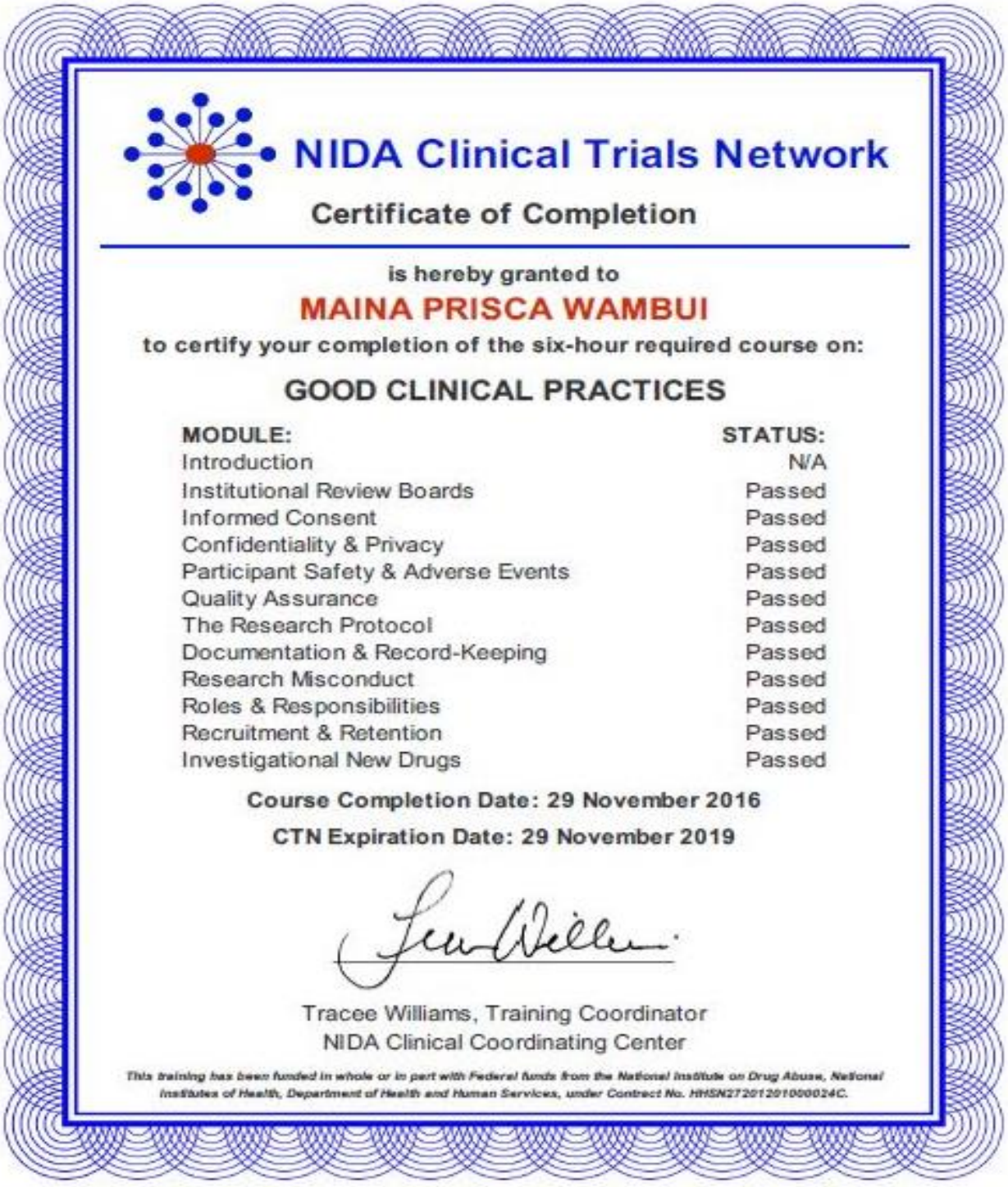
CTN Expiration Date: 29 November 2019

Tracee Williams, Training Coordinator

NIDA Clinical Coordinating Center

This training has been funded in whole or in part with Federal funds from the National Institute on Drug Abuse, National Institutes of Health, Department of Health and Human Services, under Contract No. HHSN272012010000024C.

Appendix Ten: Maina Prisca GCP certificate



NIDA Clinical Trials Network

Certificate of Completion

is hereby granted to

MAINA PRISCA WAMBUI

to certify your completion of the six-hour required course on:

GOOD CLINICAL PRACTICES

MODULE:	STATUS:
Introduction	N/A
Institutional Review Boards	Passed
Informed Consent	Passed
Confidentiality & Privacy	Passed
Participant Safety & Adverse Events	Passed
Quality Assurance	Passed
The Research Protocol	Passed
Documentation & Record-Keeping	Passed
Research Misconduct	Passed
Roles & Responsibilities	Passed
Recruitment & Retention	Passed
Investigational New Drugs	Passed

Course Completion Date: 29 November 2016

CTN Expiration Date: 29 November 2019

Tracee Williams, Training Coordinator
NIDA Clinical Coordinating Center

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Appendix Eleven: Mbagathi County Hospital Research Authorization