**ASSESSMENT OF THE POTENTIAL OF COMFREY (SYMPHYTUM PERIGINUM) AS A SOURCE OF VITAMIN A FOR MALNOURISHED CHILDREN (8 – 16 YEARS) : A CASE STUDY OF KIRIGITI GIRLS APPROVED SCHOOL IN KIAMBU, KENYA**
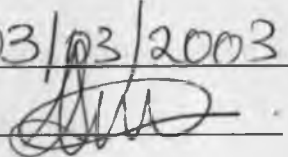
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THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

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IN THE DEPARTMENT OF FOOD TECHNOLOGY AND NUTRITION, FACULTY

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University of Nairobi, 2002

# DECLARATION

I, WAMBUI GATIGWA hereby declare that this thesis is my original work and has not been presented for a degree in any other university.

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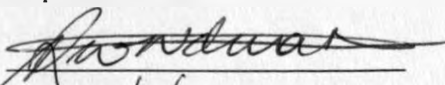
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## DEDICATION

This work is dedicated to my husband, Gatigwa Kimana who not only supported and encouraged me throughout the study period but took the time to familiarize himself with the study and to our son Kimana Gatigwa who was very patient and understanding. Without them, this work would not have been possible.

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| ACC/SCN | Administrative Committee on Coordination/Subcommittee on Nutrition of the United Nations. |
| AIDS | Acquired Immune Deficiency Disease |
| ANP | Applied Nutrition Program |
| β-Carotene | Beta-carotene |
| β-CLB | Beta-carotene Low Biscuit |
| β-CRB | Beta-carotene Rich Biscuit |
| BMI | Body Mass Index |
| CPHR | Center for Public Health Research |
| DGLVs | Dark Green Leafy Vegetables |
| DFTN | Department of Food Technology and Nutrition |
| dl | Deciliter |
| DNA | Deoxyribonucleic acid |
| DSM | Dry Skimmed Milk |
| EFA | Essential Fatty Acids |
| FAO | Food and Agriculture Organization |
| FFA | Free Fatty Acids |
| G | Gram(s) |
| GLV | Green Leafy Vegetables |
| GOK | Government of Kenya |
| Hb | Hemoglobin |
| HPLC | High Performance Liquid Chromatography |
| IU | International Units |
| IVACG | International Vitamin A Consultative Group |
| KEMRI | Kenya Medical Research Institute |
| Kg | Kilograms |
| Mol | Moles |

| | |
|---|---|
| ml | Milliliters |
| MSF | Medicine Sans Frontier |
| NCHS | National Center for Health Statistics |
| PEM | Protein Energy Malnutrition |
| PLWHA | People Living with HIV/AIDS |
| RBP | Retinol Binding Protein |
| RDA | Recommended Daily Allowance |
| R.E. | Retinol Equivalent |
| SD | Standard Deviation |
| SPSS | Statistical Package for Social Sciences |
| STI | Sexually Transmitted Infections |
| µg/dl | Micrograms per deciliter |
| UNICEF | United Nations Children Fund |
| UNHCR | United Nations High Commission for Refugees |
| VA | Vitamin A |
| VAD | Vitamin A Deficiency |
| VADD | Vitamin A Deficiency Disorders |
| WFP | World Food Program |
| WHO | World Health Organization |

# OPERATIONAL DEFINITIONS

Body Mass Index
(BMI)
- This is a measure of nutritional status used to judge the level of undernourishment or over nourishment.

  The weight for height indices used are tabulated as follows ;

  $$BMI = \frac{weight(kg).}{[height\ (m)^2]}$$

Control Group
(controls)
- Children included in the experimental study who consumed the βCLB.

Experimental Group
(cases)
- Children included in the experimental study who consumed the βCRB

Githeri
- A meal comprised of dried boiled maize and beans

Healthy skin
- Quality of skin in reference to general tone and appearance of the Skin exclusive of presence of wounds and sores.

Malnutrition
- For the purpose of this study, it is a state of nutrition where the BMI is < 18.5.

Post-test/post
intervention/
- Period after the intervention (service of the biscuits was done).

Pre-test/ baseline /
pre supplementation
- Period before the intervention (service of the biscuits was done) also referred to here as 'at baseline' or 'at recruitment'

Ugali
- A stiff maize meal made of milled maize and water

## ACKNOWLEDGEMENTS

# ABSTRACT

In February-September 2001, an interventional study employing the pre-test, post-test case-control study design was carried out among children aged 8 to 16 years in Kirigiti Girls Approved School in Kiambu District, Central Province, Kenya.

The main objective of the study was to determine the potential of comfrey (*Sympytum periginum*) in the alleviation of vitamin A deficiency. A $\beta$-carotene rich biscuit ($\beta$CRB) was made using comfrey and a relatively low $\beta$-carotene biscuit ($\beta$CLB) without comfrey was also made and their beta-carotene content determined. The comfrey rich biscuit had a significantly higher beta-carotene content (1060 $\mu$g/100 grams) than the comfrey free biscuit, which had (170 $\mu$g/100 grams).

Seventy-seven (77) children admitted at the school were randomly allocated to two study groups; a test group and a control group. Both groups were then subjected to an interview, physical examination and anthropometry. Pre and post-test morbidity experience was also recorded within which period stool samples were collected for examination of ova cysts and blood drawn for haemogram and biochemical estimation of serum $\beta$-carotene and serum retinol. Proximate composition of meals taken at the school and dietary intake were determined. The children were then dewormed and fed 95g of the respective biscuits for 31 days after which morbidity experience, anthropometry, stool and blood collection were repeated.

A seven-day morbidity recall at baseline indicated that the proportion of children who had respiratory tract infection, and skin disorders were 19.5% and 16.9% respectively.

At baseline, 79.2% of the children had dental caries with 39% of the children in the experimental group. The pretest clinical assessment data indicated that 15.6% of the children from the βCRB group and 18.2 % from the βCLB group had nyctalopia. After supplementation, nyctalopia significantly reduced in both groups (p<0.01). No significant reduction was noted in the number of children with wrinkled cornea (p<0.05) in both groups. Flu, coughs, fever and headaches, dermatitis, brown hair, skin disorders and parasitic infestation reduced after supplementation in both groups with significance recorded in the first six conditions (p<0.05) but there was no significant difference observed between the two groups at pre and post-test. In the βCRB group, the mean baseline BMI was 16.17±1.67 and in the post-test period it was 16.9± 1.67 while the βCLB group recorded indices of 16.3±2.18 and 17.1± 1.97 respectively. There was however, no significant difference.

There was no significant difference (p>0.05) in serum β-carotene and retinol levels at baseline but at post-test, the serum β-carotene significantly increased (p<0.05) in the cases from 0.0327 ± 0.069 to 0.096 ± 0.036 than in the control group from 0.050 ± 0.049 to 0.076 ± 0.035. The difference in post intervention serum β-carotene levels between the groups was highly significant (p=0.000). Despite the increase in the β-carotene, the serum retinol levels did not change significantly.

The data strongly indicates that comfrey *(Symphytum periginum)* can be a source of vitamin A But further studies are required to determine whether a higher dose of β-carotene or a longer supplementation period using βCRB is required to observe changes in plasma retinol levels.

# CHAPTER ONE

# INTRODUCTION

## 1.1. Background

Malnutrition is widespread in the world today and more so in developing countries. It is the underlying cause of over half the deaths for children under 6 years of age (Latham, 1997). Malnutrition arises chiefly due to poor dietary intake (both qualitatively and quantitatively) and low bioavailability of nutrients required by the body. Iodine, zinc, iron and Vitamin A deficiencies, also referred to as micronutrient deficiencies, are the main nutritional problems facing developing countries (Latham, 1997). They have devastating short and long-term effects. These deficiencies have greater effects on health and affect more people than previously thought, (UNICEF, 1998).

Of special importance is Vitamin A especially in children aged below five and in women of childbearing age. Vitamin A deficiency is one of the widely occurring conditions in the world today affecting more than 250 million people world-wide, more than half of who are young children aged between 6 months and two years. Of these, perhaps half will become blind or have serious visual impairment, and a large proportion will die (Latham 1997). The deficiency of this vitamin causes among other conditions; blindness, increased morbidity and mortality rates, irreversible damage to the immune system and results in an imbalance that affects the growth and development of the child. There is also an increased risk of certain cancers including cancer of the colon arising from the adverse effects of epithelial tissue.

The magnitude of vitamin A deficiency (VAD) effect in development, productivity and survival has been acknowledged and various interventions/strategies have been considered/implemented. The most commonly used type of intervention is the administration of vitamin A drops for infants and capsules for children and adults.

In Kenya, although a lot of food supplementation has been done by FAO, UNICEF, GOK, MSF and WFP in certain areas, this has been general and basic and not intended to improve nutritional status of the affected populations to optimal levels. Although the issue of vitamin A supplementation was tabled to the GOK in 1994, it wasn't until 1999 when the actual Vitamin A supplementation began. In Kenya, the VAD situation has become worse because the prevalence in 1994 for children aged 0-72 months with serum retinol < 20 µg/dl was 60%. Currently it stands at 84.4% (CBS, 2001). This means that whatever is being done is not sufficient to stabilize or improve the VAD situation, moreover not only does the population continue to increase but the emergence of HIV/AIDS which has a direct bearing on the immune system where Vitamin A is needed to boost immunity has presented a major challenge. Adequate vitamin A for women of childbearing age is critical because deficiencies in this group translates to deficiencies during pregnancies and the infants born to them are deficient as well. The risk of morbidity and mortality are increased. The political and economic scenario in Kenya has also hampered the implementation of effective and consistent Vitamin A intervention programmes.

The administration of vitamin A capsules relies on the existence of a well-established health care system. The Ministry of Health is currently distributing vitamin A capsules to children and lactating women. However, this is not sufficient because it only reaches the infant who is accessing the health services for immunization. The alternative approach would be to use locally available foods that are rich in β-carotene as part of a staple diet of the Kenyan population.

Although Vitamin A containing foods are widely available, previous studies have established that there can be VAD despite availability of vegetables, which are reportedly the major source of Vitamin A precursors (Muroki, 2000). It is postulated that β-carotene in vegetables is embedded in protein-carbohydrate matrix, which reduces it's bioavailability (McLaren, 2001).

Another problem with vegetables is that children loathe eating them (McLaren, 2001). The bioavailability of β-carotene in vegetables could be increased through the use of softer and less fibrous vegetables or through the preparation of palatable dishes rich in β-carotene. These should ideally be prepared into a form acceptable to them such as a juice or a baked biscuit. One vegetable, which can be prepared in this way is *comfrey (Sympytum periginum)*. When blended or used as flour, it is likely that the β–carotene will be more available. Comfrey is soft with low fiber content. Furthermore, it does not have a strong typical odor. The astringent taste often noticed in some products is eliminated when comfrey is incorporated in the baked product. The product is more palatable than when other green leafy vegetables are used, e.g., kale and spinach. By using whole green fresh vegetables as a major ingredient in a baking mixture, the inclusion of fat would not only enhance absorption, but would also add nutritional value. Baking will also make the product easy to ingest as it can be reconstituted using boiled water or milk for children who cannot chew.

**Comfrey**, (*Symphytum periginum*), is a very valuable source of micronutrients (Hill, 1976). Research carried out by the Department of Food Technology and Nutrition (DFTN), University of Nairobi on comfrey has shown that comfrey is especially rich in β-carotene (up to 48 milligrams per hundred grams). The use of comfrey powder and comfrey biscuits would not be wrought with logistic and non-compliance regulations as would vitamin A capsules.

Furthermore, consumption of dried comfrey poses no risk of hypervitaminosis A (Dekker, 1994) save for hypercaroteaemia, which may have very little effect on health.

Comfrey can be used to process a βeta-carotene rich biscuit (βCRB) as a source of vitamin A. This would ensure availability of a ready-to-eat vitamin A rich food that can be taken as a snack food as often as one would like. It would also be extremely invaluable in VAD intervention because supervision to prevent overly large doses would not be required. The biscuit would provide other nutrients like protein, carbohydrates, iron and calcium.

It's enhancement of the body immune system would make it possible to slow down infection rate particularly in AIDS cases or enable the body to fight off infections.

## 1.2. Justification

1. Vitamin A deficiency is very common with a prevalence of up to 94.4% among children in some communities in Kenya

2. Many Kenyans live in rural area and are peasant farmers able to grow their food including vegetables rich in Vitamin A.

3. A strategy of addressing VAD is to promote the production of vegetables that have high levels of β-carotene in a readily bioavailable form and which can be made into a delicious meal.

## 1.3. Research Objectives

### 1.3.1. Main objective.

The main objective of this research was to assess the potential of *β-carotene Rich Biscuit (βCRB)* made with comfrey as a source of supplemental vitamin A in malnourished children (aged 8-16 years).

### 1.3.2. Specific objectives

1. To determine the content of β-carotene in comfrey enriched biscuits and biscuits without comfrey.
2. To determine the pre and post intervention retinol and β-carotene serum levels of the children.
3. To determine the impact of short-term *βCRB* supplementation on morbidity of the study population.

## 1.4. Hypothesis

1. There is no difference in the post-intervention serum β-carotene levels of children (aged 8-16 years) fed on comfrey rich biscuit and those fed on comfrey free biscuit.

2. There is no difference in the post- Intervention serum retinol A levels of children (aged 8-16 years) fed on comfrey containing biscuit and those fed on comfrey free biscuit.

## 1.5.    Expected Benefits of the Study

1. βCRB may be used as part of a nutritional supplementation program in emergency situations and in the urban and rural areas to curb further vitamin A deficiency in high risk groups.

2. βCRB may be incorporated in well- infants' diet as part of food supplementation.

3. The health of children aged 8 –16 years may greatly improve if the biscuit is incorporated in their normal diets.

4. βCRB may be effective in the reducing the severity of opportunistic infections if the biscuit is incorporated in the normal diets of affected individuals.

5. Interested parties may use the information for vitamin A and β-carotene supplementation and intervention studies.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1. Overview of Malnutrition

Adequate nutrition is the intake and utilization of enough energy and nutrients together with disease control, to maintain normal growth, well-being, health and productivity. Under-nutrition ia a form of malnutrition caused by the consumption of too little macro and (/or) micronutrients. Malnutrition is classified as protein-energy malnutrition and micronutrient deficiency. It should, however, be noted that macronutrient deficiency can in some cases occur. The clinical manifestations of malnutrition include stunting, underweight, and wasting. Micronutrient deficiency, include deficiencies of vitamin A, iron, iodine, zinc and folic acid and frequently accompany the macronutrient deficiency (WHO, 1999).

### 2.1.1. The vicious cycle of malnutrition

Malnutrition often begins at conception (Allen, 2001). When pregnant women consume inadequate diets, have excessive workloads or are frequently ill, they give birth to smaller babies who are already nutritionally disadvantaged. The breast milk of these women is also low in most nutrients and more so vitamin A (WHO 1999). Their children are therefore more likely to die as infants. If they survive, by the second year of life they may have permanent damage (WHO, 1999).

The effects of early childhood malnutrition persist into the school years and even adulthood, lowering productivity and quality of life (WHO, 1999). Small adult women who were malnourished as children are more likely to produce small babies who grow into small adults

and who in turn produce small babies and the cycle of malnutrition and illnesses continues (Allen, 2001). It is therefore necessary to target vulnerable groups and break this vicious cycle because in Kenya alone, this cycle translates to billions of Kenya shillings lost in life and under-productivity and subsequent superficial medical bills (Odour, 2000).

### 2.1.2.   Common disorders of malnutrition, and their effect on vitamin A status.

*Protein-energy malnutrition (PEM)* is the most common nutritional problem in most countries in Asia, Latin America, the Near East and Africa. Children below five years of age suffering from severe PEM and are often grossly wasted, stunted, underweight or oedemic or will have a combination of two of the aforementioned. The significance of PEM in relation to vitamin A is that where there is PEM, there will most certainly be VAD because the metabolism of Vitamin A requires protein (Latham, 1997).

It is estimated that 38% of all malnourished children in the world are stunted, 8% are wasted and 25% are underweight (Odour, 2000). Children (aged 8-16 years) are even more so affected because their deficiency symptoms are not as pronounced as the known high-risk groups and yet this is where one of the greatest demands on nutrients arises due to the growth spurt.

*Iron deficiency disorders.* Iron deficiency is the most prevalent important nutritional problem of humans. Iron is essential for the manufacture of haemoglobin in the blood. It threatens over 60% of women and children in most non-industrialized countries (Latham 1997). The prevalence rate of iron deficiency anemia in Sub-Saharan Africa is 42% and increasing (Latham, 1997). The most common cause of anemia is iron deficiency although not necessarily a dietary deficiency of total iron intake.

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(Deficiencies of folates, vitamin $B_{12}$ and protein may also cause anemia while ascorbic acid, vitamin E, copper and pyridoxine are also needed for production of red blood cells. *Vitamin A is associated with iron because VAD causes anemia)*, (Latham, 1997). Both vitamin C and vitamin E may have a bearing on vitamin A status. Ascorbic acid improves availability of iron while vitamin E deficiency has been associated with VAD (Latham, 1997).

### 2.1.3. *Causes of malnutrition*

The causes of malnutrition including vitamin A deficiency are multifaceted as illustrated in Fig. 1. Disease and inadequate dietary intake are the immediate causes of malnutrition in most individuals (Allen, 2001). Their role in the vicious cycle of malnutrition and their relation to other contributing factors are discussed below.

### 2.1.3.1. *Inadequate food intake and disease*

Inadequate food intake in individuals will very often lead to disease caused by lowered immunity through nutrient deficiencies. Underlying these causes are barriers in household and the family. The efficient utility of resources is dependent on the economic, political and social organization of the community. When there is little or no efficiency in the above-mentioned areas, inadequate education that follows leads to poor household food security, inadequate maternal care and insufficient health services and poor sanitation. These can lead to inadequate dietary intake, which is the immediate cause of malnutrition or can precipitate malnutrition through decreased foodstuff intake, poor nutrient utilization or nutrient loss.

Poor nutrient intake can also cause disease, which in turn results in malnutrition. Thus, malnutrition and disease constitute a vicious cycle (Fig. 1).

### 2.1.3.2. Food availability and access

Poor food accessibility is caused by the inability of families to produce or acquire enough food containing needed energy and nutrients and the inability of families to access land and agricultural inputs, marketing and distribution of foods, income and other factors (WHO 1999). These factors lead to the overall decreased availability of food both in terms of quality and quantity, which in turn leads to malnutrition (WHO, 1999).

### 2.1.3.3. Water/sanitation and inadequate health services

The utilization of health services is determined by their accessibility in terms of social acceptability, economic access (affordability), suitability within accessible distance in terms of time and terrain (WHO, 1999). During illness, there is a higher metabolic demand on the body and if the illness is prolonged or not treated, there is a greater loss of weight (Nduati, 2002). Health services are also a source of information on prevention of disease and coping mechanisms e.g., many mothers are not aware of the need to feed children more frequently after illness to prevent subsequent malnutrition.

Indicators of inadequate health services include low immunization coverage; lack of prenatal care; inadequate management of sick and malnourished children; and inadequate water and sanitation facilities, all which have a negative impact on vitamin A status (WHO, 1999).
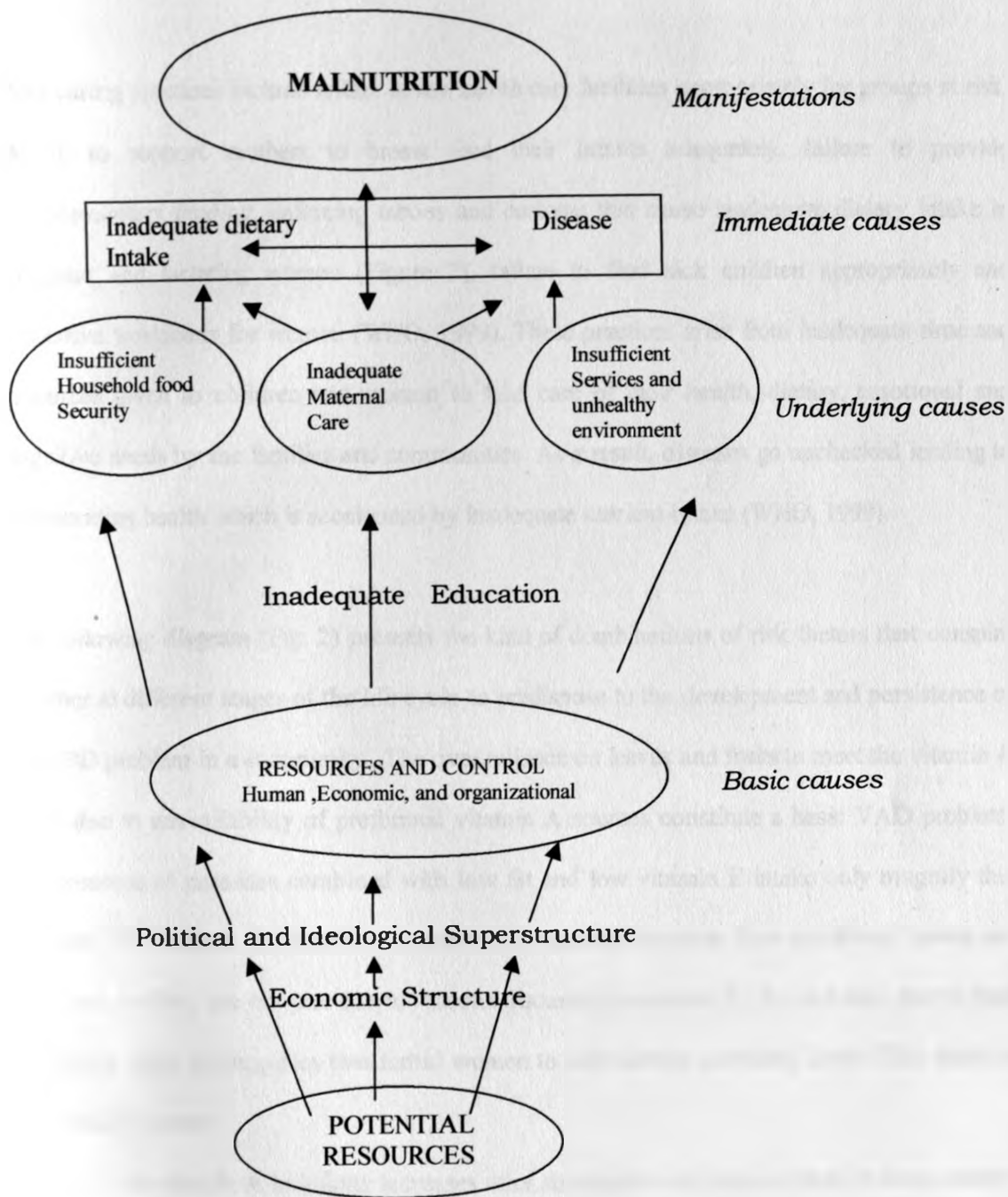
Source: (UNICEF, 1990).

Fig. 1.     The causes of malnutrition are multi-sectorial

### 2.1.3.4. Maternal and child- care practices

Poor caring practices include failure to use health care facilities appropriately for groups at risk, failure to support mothers to breast feed their infants adequately, failure to provide complementary feeding, enforcing taboos and customs that cause inadequate dietary intake in pregnant and lactating women (Figure 2), failure to feed sick children appropriately and excessive workloads for women (WHO, 1999). These practices arise from inadequate time and resources given to children and women to take care of their health, dietary, emotional and cognitive needs by the families and communities. As a result, diseases go unchecked leading to deteriorating health which is accelerated by inadequate nutrient intake (WHO, 1999).

The following diagram (Fig. 2) presents the kind of combinations of risk factors that conspire together at different stages of the life cycle to predispose to the development and persistence of a VADD problem in a community. The over reliance on leaves and fruits to meet the vitamin A needs due to unavailability of preformed vitamin A sources constitute a basic VAD problem. The presence of parasites combined with low fat and low vitamin E intake only magnify this problem. For women the situation worsens in pregnancy because their nutritional needs are increased but they are not met due to reasons discussed in section 2.1.3. and also due to food taboos sin some communities that forbid women to take certain nutritious foods. This leads to low fetal VA status.

The need for vitamin A in infants increases after six months and when a child is born already deficient and their diet is also deficient in Vitamin A, their health is compromised due to lowered immunity. (Semba, 1995). This problem arises due to inadequate milk production necessitating earlier weaning or bottle-feeding. In the absence of proper maternal care and delayed or no immunization, the risks of infections is increased and VADD sets in.

This child grows up deficient in vitamin A with the accompanying deficiency disorders and matures and the VADD cycle continues (McLaren, 2001).

```
┌─────────────────────────────┐              ┌─────────────────────────────┐
│         ADULTS              │              │        PREGNANCY            │
│                             │              │                             │
│ Over-reliance on leaves and │     ───▶     │ Increased needs             │
│ fruits                      │              │                             │
│ Low preformed vitamin A     │              │ Food taboos                 │
│ Low fat                     │              │                             │
│ Low vitamin E               │              │    ──▶ Low fetal VA status  │
│ Parasites                   │              │                             │
└─────────────────────────────┘              └─────────────────────────────┘

           ┌─────────────────────────────────────────┐
           │            YOUNG CHILD                   │
           │       Increased needs for growth         │
           │                                          │
           │  Breast protective    Bottle hazardous   │
           │  Immunization         Infections         │
           │  Mother care          Parasites          │
           │                       Neglect            │
           └─────────────────────────────────────────┘

                    Vitamin A Deficiency Disease (VADD)
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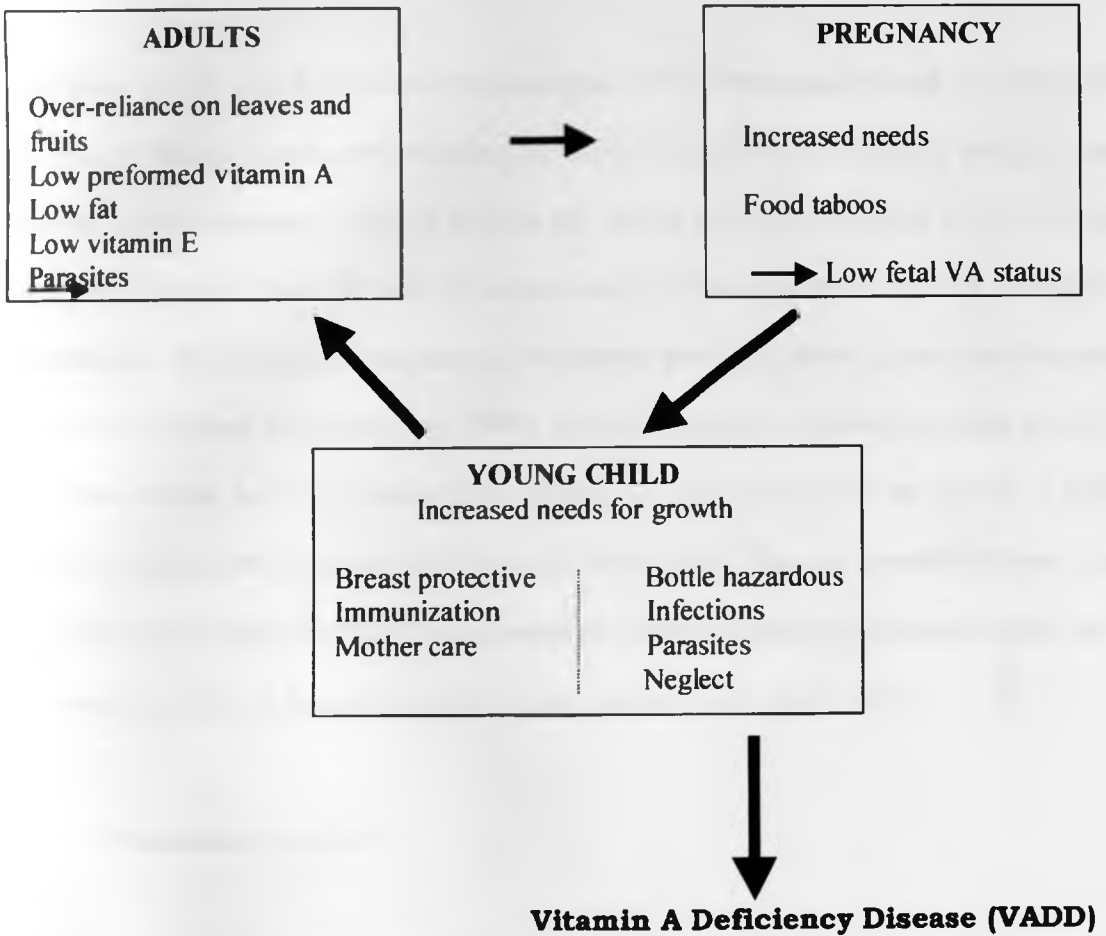
Source:   McLaren and Frigg, 2001

*Figure 2:*        *Vitamin A deficiency (VADD) Disorders Cycle.*

## 2.2. Vitamin A

### 2.2.1. Background

Vitamin A is a fat - soluble vitamin (Satyanarayana, 1999). Preformed vitamin A or retinol is a fat-soluble vitamin found only in animal products. Carotenes or carotenoids can act as pro vitamin A (Satanarayana, 1999). McCollum and Davies discovered Vitamin A in 1913 when experiments showed that if the only fat present in diets of young animals was lard, their growth was retarded. When butter was substituted, the animals grew and thrived. It was later discovered in 1919 by Stunbock (Satyanarayana, 1999), that many products of plant origin had nutritional properties similar to those presented by vitamin A. They were found to contain a yellow pigment carotene, which converted to vitamin A in the body. There are over 600 different types of carotenoids in plants, but the most important for human nutrition is β-carotene, which can be converted to vitamin A by enzymic action in the intestinal wall  (Antia, 1973).

### 2.2.2. Functions of vitamin A

Vitamin A is necessary for a variety of functions such as vision, proper growth and differentiation, reproduction and maintenance of epithelial cells (Antia, 1973). Retinol and retinoic acid function almost like steroid hormones regulating the protein synthesis and thus are involved in the cell differentiation and are essential for proper growth (Lee, 1996). Retinol and retinoic acid are required to prevent keratin synthesis, which is responsible for horny surface. Furthermore, retinyl phosphate is essential for the formation of mucopolysaccharides compounds of mucus secreted by epithelial cells to maintain moist surface. Vitamin A is

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considered to be essential for the maintenance of a proper immune system to fight against various infections. It is also necessary for the normal development and growth of bones and teeth (Somer, 1992).

However, a certain amount has to be consumed daily for the body to perform optimally. The specific amounts required for different age groups by sex are referred to as recommended dietary intakes and are presented in table 1.

*Table 1: Recommended dietary intakes of vitamin A ($\mu g$ re/day)*

| AGE AND SEX GROUPING | FAO/WHO |
|---|---|
| 0-1 | 350 |
| 1-6 | 400 |
| 6-10 | 400 |
| 10-12 | 500 |
| 12-15 | 600 |
| 15 – 18 + (MALE) | 600 |
| 15 – 18 + (FEMALE) | 500 |
| PREGNANCY | 600 |
| LACTATION | 850 |

1: Age and sex grouping  2: FAO/WHO 1988

Source: Mc. Laren 1994

Vitamin A status can be grouped into four main categories depending on serum retinol levels: deficient, borderline deficient, normal and excessive/toxic, depending on the retinal serum levels (Lee, 1996). Serum retinol levels < 20$\mu g$/dl (0.70 $\mu mol$/L) are classified as deficient, levels between 20$\mu g$/dl (0.70 $\mu mol$/L) and 25$\mu g$/dl (0.70 - 0.88 $\mu mol$/L) are classified as borderline deficient while levels > 25$\mu g$/dl constitute a normal level (McLaren, 2001).

Toxicity is observed in vitamin A serum levels > 100μg/dl also referred to as hypervitaminosis A (Lee, 1996).

### 2.2.3. *Metabolism of vitamin A*

Dietary retinyl esters are hydrolyzed by pancreatic or intestinal brush border hydrolases in the intestine, which release retinol and fatty acids (Fig. 3). Carotenes are hydrolyzed by β-carotene 15-15'-dioxygenase of intestinal cells to release 2 moles of retinal, which is reduced to retinol.
In the intestinal mucosal cells, retinol is reesterified to long chain fatty acids, incorporated into chylomicrons and transferred to the lymph. The retinol esters of chylomicrons are taken up by the liver and stored (Satayanarayana, 1999). When needed, vitamin A is released from the liver where it is stored, as free retinol. Zinc and iron play an important role in the mobilization and utilization of stored retinol (Allen, 2001). Retinol is transported to the extra-hepatic tissues by the plasma retinol binding protein, a specific carrier protein (RBPmol. wt. 21,000) in association with transthyretin, earlier known as pre-albumin (Satayanarayana, 1999).

Protein deficiency may influence vitamin A status by reducing the synthesis of RBP. The retinol-RBP complex binds to specific receptors on the cell membrane of peripheral tissue and enters the cells (Satayanarayana, 1999). Many cells of target tissues contain a cellular retinol-binding protein that carries retinol to the nucleus and binds to the chromatin (DNA). It is here that retinol exerts its function in a manner analogous to that of a steroid hormone. Retinol and retinoic acid are involved in the synthesis of transferrin, the iron transport protein.
Iron deficiency alters the distribution of vitamin A where VAD impairs iron mobilization from stores (Allen, 2001). Vitamin A supplementation therefore improves hemoglobin concentration, which is important for proper metabolic processes (Allen, 2001).

Figure 3 gives a summary of vitamin A absorption, transport and biochemical functions.
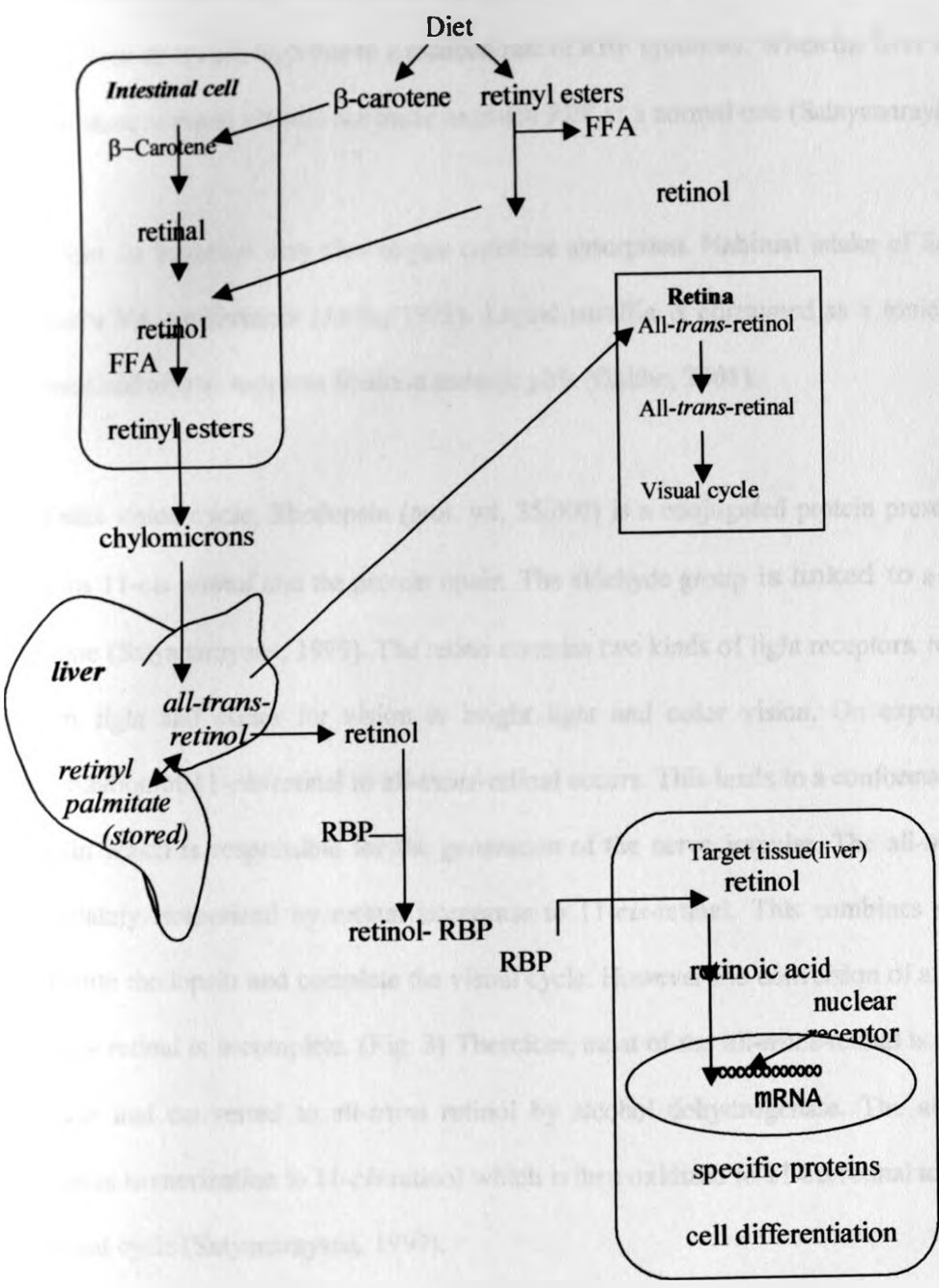


Source: Satyanarayana, 1999

*Fig 3:*     *Absorption, transport and function of vitamin A*

The availability of stored vitamin depends on the nutritional status of a child (Latham, 1997). Severely malnourished, protein-deficient children will have subnormal levels of serum retinol, even if liver stores are high due to a reduced rate of RBP synthesis. When the liver is diseased, it cannot store as much vitamin nor make as much RBP as a normal one (Satayanarayana, 1999).

Deficient fat ingestion may also impair carotene absorption. Habitual intake of liquid paraffin increases VA requirement (Antia, 1973). Liquid paraffin is consumed as a tonic for stomach ailments and also to suppress libido in teenage girls (Gaitho, 2001).

In Walds vision cycle, Rhodopsin (mol. wt. 35,000) is a conjugated protein present in rods. It contains 11-*cis retinal* and the protein opsin. The aldehyde group is linked to ε-amino group of lysine (Satyanarayana, 1999). The retina contains two kinds of light receptors, rods for vision in dim light and cones for vision in bright light and color vision. On exposure to light, isomerization of 11-*cis*-retinal to all-*trans*-retinal occurs. This leads to a conformational change in opsin which is responsible for the generation of the nerve impulse. The all-*trans*-retinal is immediately isomerized by retinal isomerase to 11-*cis*-retinal. This combines with opsin to regenerate rhodopsin and complete the visual cycle. However the conversion of all *trans*-retinal to 11-*cis* retinal is incomplete. (Fig. 3) Therefore, most of the all-*trans*-retinal is transported to the liver and converted to all-*trans* retinol by alcohol dehydrogenase. The all-*trans*-retinol undergoes isomerization to 11-*cis* retinol which is then oxidized to 11-*cis* retinal to participate in the visual cycle (Satyanarayana, 1999).

*Dark adaptation time:* When a person shifts from a bright to a dim light, rhodopsin is depleted and vision is impaired. However, within a few seconds known as dark adaptation time, rhodopsin is resynthesized and vision is improved.

Dark adaptation time is increased in persons with VAD. Vitamin A also has an important role in the bleaching of rhodopsin as it stimulates the nerves involved in photon absorption and color vision (McLaren, 2001).

### 2.2.4. *Sources of vitamin A*

Animal sources contain (preformed) vitamin A. The best sources are kidney (Allen, 2001), liver, egg yolk, full-cream milk (when fortified), cheese, and butter. Fish (cod or shark) liver oils are also very rich in vitamin A (Piwoz, 2001).

### 2.2.5. *Carotenoids*

Vegetable sources contain the pro vitamin A substances referred to as carotenes. They belong to the carotenoids group of naturally occurring pigments. Carotenoids are nonpolar and extremely hydrophobic with virtually no solubility in water. They are therefore restricted to hydrophobic areas in cells, such as the inner core of membranes.

Carotenoids also occur in algae, moulds, mushrooms, and bacteria and in all classes of animals including mammals. However, no animal is able to synthesize carotenoids (McLaren, 2001) because animals greatly differ in the way that carotenoids from their diet accumulate in their tissues, especially in adipose tissue (Bauerenfiend, 1981). The reason for these differences is not understood. Man indiscriminately accumulates carotenoids. These remain in situ when fat is metabolized as in starvation.

The carotenoid β-carotene, is of special importance to man as it has the highest vitamin A retinol activity of all carotenoids and apart from being a provitamin A, it functions as an antioxidant and reduces the risk of cancers initiated by free radicals and strong oxidants. β-carotene is also found to be beneficial to prevent heart attacks. This is also attributed to the antioxidant property (Satyanarayana, 1999). It is found in yellow and dark green vegetables and fruits. Good sources of carotenes are sweet potatoes, carrots, spinach, pumpkins, mangoes, papaya, red palm oil (McLaren, 2001). In Africa however, and developing countries, these foods are only seasonally available apart from sweet potatoes which is a perennial crop, or may be found only in particular geographic regions especially in areas where there are rains and farming methods are varied (ACC/SCN 1994).

The following relationship between vitamin A and β-carotene has been established as reported by the joint FAO/WHO Expert Group. The biological activity of vitamin A is now usually expressed as retinol equivalents (RE) rather than in international units (IU) (Salkeld, 1999).

1 retinol equivalent $= 1 \mu g$ retinol

$= 6 \mu g$ β-carotene

$= 12 \mu g$ other carotenoids

$= 3.33$ IU of vitamin A activity from retinol

$= 10$ IU of vitamin A activity from β -carotene

The concept has helped in assessing the equivalence of different sources of vitamin A activity. The actual values remained untested, despite a strong call from the committee for research, and led to a false sense of security until challenged in the mid 1990's (McLaren, 2001).

In the mid 1980's evidence began to accumulate that widespread sub clinical vitamin A deficiency could lead to a significant increase in the risk of mortality and morbidity in young children. It began to become clear that while consumption of dark green leaves and yellow fruits could protect against severe VAD leading to xerophthalmia, it might not be capable of preventing sub clinical vitamin A deficiency in a substantial proportion of the young child population of developing countries (McLaren, 2001). This is attributed to the bioavailability and the bioconversion of carotenoids.

Bioconversion relates to the efficiency with which the carotenoids in question are converted to vitamin A in the body. On the other hand, bioavailability refers to the accessibility by the body to carotenoids hence it is possible to consume carotenoids rich foods with carotenes entering the body system (bioavailability), but for various factors the body may not be able to convert it to vitamin A for utilization (bioconversion) (Britton, 1999). In 1999, a group at the Department of Human Nutrition at Wageningen University in the Netherlands, introduced a mnemonic; SLAMENGHI to assist in the discussion of the bioavailability and bioconversion of carotenoids. The following is the mnemonic they tabled.

### 2.2.5.1. "S": Species of carotenoids

The naturally occurring configuration of carotenoids in plants is usually the all-trans isomer. It is more readily absorbed in man than the 9-cis form. A significant proportion of 9-cis, β-carotene is converted to the all-trans form before entering the blood stream (McLaren, 2001).

### 2.2.5.2. "L": Molecular linkage

The esters of carotenoids are common in fruit and vegetables but their absorption has been little studied. Esters of lutein are reported to be more bioavailable than esters of carotenoids (McLaren, 2001).

### 2.2.5.3. "A": Amount of carotenoids consumed

In a meta-analysis, which included 31 studies in which a daily β-carotene supplement of <50mg lasted <1 year it was found that the duration of β-carotene supplementation was a significant predictor of β-carotene response (Castenmiller and West, 1998). One of the most time-significant studies involving beta-carotene supplementation in children where positive vitamin A status rose to a level that was apparently similar to that achieved with oral vitamin A supplementation, was the study carried out in Tamil Nadu in India where regular provision of papaya and drumstick leaves (coupled with nutrition education) were used over a period of 1 year (IVACG, 1993).

### 2.2.5.4. "M": Matrix in which the carotenoid is incorporated

In green leaves carotenoids exist within chloroplasts as pigment-protein complexes, which require disruption of the cells for the carotenoids to be released. In other vegetables and fruits, carotenoids are sometimes found in lipid droplets from which they may be released. The Indonesian study by De Pee found that β-carotene from a simpler matrix other than that found in DGLVs produced a strong improvement in serum retinol status (De Pee, 1996). Cooking assists in the release, but if excessive may lead to oxidative destruction of the carotenoids.

The carbon-carbon double bonds of the carotenoids are subject to oxidation by oxygen in the air. Heat may bring about structural changes including isomerization of the all-trans carotenoids to *cis* forms. The effect of food matrix and processing on bioavailability of carotenoids is shown in Table 2.

*Table 2:     The effect of food matrix and processing on bioavailability of carotenoids*

**Very high bioavailability**

| | |
|---|---|
| Formulated natural or synthetic carotenoids. | Formulated carotenoids in water dispersible beadlets. |
| Natural or synthetic | Carotenoids – oil form |
| Papaya, peach, melon | Fruits |
| Squash, yam, sweet potato | Tubers |
| Tomato juice | Processed juice with fat-containing meal |
| Carrots, peppers | Mildly cooked yellow/orange vegetables |
| Tomato | Raw juice without fat |
| Carrots, peppers | Raw yellow/orange vegetables |
| Spinach | Raw green leafy vegetables |

**Very low bioavailability (<10%)**

Source: Boileau, Moore, Erdman Jr., 1999

The lowest bioavailability is in green leafy vegetables such as spinach. Yellow vegetables like carrots and tomatoes have higher bioavailability apparently because of less fibre and less complex matrix. Processed tomato juice with fat-containing meals has a higher bioavailability than a tomato. This can be attributed to the fat and the processing.

Fruits such as papaya, peach and melon have higher availability than tubers. The highest availability is in formulated synthetic or natural carotenoids followed by carotenoids in oil form, which have polar groups, e.g., retinol palmitate esters.

DGLVs are a staple food for most communities at risk of VAD and are eaten more frequently with the main starchy staple and are more readily available than other sources with higher vitamin A bioavailability.

### 2.2.5.5. "E": Effectors of absorption and bioconversion

Dietary components may influence absorption. A minimum of 5 g fat daily is required for adequate micelle formation. This appears to be sufficient for absorption of $\alpha$-carotene, $\beta$-carotene and vitamin E, but lutein esters need more (Roodenburgl, 2000). Adequate protein and zinc intake assists maintenance of vitamin A status. Vitamin E, as an antioxidant, protects vitamin A from being oxidized. Fibre, chlorophyll and non-provitamin carotenoids, which are commonly present in the diet, tend to reduce bioavailability. There is evidence that alcohol ingestion interferes with the conversion of $\beta$-carotene to vitamin A.

### 2.2.5.6. "N" Nutrient status of the host

Absorption of carotenoids is influenced by vitamin A status. If it is low, conversion of carotenoids to vitamin A is likely to be increased. There is evidence that zinc deficiency impairs the efficiency of $\beta$-carotene conversion to vitamin A.

### 2.2.5.7.  "G":  Genetic factors

There are some genetic defects that cause vitamin A deficiency. The three known to date are; the enzymatic failure to cleave β-carotene in the small intestinal mucosa (McLaren and Zekian, 1971), heterozygotic reduction of plasma RBP (Matsuo, Matsuo and Shiranga, 1988), and mutations in the gene for retinal binding protein (Biesalski, Frank and Beck, 1999).

### 2.2.5.8.  "H":  Host factors

The serum response to β-carotene is higher in women than in men but the reason is not known. In several ways, men are more susceptible to develop VADD than women. Age does not appear to be a factor. Diseases that interfere with intestinal absorption, especially of lipids, are likely to impair carotenoids bioconversion. In developing countries, intestinal parasites such as *Ascaris lumbricoides* and *Giardia lamblia* are of great importance.

### 2.2.5.9.  "I":  Mathematical interactions

This refers to the possible synergistic or even antagonistic effect that two or more factors might have when acting together. At present this is a theoretical concept only, as data are lacking, but it will have to be borne in mind as knowledge progresses.

From the above mnemonic, it is clear that there isn't enough data available to credit or discredit the bioavailability and bioconversion of β-carotene to vitamin A and the fact that DGLVs are a staple food for most communities and some have  a much lower incidence of VADD indicates that a lot more work needs to be done.

It is therefore important to continue research with leafy vegetables, as information available is contradictory as there are two schools of thought: one argues that green leafy vegetables improve vitamin A status and the one holds the opposite.

### 2.2.6. *Manifestations of vitamin A deficiency.*

The symptoms of VAD are not immediate, since the hepatic stores can meet the body requirements for quite some time: 2-4 months. However, once they manifest, if no intervention is done, the condition progresses in various stages (shown in Fig.4). These are accompanied by various clinical manifestations shown in Table 3 (pg. 28).

The deficiency manifestations are related to the eyes, skin and growth. The earliest symptoms of VAD are difficult to detect, but night blindness is a good indicator. At this stage, the individual has reduced ability to adapt to dim light. According to WHO classification, this is XN (Table 3). When the prevalence rate of plasma vitamin A is <10mg/dl in more than 5%, it becomes a significant health concern (WHO, 1982). This however can only be determined by analyzing blood samples drawn from a population. The high cost of this exercise in terms of equipment and personnel makes it an almost impossible determiner of VAD although it would be the most effective in terms of prevention as it is often the first sign of VAD before physical manifestations occur.

The various stages are discussed in the subsequent section. These stages are classified based on progression of VAD and clinical assessment of the presenting symptoms (Table 3).
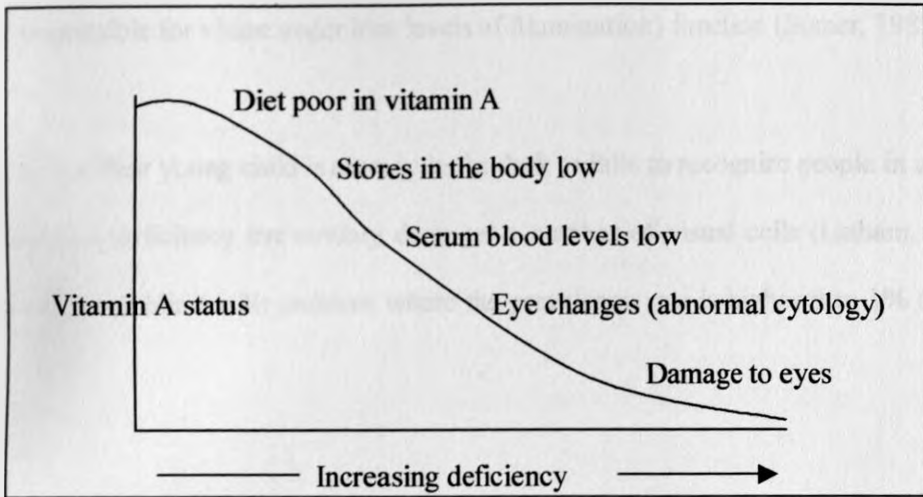
Source: WHO, 1999

*Fig. 4:*     *Stages in the development of VAD*

### 2.2.6.1.     *Xerophthalmia*

Xerophthalmia is the general term applied to all ocular manifestations of impaired vitamin A metabolism, from night blindness to corneal scar. The classification of xerophthalmia is presented in Table 3.

### 2.2.6.2.     *Night blindness / Nyctalopia*

Most communities with VAD have a local term for night blindness. Even at an early stage well before any physical changes in the eye can be seen (WHO, 1999). Night blindness comes as a

result of VAD interference with rhodopsin production, which impairs rod (the sensory receptors of the retina responsible for vision under low levels of illumination) function (Somer, 1982).

Parents may notice their young child is clumsy in the dark or fails to recognize people in a dimly lit room. Prolonged deficiency irreversibly damages a number of visual cells (Latham, 1997). Night blindness is a public health problem where the prevalence rate is higher than 1% (WHO, 1982).

*Table 3:*     *Classification of xerophthalmia*

| Ocular signs | Classification |
|---|---|
| Night blindness | XN |
| Conjunctival xerosis | X1A |
| Bitot's spots | X1B |
| Corneal xerosis | X2 |
| Corneal ulceration/ keratomalacia | |
| < 1/3 corneal surface | X3A |
| Corneal ulceration/Keratomalacia | |
| ≥ 1/3 corneal surface | X3B |
| Corneal scar | XS |
| Xerophthalmia fundus | XF |

Source: WHO 1982

### 2.2.6.3. Conjunctival xerosis

Conjunctival xerosis is the next sign of VAD and the first physical sign. The conjunctiva dries up and patches of xerosis give the appearance of sandbanks at receding tide. The conjunctiva loses its shiny luster and often becomes thickened, wrinkled and sometimes pigmented (keratinizing metaplasia). The epithelium of the conjunctiva is transformed from the normal columnar to the stratified squamous type with a resultant loss of goblet cells (Latham, 1997).

### 2.2.6.4. Bitot's spots

Bitot's spots are usually triangular-shaped, raised whitish plaques that occur in both eyes (Sommer, 1995). They sometimes accompany conjunctival xerosis and when examined closely, they look like fine foam with many tiny bubbles. This foamy, sticky cheesy material comprises keratinized epithelial cells and can be wiped away. A prevalence rate above 0.5% indicates a public health problem (WHO, 1982). Bitot's spots in the absence of xerosis may have another cause (Latham, 1997).

### 2.2.6.5. Corneal xerosis

Corneal Xerosis is the drying of the corneal surface, which first appears hazy and then granular on simple eye examination. The drying is followed by a softening of the cornea, often with ulceration and areas of necrosis to produce corneal ulceration/keratomalacia. The corneal ulcers are usually circular and punched out appearance. They may initially be small covering a third of the eye or less, but they may extend centrally to involve much of the cornea.

Ulceration may lead to perforation of the cornea, prolapse of the iris, the loss of ocular contents and perhaps destruction of the eye, a condition termed keratomalacia (Sommer, 1995). Although the lesions usually occur in both eyes, the corneal ulceration may be more advanced in one eye. With severe manifestations the child is usually seriously ill, sometimes with a high fever (Latham, 1997). Corneal Xerosis becomes a public health concern when the prevalence rate is in excess of 0.01%.

## 2.2.6.6. *Corneal scar*

When a corneal ulcer is treated while it is still small, it will heal forming a corneal scar. The size of the scar and the limits it imposes on future vision will depend on how large or advanced the corneal ulceration was and its location (Latham, 1997). A prevalence of 0.05% indicates a significant level of VAD.

## 2.2.6.7. *Xerophthalmia fundus*

Xerophthalmia of the fundus is sometimes seen early in the disease under examination with an ophthalmoscope. The retina has white dots around the periphery of the fundus (Latham, 1997). It is a rare condition where impairment of the rods leads to structural damage of the retina.

## 2.2.7. *Aetiology of vitamin A deficiency.*

Vitamin A deficiency is the most common cause of blindness in many endemic areas. It is estimated that over 100 million young children in the world are affected by VAD. It results from low body stores of vitamin A (WHO, 1999).

Xerophthalmia occurs almost entirely in children living in poverty. It is extremely rare to find cases in more affluent families even where xerophthalmia is prevalent. Communities where there are high rates of infectious and parasitic diseases such as measles and helminthes, prolonged or severe diarrhea and other infections, also have reduced blood levels and stores of vitamin A (WHO, 1999).

Children who have a brother or sister with eye signs of VAD are ten times more likely to have severe VAD. Mothers of these children are five to ten times more likely to have night blindness. This is because the mothers are more likely to fall short of nutritional requirements as they usually eat after the family has eaten and are forced to skip meals when the food is finished. Children between six months and six years and pregnant and lactating mothers are the group most at risk of suffering from VAD (WHO, 1999). Children from the same neighborhoods and communities as someone with VAD are twice as likely to have or develop severe VAD.

Families affected by land shortages, inequity and low levels of female literacy and grossly inadequate food security and health are also a high-risk group for VAD (WHO, 1999).

Families living in certain environments are also at high risk for VAD, including communities where the availability of vitamin A rich foods is low, where infant mortality levels are high (above 100%), under five mortality levels are high (over 75%), or where there are high prevalence rates of underweight, stunting, wasting, or where there are high measles case fatality (>1%) (WHO, 1999).

The most important effect of vitamin A supplementation is the prevention of death and blindness and the reduction of severity of morbidity (McLaren, 2001). It is estimated that by giving adequate vitamin A in vitamin A deficient populations, child mortality from measles can

be reduced by 50 percent, and child mortality from diarrheal disease by 40 percent (WHO, 1999). Overall mortality in children 6 to 59 months of age can be reduced by 23 % while perinatal mortality rates can be reduced by 44% (Allen, 2001). VAD can vary dramatically by season due to a shortage of vitamin A-rich foods and seasonal increases in diarrhea and measles. In Ichag, a village in West Bengal, during the months of April, May, and June, the percentage of children with Bitot's spots and night blindness increased by 2 to 3 times compared with children examined during October, November, and December (WHO 1999).

## 2.2.8.    Vitamin A and morbidity.

Increased morbidity brings with it heightened nutrient requirements and reductions in the efficacy of absorption and utilization of nutrients (Semba, 1999). Retinoids are necessary for the maintenance of the immune function, which depends on cell differentiation and proliferation in response to immune stimuli. Retinoic acid is important in maintaining an adequate level of natural killer cells that have antiviral and anti-tumor activity (McLaren, 2001).

Nutritional deficiencies may lead to oxidative stress and immune suppression which in turn lead to increased HIV replication and hastened disease progression (Semba, 1999). β-carotene has anti-oxidative properties, which can be effective in reducing the rate of oxidative stress and immune suppression (Gillespie, 1999).

In Kenya, over 2,000,000 people are living with HIV/AIDS. Of these, 51% are women and over 200,000 are aged between 15 and 24 years (IATF, 2000). Micronutrients and especially vitamin A may play a significant role in delaying the onset of HIV and death because they contribute in helping the body resist opportunistic infections. Without sufficient vitamin A, the body's

resistance to opportunistic and infectious diseases is considerably lowered (ACC/SCN. 1998). By administering substances that enhance the increase of RBP, which is reduced in PLWHA and general morbidity (ACC/SCN, 1998), it may be possible to improve the immune system of the body by administering substances that enhance its performance, e.g., Vitamin A (Piwoz, 2000).

Malabsorption of vitamin A can occur with diarrhea, intestinal infections and parasitic infestations while gastroenteritis, measles, diarrhea and respiratory infections suppress Vitamin A absorption (Tomkins, 1989). Intestinal parasites play a major role in the bioavailability of β-carotene (Allen, 2001). In particular, *Giardia lamblia, Ascaris lumbricoides*, (round worm) and *Ankylostoma duodenale* (hookworm) have been shown to reduce vitamin A absorption and in some incidences to be associated with VADD (Curtale, 1995). The impact may be greater on absorption of carotenoids than on the absorption of preformed vitamin A.

Most of the population becomes infested where sanitation is lacking. Deworming programmes fail to have a long-term impact in the absence of improvements in sanitation and the breaking of the cycle of transmission (McLaren, 2001).

## 2.3. Efforts to Alleviate VAD

### 2.3.1. VAD intervention

In Kenya, there have been many nutritional interventions done. To date, the most widely used VAD intervention is the vitamin A supplementation, which comes in the form of drops for children and capsules for adults. These are widely distributed in health clinics and in communities where related programmes are carried out (Gotink, 2000).

Despite these intervention programmes, statistics show that there has been no relative difference in children's health status for the past 30 years (Gotink, 2000). Apparently ongoing programmes have been unable to cope with growing populations. Problems arising include unsuitability of some intervention programmes in addressing VAD, inadequate intervention programmes and logistic delays in implementation of the programmes (Hancock, 1989). The unsuitability of interventional programmes, which are not deficiency- specific, may be appropriate in a nutritional crisis appropriate for intervention but they are not sustainable (Gotink, 2000). Moreover, VAD constitutes a significant health problem in Kenya and deteriorating health indices indicate a grave future for the nation unless this trend is reversed (Mwaniki, 2002)

### 2.3.1.1. Food based interventions

There are a number of food-based interventions. These include fortification of cereals, legumes, oil, and powder mixtures with vitamin A (WHO, 2001). Whereas these may be suitable for adolescents and adults, there are many serious limitations for children. The presence of beans in semi-cooked form makes it impossible for the meal to be of much use to young children, as their digestive systems cannot cope (Mbugua, 2000). Older children find the food unpleasant and often suffer flatulence when legumes are consumed.

Further, the logistics of getting the interventions to groups at risk especially those who are faced with drought have often been costly. They have also often been inappropriate and delayed.

The main cause for this has been politics and organizational bureaucracy (Hancock, 1989).

Many deteriorate rapidly under these conditions resulting in death or with permanent damage to their health (Hancock, 1989). The wrong assumptions made that those at risk will have the knowledge, the firewood, water and physical energy to cook the food provided have further compounded the problem. According to ACC/SCN, (1994) a family cooking on a simple wood stove requires roughly 5 kg. of firewood per day.

Due to the form in which most interventions come, preservation of nutrient content has been a problem because of extreme environment conditions like storage of dry foods in dry and humid conditions, which causes nutrient instability and spoilage. Storage conditions and time lapse between dispatch from source to delivery for consumption further aggravates the situation. Rancidity in oil-containing foods are problems often encountered. Microorganism colonization has also been of concern especially in inadequately dried cereal and bean powders (Hancock, 1989).

The various foods used for supplementation, complementary feeding and for emergency situations practically all have no vitamin A. The basic food commodities distributed to groups at risk in Kenya by aid organizations such as UNHCR, WFP, MSF, Red Cross and the government of Kenya collectively include; cereals, edible oil/fat, pulses, spices, coffee, tea, sugar, salt, high protein biscuits and dry skimmed milk. Fresh meat, fruits and vegetables are rarely available (ACC/SCN, 1994).

Whereas there are problems in fortifying them, baked products with ingredients high in provitamin A substances or preformed vitamin poses few problems. Such products (as the β-carotene rich biscuits) could be used as a first-line commodity while other foods are bing procured and assembled (ACC/SCN, 1994).

### 2.3.2. Kitchen gardens and nutrition education

Apart from vitamin A capsules and drops, other efforts being made to address VAD include promotion of kitchen gardens where dark green leafy vegetables are grown and nutrition education through health clinics and community health workers (IVACG, 1994).

In Thailand, communities were encouraged to grow the ivy gourd plant (coccinia indica), which is rich in vitamin A in a study carried out by Chittchang. Within a relatively short time, significant changes in dietary habits and nutritional benefits were noted (IVACG, 1995). Similar studies done in Indonesia, Guatemala, Bangladesh, Haiti, India, Niger and Vietnam, using among others, different green leafy vegetables such as spinach, fenugreek, safflower, dock, amaranth and dill (Joshi, 1995) yielded similar results (IVACG, 1995).

The communities were able to adapt to these new diets because they are fundamentally vegetarians and nutritional education provided before the commencement of implementation of programmes provided an understanding for need of change.

### 2.3.3. Green leafy vegetables and fruits

Fresh fruits and green leafy vegetables are good sources of provitamin A: Carotene (McLaren, 2001). Processing the food or reducing particle size makes carotenoids more available. Also, cooked or processed vegetables have been shown to have more bioavailability of β-carotene than raw vegetables (Thokozile, 2001).

DGLVs are eaten with the main starchy staple more than any other sources with higher bioavailability of vitamin A in most communities at risk of VAD. As other sources of vitamin A are from animal origin and are not easily available to groups at risk because of their cost and in some cases availability, the role of vegetables as the main source of vitamin A for these groups cannot be understated. Since the bioavailability of vitamin A is low in vegetables, it is extremely important to explore ways in which benefits from DGLVs can be maximized to provide dietary requirements (WHO, 1999).

### 2.3.3.1. *Bioavailability of vitamin A from dark green leafy vegetables (DGLV) and green leafy vegetables ( GLVs): case studies.*

In a 12-week supplementation study in Indonesia, it was observed that an additional daily portion of dark green leafy vegetables given to lactating women did not improve vitamin A status (as measured by serum retinol, breast milk retinol, and serum $\beta$-carotene). In those who received a similar amount of $\beta$-carotene on a wafer there was significant improvement (De Pee, 1996). However, in the study carried out among pregnant women in South Nepal, deaths among women receiving either low-dose vitamin A or beta-carotene supplements dropped dramatically, by an average of 44% (Allen, 2001).

Later work done in 1998 by De pee showed that in central Java vegetables played a lesser part in maintaining vitamin A status than had been thought and orange fruits were more effective in improving vitamin A status than dark green leafy vegetables as more studies carried out in the same year by De Pee revealed. The calculated $\beta$-carotene: retinal equivalence in leafy vegetables was 26:1 (West, 2001).

This however contradicted the study in Guatemala where supplementation of diets in children aged 7-12 years failed to show any benefits (Bulux, 1994).

In another study carried out in Ghana that consumption of DGLV with added fat significantly increased serum retinol levels in children, but even so just over 50% remained with inadequate vitamin A status (Takyi, 1999).

Another study was carried out in China on the effect of vitamin A body stores of children 3-7 years old of two vegetable diets using the double stable isotope method. Vitamin A labeled with different kinds of deuterium (D4 and D8) was administered before and after the intervention to estimate changes in the total body vitamin A stores that occurred. One group received their daily customary intake of 56g of green-yellow vegetables and 224g of light-colored vegetables, and another received 238g of green-yellow vegetables, and the other received 34g of light colored vegetables. Only the latter diet maintained adequate Vitamin A stores (Tang, Gu, Hu et al, 1999). It was calculated from the isotope studies that pro vitamin A carotenoids (mainly β-carotene) provided an estimated vitamin A equivalent of 27:1 on average. It should be noted that to achieve this effect more than ¼ kg of vegetables was consumed each day by these children as young as 3 years. A study done on the bioavailability of β-carotene from oriental vegetables found higher bioavailability, but still much less than from purified β-carotene beadlets (Tang, 1999).

Another study was carried out in Kenya to assess the potential of symphytum asperrimun (comfrey) as a source of vitamin A for school children. The 85 subjects were males ( aged between 8 and 16 years) in a reception center for juvenile delinquents.

Comfrey leaves were harvested, de-ribbed, cleaned, blanched and oven dried. The dried leaves were then crushed into powder and fried with vegetable oil for five minutes. 85 gram portions of the resulting cake were measured and well mixed with individual school rations of the experimental subjects. The intervention was carried out once per day (lunch time) for twenty-one days without a break. At post-test, the experimental group recorded significantly higher levels of serum β-carotene but almost similar serum retinol levels to the non-experimental group (Chania, 1998).

This study indicated that comfrey leaves had high levels of β-carotene compared to other dark-green vegetables (especially when they were blanched whole and then dried), and lower vegetable matrix (Chania, 1998).

# CHAPTER THREE

## STUDY SETTING AND METHODOLOGY

### 3.1.    Study Area

Kĩambu district, where the study was carried out, is in the Central Province of Kenya and it has a total area of 1,323.9 square kilometers. The majority of the population is from the Kikuyu ethnic group (96%). The population density per square kilometer is 562. It has a population of 744,010 with females making up for 53% of this figure.

There are 5 locations in Kĩambu district namely; Kĩambaa, Gĩthũnguri, Limuru, Lari and Kikuyu, and 8 sub locations.: Kĩambaa, Ndumberi, Ruaka, Tin'gan'ga, Rĩabai, Kamĩti, Kĩambaa Settled Area and Waguthu.

The Kirigiti Girl's Approved School is in Kĩambaa Division, Rĩabai location, Rĩabai sub-location, Rĩabai village. Rĩabai sub-location has two villages: Kĩhingo and Rĩabai. Rĩabai village has an area of 3.3 square kilometers with a population density of 3,130 per square kilometer. It has 2,770 households and a total population of 18,574. Females are 9,593 while the males are 8,981 (CBS, 2001).

The unemployment in the area is high and this situation has been made worse by the on-going retrenching program undertaken by the government. Subsistence farming is the main economic activity although a lot of the people in the area are engaged in small businesses not carried out in the area. The main foods grown in the area are maize, *mathũkũma* (kale), beans, bananas and potatoes. A small percentage area of land is under coffee, which was the main income earner in the area 10 years ago. The researcher gathered that this changed due to low returns and delayed payments to the farmers. Animal products contribute little to most family diets because the milk

and meat from cattle raised in the area and products from poultry rearing and keeping are mainly for income generation rather than family consumption. Money earned from the sale of these products is used to buy items such as other foods, condiments like salt and sugar, fats and kerosene. Some is also used to pay for services and education.

The houses in the area are mainly wood and iron sheets although it has quite a high number of stone houses as well. Piped water makes up the main water supply because wells dug up in the area yield water that is unsuitable for human consumption although it can be used for other domestic purposes. General hygiene is taken seriously and most residents take a bath every day on average. Household refuse is thrown in pits while organic food waste is either fed to the animals or thrown in the compost heap. Every household has access to a fecal waste facility either shared or individually owned. The majority of these are latrines although Water closets (W.C) are also used especially in the stone houses.

Kirigiti is a fast growing shopping center situated one kilometer away from the school. This mainly comprises small businesses like shops selling consumable and non-consumable products and a few services like basic health care and housing (CBS, 2001).

The Kiambu Provincial Hospital is 5 kilometers away from most residents and although the buildings are good, the facilities, staffing and services are inadequate and sometimes lacking altogether. The main asphalt Nairobi-Kiambu road runs 2 kilometers from the school and the school is built along the main Kiambu-Kamiti road. Kiambu town, which is the administrative and main economic center of the district, is situated 5 kilometers from the school and it is here where the district hospital is located.

### 3.1.1. *Study site and study subjects*

The study was conducted at the Kirigiti Girls' Approved School. It is a re-habilitation center and the only reception center for juvenile female wards of the state in the country.

The children are placed there by the juvenile courts all over the country or the Children's Welfare Department in the Ministry of Planning and Social Services as their cases are decided and there is no official registration date. The study subjects are received at the point of release by the two aforementioned bodies.

The Kirigiti Girl's Approved School has seven formal levels of classes: standard two to eight and two vocational training classes where Home Economics and Dressmaking are taught separately. Children are distributed into these classes as they join the school according to their previous level of education before conviction or after an I.Q. assessment regardless of their age.

At the time of the study, there were 228 children at the school aged between 8 and 18 years. They were distributed as follows: Standard two 10.8%, Standard three 13.2%. Standard four 15.3%, Standard five 14.9%, Standard six 12.3%, Standard seven 11.4%, Standard eight 7.5%, Home Economics class 8.0%. Dressmaking class 13% and those awaiting placement were 0.9%.

## 3.2. Sampling

### 3.2.1. Sample size determination.

The calculation of the required sample size was based on the prevalence of mild VAD in Kiambu District, which was 30% for children aged between 6 and 72 months. This is because there was no baseline data for the specific study age group. This assumption and a confidence interval of 95% were taken into account for the sample size determination.

The following formula for comparative studies was used (Fischer, 1991).

$$n = \frac{z^2 (pq)}{d^2}$$

where: n = sample size.

z= the standard normal deviation set at 1.96 which corresponds to 95% confidence interval.

p= the proportion of VAD in the area.

q= 1-p estimate of proportion of non vitamin A deficient children in the study area.

d= degree of accuracy desired

$$\frac{(1.96)^2 (.3 \text{ X } .7)}{(0.1)^2}$$

The sample size (n) obtained was = 81 subjects

This figure included a possible attrition rate of 8% ( a total of 6 children)

Based on this calculation, the study subjects were drawn from classes two, three and four and the cut off criteria was age. All the children in these classes, save three were included in the study. This made a total of 77 children.

### 3.2.2    Study design, methodology and sampling procedure

The study was interventional and experimental in nature. The Pre-test, Post-test research design was employed on 77 study subjects aged between 8 and 16 years.

The sampling frame comprised of 197 girls in the Kirigiti Girl's Approved School in Kiambu District, Kenya aged 8-16 years (Fig. 5). The study subjects were drawn from classes 2-4 which had 80 students. Those who were over 16 years old were excluded from the study. The school was purposively selected because it was believed that the children would have high risk for VADD among other reasons that include;

1. Uniformity in the lifestyles of the experimental group and the control group.

2. Similar feeding patterns of the two groups.

3. Similar exposure to morbidity conditions.

4. Acceptable age variation.

5. Same gender.

6. Similar vulnerability to VADD due to their age and their deprived backgrounds and their micronutrient deficient feeding pattern at the time of the study.

The sample size took into account the financial constraints, plausibility of results and the possibility of interpersonal result differences. Individual numbers from 1 to 77 were placed in unmarked envelops which were then sealed. The envelops were shuffled and all the study children were asked to line up and each picked an envelop of choice.

Site ➝ Kirigiti Girl's Approved School
(Purposively selected)       (228 girl children)

Systematic selection ➝ All children ages 8 –16 years
(Purposive sampling)       (197 children)

Selection of subjects ➝ All children ages 8 –16 years in classes 2-4
(Purposive sampling)       (77 children)

Collection of baseline information

Identifying children by numbers
(Random allocation Nos.1 –77)

De-worming

Determining which number (odd or even) to be either of the groups

Allocation of groups

Collection of blood samples for baseline serum vitamin A and β-carotene analysis.

Collection of stool for pre-test parasitic load assessment.

Pre-test clinical assessment and anthropometric measurements (taken to determine infections and nutritional status respectively).

Determining which of the groups will be experimental or control

**Experimental Group**
(βCRB group: all odd numbers)

**Control group**
(βCLB group: all even numbers)

Service of biscuits (31 days)

Service of biscuits (31 days)

Collection of blood samples for post-test serum vitamin A and β-carotene analysis.

Collection of blood samples for post-test serum vitamin A and β-carotene analysis

Collection of stool for post-test parasitic load assessment.

Collection of stool for post-test parasitic load assessment

Post-test clinical assessment and anthropometric measurements taken to determine infections and nutritional status respectively.

Post-test clinical assessment and anthropometric measurements taken to determine infections and nutritional status respectively.

Analysis

Analysis

*Figure 5:*     *Study design and sampling procedure*

This exercise generated two groups: one of 39 children, and the other of 38 children each randomly placed even or odd based on the number each was holding (Fig. 5).

To determine which of these two groups would be experimental and which would be the control, two papers, one placing odds as control and even as experimental and the other placing even as control and odd as experimental were placed in individual unmarked envelopes, which were then sealed. A teacher in the school was then asked to pick one of the two envelops to determine which group would be the experimental group and which one would be the control group. This exercise placed the odd number holders in the experimental group and the even number holders in the control group.

## 3.3.    Study Instruments and Data Collection Procedures

### 3.3.1.    Determination of β-carotene content in the comfrey rich biscuit.

The determination of β-carotene content in the comfrey rich biscuit (β-CRB) and the comfrey free biscuit (β-CLB) was done at the department of Food Technology and Nutrition laboratory using the column method.  The samples were baked and processed at the Mealz Jamaica Restaurant in Nairobi, Kenya and a spectrophotometer was used to determine the β-carotene content after drying and crushing the samples.

### 3.3.2.    Training interviewers and recruiting assistants

One field assistant with secondary level of education was recruited and trained for one week on questionnaire interpretation, social etiquette and dietary recall measurements. Training on anthropometric data collection technique was also conducted. This included the measuring and recording of weight, height and mid-upper arm circumference (MUAC) of the children

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Field ethics such as professionalism in carrying out specified duties, confidentiality of experience and collected data, suitable dress code, pleasant disposition and data collection through observation were also incorporated in the training program.

The drawing of blood; from the children, was done by three qualified personnel from the Center for Public Health Research (CPHR), of the Kenya Medical Research Institute (KEMRI), after consultations and briefing on specific study requirements.

Services of a qualified pastry chef, who was intensively trained on the preparation of the βCRB and the βCLB were employed to assist in the production of the biscuits. Training and consultations were an on-going process throughout the research period.

### 3.3.3 Questionnaire

A structured questionnaire consisting of questions on the history of the child, the dietary history and social background was administered to the children (Appendix 2).
Clinical and morbidity information was also recorded on this form. This information was obtained from the children, the manager, the cateress and the school nurse.

### 3.3.4. Child age determination

Information on the ages of the children was obtained from the children and verified with the school admission records. The information in the records had been provided by the court stewards and given to the school manager.

### 3.3.5. Food intake

The amount of food consumed by the children was weighed using a Salter dietary scale for all the meals eaten in a day.

These measurements were taken for one week. The amount of nutrients taken was determined by proximate analysis done at the Department of Food Technology and Nutrition laboratory at the University of Nairobi, Kabete Campus.

### 3.3.6. Anthropometric measurements

All study subjects had their weight, height and mid-upper arm circumference (MUAC) taken by the researcher with the assistance of a field assistant.

### 3.3.6.1. Body weight

Body weight was measured to the nearest 500 grams using a Soehnle bathroom scale. The calibration was checked before the weighing session and checked or readjusted after every 5[th] child weighed with a 1 kilogram standard weight. The study subjects wore only the school tunic, which was made of light cotton polyester fabric. Each child was weighed twice with each reading and the average from the two recorded.

*3.3.6.2. Height*

The height was taken after the child was weighed. Roche paper wall height meter was used and the height was taken to the nearest 0.5cm. Bare-footed, the child stood straight with back against the wall with the back of heels, calves, backside, shoulder blades and back of head touching the wall. The child was asked to look straight ahead with hands hanging freely on the side.

*3.3.6.3. BMI*

BMI was calculated as ratio of body weight (kg) and height in meters ($Kg/m^2$):

$$BMI = \frac{weight\ (kg)}{height\ (m^2)}$$

The cut off value used in this study to classify children as undernourished was <16.0 (Latham, 1997).

### 3.3.7. Biscuit preparation

The biscuits were baked at Mealz Jamaica Restaurant in Nairobi Kenya under high standards of hygiene. The main equipments used were a blender to macerate the comfrey leaves, a weighing scale, a dough mixer, baking trays, biscuit rings to standardize the size of the biscuits, oven and cooling racks for the biscuit preparation.

## 3.4. Implementation of Research Activities

In March 2000, proximate analysis and beta-carotene content for comfrey rich biscuits were done. The results and available scientific information on comfrey formed the basis of the study proposal.

### 3.4.1. Comfrey leaf production

In July, arrangements for comfrey leaf production with a farmer in Kiambaa, Kiambu District were finalized. These included details on the growing, harvesting, pre-preparation and packaging of the comfrey leaf before processing and production. This took one week of training and consultations.

### 3.4.2. Ethical clearance and consideration

In September, the researcher applied for research proposal approval to the Board of Graduate Studies, University of Nairobi and approval was granted in October 2000. A Research Clearance Permit to enable the researcher to carry out the study was issued from the Ministry of Education, Science and Technology in September 2000. In October, ethical clearance was sought from the Kenyatta National Hospital Ethical and Research Committee. This was granted in late December 2000. The clearance to carry out the research at the Kirigiti Girl's Approved School was granted in February 2001, by the Director of Children's Services in the Office of the Vice President and Ministry of Home Affairs, Heritage and Sport.

Informed consent was obtained from all the study subjects who were very cooperative and the school manager who at this point was the guardian. The blood collection was carried out in a room specifically allocated for the study under hygienic conditions using sterilized needles, syringes, vacutainer tubes and gloves. Methylated spirit swabs were also used before and after drawing of blood. Measures were taken to ensure that the subjects experienced as little discomfort as possible especially in the drawing of blood.

New polypots and surgical tongue depressors were used for stool collection to ensure the children did not come into direct contact with the stool. Morbidity emerging at the time of the study was brought to the attention of the management.

### 3.4.3. Data collection logistics and protocol

In the same month, the researcher put together all the required equipment and data collection tools. Authorization to use Mealz Jamaica restaurant facilities for the biscuit production was given by the owner of the restaurant. General information about the study area was collected from the school headmaster, school manager and the local people. The researcher paid a visit to the office of the District Officer in Kiambu in fulfillment of protocol procedures.

Anthropometric measurements were taken on 11th February 2001. Part of the questionnaire that dealt with the history, clinical, medical, dietary and personal information of the child was completed at this time. Clinical assessments were also done. All questionnaires were then closely checked and crosschecked for completeness on collected information. Blood for baseline β-carotene and vitamin A analysis was drawn on the 15th February 2001.

Stool samples from the study subjects for baseline worm load investigation were also collected on the same day.

### 3.4.4. *Validation and reliability*

The researcher not only closely supervised the data collection, processing of the comfrey, production of the biscuits and administration, blood and stool collection, but was also directly involved in carrying out all mentioned activities.

Validation of all data and quality control of the questionnaire was also done at every stage.

## 3.5. Planting, Harvesting, Packaging and Processing of Comfrey Leaves

The comfrey leaf was grown at a time when there was intermittent rainfall. It was grown on red loamy soil and the plants were therefore watered daily. Only the mature comfrey leaf was harvested from the bottom of the stem and washed with clean running tap water to remove dirt particles and packed in black polythene bags, after water was allowed to drain for five minutes, to minimize nutrient loss due to exposure to light. They were then transported by car whose inside temperature was always set to $20^0C$ for this purpose.

At the processing center, the comfrey leaves were de-ribbed (Fig. 6), shredded and macerated using a National Food Processor model MK – 155N, in batches of 100grams with 50 milliliters of water for 2 minutes. The macerate was stored in a tightly sealed cool box at $8^0C$ ready for use.

### 3.5.1. Preparation of βCRB (β -carotene rich biscuit)

The β -carotene rich biscuit was processed as follows, using the recipe in Table 4: The macerated comfrey leaf was added to a mixture of whisked fat and sugar with flour and baking powder. This mixture was then kneaded to a soft consistency using a Macdams 20 liter mixer Model SM40 set at speed 2 for 5 minutes. The mixture was then placed in individual burger rings placed on baking sheets after weighing each scoop. Each individual biscuit mixture scoop weighed 120 grams. The biscuits were then baked in a preheated Macbak double tier model oven for 60 minutes at 150° centigrade. Ninety-six biscuits were processed per baking session.

The biscuits were then removed from the oven and left to cool in a cold room on cooling racks for 45 minutes. Samples for laboratory analysis were picked immediately after baking and delivered to the laboratory for proximate and β -carotene content analysis. The remaining biscuits were then packed in a bioxypolyproplene (BOPP) bag, 6 biscuits per pack and tightly sealed. Twenty of these 6-packs were then repacked in carton boxes, which were sealed and stored in a dark, dry, cool ( $15^0$C) store ready for consumption.

### 3.5.2. Preparation of the βCLB (β-carotene low biscuit)

The recipe for βCLB was exactly the same as that of the comfrey biscuit (Table 4) except that it did not have the comfrey leaves. It was baked at 180° centigrade for 30 minutes. It also had green permitted food coloring (: blend of brilliant blue FCF Tartrazine NaCL with a 20% die

content) added so that it closely resembled the experimental biscuit. The storage conditions were also the same.

*Table 4:* **Biscuit recipes**

| β -**CRB** ingredients | β -**CLB** ingredients | Amount |
|---|---|---|
| Sugar | Sugar | 75 grams |
| Shortening | Shortening | 75 grams |
| Wheat flour | Wheat flour | 100 grams |
| Baking powder | Baking powder | $^1/_8$ teaspoon |
| Water | Water | 50ml |
| Comfrey leaf | - | 100 grams |
| - | Permitted food color | A pinch |

*Note: The shortening used was unfortified vegetable Joma brand. The wheat flour was also unfortified.*

Harvesting of comfrey leaf

Fresh comfrey – washed
De-ribbed and blended

| **INDREDIENTS** | **INGREDIENTS** |
|---|---|
| Flour, fat, sugar, green food color, water | Flour, fat, sugar, comfrey, water |
| Whisked fat, sugar and Other ingredients added till dough texture is of solid consistency. | Whisked fat, sugar, and **addition of comfrey.** Other ingredients added to solid consistency. |
| 120g scoop of mixture placed in burger ring | 120g scoop of mixture placed in burger ring |
| Oven baked for 30 mins. Oven temperature 180°C and cooled. | Oven baked for 60 mins. oven temperature 150° C and cooled |
| 2 samples from each ground to determine nutrient* content. The rest packed and stored. | 2 samples from each ground to determine nutrient* content The rest packed and Stored |

Note: Proximate analysis was done for the two biscuits and the β–carotene content in each determined. The biscuits were packed in BOPP paper to prevent oxidation and then repacked in opaque carton boxes to prevent exposure to light both of which may have a negative effect on the Vitamin A content.

Fig 6:      *Harvesting, Packaging And Processing of Comfrey Leaves.*

### 3.5.3. *Analysis of the βCRB and βCLB biscuit samples.*

The proximate analysis and the determination of β-carotene for the βCRB and the *β CLB* were done at the Food and Technology Laboratory at the University of Nairobi, Kabete Campus as explained below. The determination of β-carotene was done using the column method.

Method

The biscuit was crushed using pestle and mortar. Two grams of the sample were extracted using 20 milliliters of acetone, until the material turned colorless. The extract was then filtered into a 100 ml volumetric flask and made up to the mark with acetone. Twenty-five milliliters of the acetone extract was then transferred into a 50 ml round bottomed flask and evaporated to dryness in a Heildoph vacuum evaporator.

The yellow β-carotene pigment was eluted through silica gel-petroleum ether column and collected into a 25 ml volumetric flask and then made to the mark with petroleum ether. The absorbance of the eluted β-carotene was read at 450nm, using a spectrophotometer. The β-carotene level was read from a β-carotene standard curve set at $0.1 - 0.85$ μg/ml.

### 3.6.   Analysis of School Meals

Whole portions of meals served at the school were collected and taken to the Department of Food Technology and Nutrition laboratory for proximate analysis and vitamin A determination.

## 3.7. De-Worming the Study Subjects

Following the pre-test stool and blood collection, the subjects were dewormed five days before the commencement of the feeding. Each subject was given one 400mg Nubend – 400 Albendazole tablet. Although the tablet was chewable, the researcher supervised its intake with water to ensure each subject had ingested it.

## 3.8. Meal Service

The children assembled at 09.30 hours every day at the feeding area after washing their hands. Those children who had picked odd numbers sat to the left of the feeding area and were given the comfrey rich biscuit while the even number group sat to the right and were given the comfrey-free biscuit.

The biscuit consumption by both groups was closely supervised by the researcher to ensure that the children ate the whole biscuit and to also ensure that there was no exchange of biscuits between the groups as it was believed such an exchange would have a negative impact on the results of the study.

The intervention was carried out for thirty-one days after which clinical assessment was done, anthropometric measurements were taken, and stool and blood samples collected for serum retinol and $\beta$-carotene analysis. This was done to determine whether the experimental biscuit had made any significant change in the children.

### 3.9. Blood Analysis: Serum Retinol and Serum β-Carotene

#### 3.9.1. Blood collection

Blood collection and sample preparation and analysis were carried out with the assistance of laboratory technicians from the CPHR. Five milliliters of venous blood was collected from each subject before and after the study. A clean veni-puncture was made using a Microlance-3 sterile, disposable, 0.6mm x 25mm needle and a 5ml disposable syringe both manufactured by Becton Dickinson, Spain.

The blood was removed from the anticubital vein after cleaning the area with a surgical spirit swab and was placed in a pre-labeled, sterile 5ml 13x75 mm silicon coated Venoject evacuated blood collection tube manufactured by Terumo Europe N.V., Belgium and covered with aluminium foil to protect the blood from ultraviolet light which affects vitamin A levels. The tube was then sealed using a sterile screw cap. The labeled samples were then placed in a cool box maintained at 8-10° C by a liquid nitrogen ice pack and transported to the CPHR where the serum was separated through the centrifuge method to be used for serum retinol and serum β-carotene determination.

#### 3.9.2. Separation of serum

The clotted blood was centrifuged at 3000 revolutions per minute for 10 minutes using an MSE model centrifuge. The clear serum was separated using Pasteur pipette and put into a sterile cryotube, labeled by name, study number and date using a permanent marker. The serum was

flushed with nitrogen gas, covered with aluminum foil and kept at a $-40^O$ C deep freezer until analyzed for retinol and β-carotene which was done within three months using High Performance Liquid Chromatography (HPLC) method of biochemical assay as described below.

### 3.9.3. Retinol analysis

The frozen serum samples were removed from the deep freezer and thawed at ambient temperature ($25^o$ C and vortexed. 250 micro-liters of homogenized serum was pipetted into a conical centrifuge tube with a Teflon sealed screw cap using a 40-200µl Finn pipette. 1.5 micro-liters of ıµg/ml of of retinyl acetate was then added to the serum sample as internal standard. This was then de-proteinized and precipitated by vortexing for 20 seconds using 100% ethanol. The extracting solvent (HPLC grade hexane) was added and the mixture was then vortexed for I minute and centrifuged at 3000 rpm for 2 minutes. One hundred micro-liters of the supernatant upper layer was removed and stored in a second aluminum foil covered 13x100 mm length tube. The extraction was repeated and the two extracts were evaporated at $37^o$ C in a water bath under a gentle stream of nitrogen gas. The 20 micro liters residue was reconstituted with 100µl of the mobile phase consisting of 95% methanol and 5% distilled water.

Thirty micro liters of reconstituted residue was injected into the A Supelcosil C 18 column measuring 25cm x 4.6 mm (internal diameter) column using SGS gastight syringe and needle. The flow rate in the column was 2ml/minute.

The retinol peak was detected at 325 nm using a Hitachi L 6000 UV variable detector. The peaks and the peak area counts were recorded by a Hitachi integrator.

The serum retinol normal range was set at 0.20-0.65 µg/ml. and the serum retinol was calculated from a standard curve by linear regression.

### 3.9.4. β-carotene analysis

The frozen serum samples were removed from the deep freezer and thawed at ambient temperature (25° C) and vortexed. Two hundred and fifty microliters of homogenized serum were accurately pipetted into an aluminium foil covered 16 x 100 mm culture tube with Teflon sealed screw top. Two hundred and fifty microliters of 100% ethanol was added. This was rapidly vortexed at intermittent intervals of 15 seconds for 1 minute. HPLC (1.5ml) grade hexane was added to each tube and they were capped. This was then vortexed for 1 minute at room temperature and centrifuged at 3000 rpm for 2 minutes at room temperature to ensure extraction of the β-carotene and to obtain a clear separation of the phases.

The upper phase was removed and stored in an aluminium foil covered 13 x 100mm tube. The lower phase was re-extracted and the resultant upper phase added to the first extracted upper phase. The combined supernatants were evaporated under a steady steam of nitrogen gas to about 37°C. The residue was reconstituted with 100µl mobile phase consisting of 78% acetonitrile, 16% chloroform, 3.5% 2- propanol and 2.5% water. 30µl of reconstituted residue was injected into the HPLC Supelcosil C 18 column, measuring 25cm x 4.6 mm (internal diameter), using SGS gas tight syringe and needle. The flow rate in the column was 2ml/minute. The β-carotene peak was detected at 450 nm using a Hitachi L 6000 UV variable detector. The peaks and area of the total carotenoids and the β-carotene was recorded by a Hitachi integrator.

Using the external standard method, the normal range for carotenoids was set at 0.5 to 2.5μg/ml while the β-carotene normal range was set at 0.1 to 0.85 μg/ml. The β-carotene was calculated from a standard curve by linear regression.

## 3.10. Stool Collection and Worm Load Determination

### 3.10.1. Stool collection

Each child was issued with a 30ml plastic polypot with a tight fitting cap for stool collection and a wooden 6-inch surgical tongue depressor/spatula manufactured by Kamfit Surgicals (Nantong Foreign Trade Corporation, China), and instructed on their use. Each polypot was clearly labeled with the child's study number before blood referred to above had been drawn and placed in individually labeled brown packing paper bags. All samples were then placed in a carton box, which was then sealed, transported to the CPHR. Processing was done immediately.

The study specifically targeted to determine the egg load of the *Ankylostoma duodenale (hookworm), Schistosoma mansoni, Ascaris lumbricoides* and *Trichuris trichiura* because it was believed that when present, they interfere with the intake of vitamins especially vitamin A and minerals in the gut.

### 3.10.2    Stool analysis

The Kato Thick Smear technique (Cheesbrough, 1981) for the detection of all common types of intestinal helminth eggs was used.

### 3.10.2.1.    Procedure

The stool sample was homogenized and with an applicator stick, 50mg of fresh stool specimen was transferred to a clean microscope slide. This was then covered with cellophane that had been impregnated with glycerin and a tincture of malachite green solution before the transfer, and pressed to spread evenly in an area of about 20x20cm. The smear was allowed to stand for an

hour at room temperature to dry and clear the feces and to facilitate egg screening.

The entire film was then examined under low magnification in duplicate. An egg count from the two slides was then done and the resultant figure was multiplied by 10 to give the number of eggs per gram of stool.

### 3.11.    Data Management, Processing and Analysis

As indicated in the methodology section, all the necessary quality control measures were taken for biscuit processing, blood samples collection, anthropometric measurements and stool specimen.

Personnel involved in the project were closely supervised throughout the study period. The data on the questionnaire was checked for completeness of information and consistency of answers. Data collected was coded and entered in the computer using the

Statistical Package for Social Sciences (SPSS) program. Frequencies, percentages, averages and means were computed using the SPSS program. Chi-square and p-values of the same variables between groups were computed using the EPI 6 program. The single and two tailed tests of significance were used to compare differences of means between groups. This was done using the SPSS program. Analysis of variance test (Anova) was used to calculate the association between morbidity and oral and ocular conditions using the SPSS program. The Microsoft Excel program was used for graphics.

## 4.1. Demographic Characteristics

### 4.1.1. Social background

This study was carried out at Kirigiti Girl's Approved School in Kiambu. A total of 77 children were randomly selected from lower primary classes 2 to 4. Baseline information obtained indicated that the children came from varied backgrounds before joining the institution (Figure 7). Some (6.3%) lived with their parents, 11.1% with guardians, 17.3% were in homes for destitute children and a similar number had been rounded up from the streets and held in police stations before being presented in a juvenile court. About a half (48%) came from remand homes. A quarter of the children (24%) had parents although not all were staying with them, and slightly over a quarter (26%) were orphans. Slightly over a third (38%) were from broken or single parent homes while about (12%) others were juvenile delinquents.
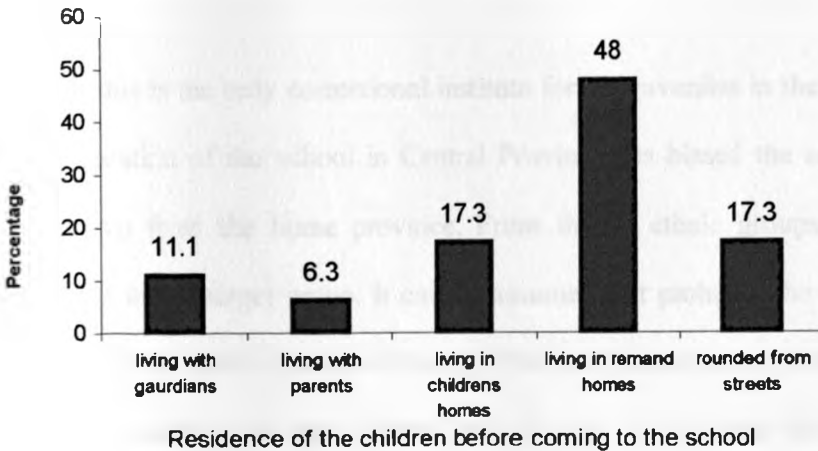
*Figure 7: Social background of the children*

### 4.1.2. Ethnicity

The children came from different ethnic backgrounds and included Kalenjin (2.6%), Kamba (9.1%), Kikuyu (58.4%), Luhya (7.8%), Luo (18.2%), Pokot (1.3%), Taita (1.3%) and Turkana (1.3%). The children had been at the school for at least three months. This data is presented in figure 8.
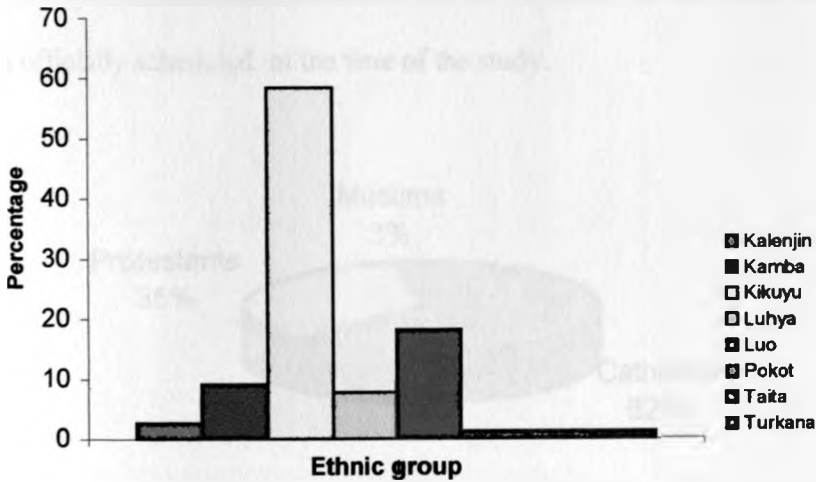


*Figure 8: Distribution of the children by ethnicity*

Although this is the only correctional institute for girl-juveniles in the country, it would appear that the location of the school in Central Province has biased the admissions with over half being drawn from the home province. From the 46 ethnic groups in the country, 8 were represented in the target group. It can be assumed that probably the process of referral is too laborious for the state to handle efficiently. This distribution pattern however, had no bearing on the study as allocation was random and the two groups had the children almost evenly distributed in ethnicity.

### 4.1.3. Religion

There were three main religious denominations representation (Figure 9): About two thirds (62.3%)of the children were Catholic, (35.1%) slightly more than a third were Protestant and (2.6%) and a few were Muslim. The distribution of the children by religion in the two groups was well balanced although religious affiliation had no effect on the study as the children did not fast nor eat different amounts or types of food during religious periods e.g. fasting during lent for the Catholics and ramadhan for the Muslims. In any case no special religious period was officially scheduled at the time of the study.
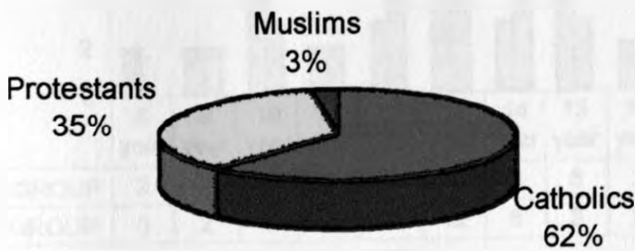


*Figure 9: Distribution of the children by religion*

### 4.1.4. Age and birth order

The mean birth order of the children was $2.34 \pm 1.48$. The majority of the children were the only children born to their parents. They had a mean age of $12.62 \pm 1.96$ with slightly more than two-thirds (68.8%) of the children falling in the 8 to 13 years category (Figure 10). The age range was 8-16 years.

A normal age distribution pattern was observed in the children. About 2.6% of the children fell in the 8-year category. The 9, 11, 14 and 16-year categories each had an equal proportion (5.2%) of children while the 10, 12 and 15 year categories which had about 11.7%.

The 14 - year category had a slightly higher number of children (11%), while the highest concentration of children, slightly over a third (33%), was observed in the 13 - year category. There was no significant difference in the age distribution between the two groups (Table 5). Age therefore had no significant bearing on the findings of the study. It can therefore be assumed that the growth rate was not significantly different within the ages and groups.

| | 8 year | 9 year | 10 year | 11 year | 12 year | 13 year | 14 year | 15 year | 16 year |
|---|---|---|---|---|---|---|---|---|---|
| BCRB GROUP | 2 | 2 | 6 | 2 | 3 | 13 | 3 | 6 | 2 |
| BCLB GROUP | 0 | 2 | 3 | 2 | 6 | 12 | 8 | 3 | 2 |
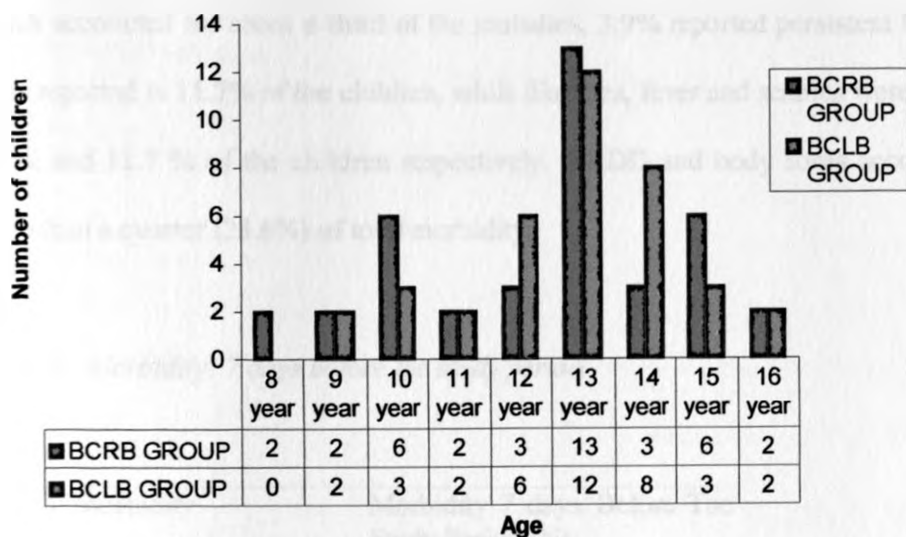
*Figure 10: Age distribution of children in the βCRB and the βCLB groups*

*Table 5: A summary of the demographic characteristics of the children*

| | Intervention Group βCRB (N=39) (N) | Non-Intervention Group βCLB (N=38) (N) |
|---|---|---|
| **RELIGION** | | |
| Catholic (N) | 26 (33.8) | 22 (28.6) |
| Protestant (N) | 13 (16.9) | 14 (18.2) |
| Muslim (N) | - | 2 (2.6) |
| **HISTORY** | | |
| Age (in years) | $12.44 \pm 2.17$ | $12.82 \pm 1.72$ |
| Duration of stay in School | + 6 months | + 6 months |
| Birth order | $2.33 \pm 1.51$ | $2.34 \pm 1.48$ |

1. (N): number of children    2. %: N=77 – figures in parentheses are percentages

## 4.2. Morbidity

There was no significant difference in the distribution of morbidity between the βCRB and the βCLB group. Morbidity, in the 7- day period prior to commencement of the study, was reported in about a half (54.6%) of the children. At recruitment (Table 6), 31.2% of the children had flu, which accounted for about a third of the maladies, 3.9% reported persistent headaches, coughs were reported in 11.7% of the children, while diarrhea, fever and scabies were reported in 7.8%, 7.8%, and 11.7 % of the children respectively. VADD and body sores accounted for slightly more than a quarter (28.6%) of total morbidity.

*Table 6: Morbidity: 7 days before the study period*

| Type of Morbidity | Morbidity 7 days Before The Study Period (N) | |
|---|---|---|
| Flu | 24 | (31.2) |
| Headache | 3 | (3.9) |
| Cough | 9 | (11.7) |
| Diarrhoea | 6 | (7.8) |
| Fever | 6 | (7.8) |
| Scabies | 9 | (11.7) |
| Wounds And Sores | 22 | (28.6) |

1. (N): number of children     2. %: N=77 – figures in parentheses are percentages

### 4.2.1. VADD

Nyctalopia was reported in about a third (33.8%) of the children (Figure 11). Slightly more than a quarter (28.6%) had wrinkled cornea and unilateral Bitot's spots was observed in close to a quarter (23.4%) of the study group.

23.4%

33.8%

■ Bitot's spot

☑ Wrinkled cornea

☑ Nyctalopia

28.6%
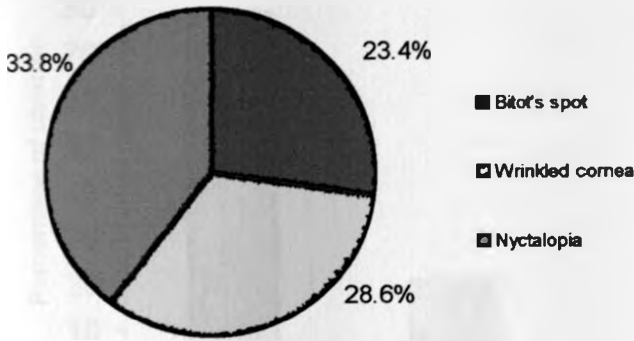
*Figure 11: VADD prevalence in the children (N = 77)*

### 4.2.2. Diarrhea

At recruitment, ten percent of the children (9.1%) had diarrhea. The frequency average, here reported as diarrhoeal episodes by frequency of movements per day, was 2.5 movements and even then not all episodes were watery. A comparative summary of baseline morbidity is presented in table 7.

## 4.3.   Clinical Examination

### 4.3.1. Dental condition

Examination on dental health was done because it affects ingestion and mastication, which can affect the vitamin A status of the child. The children did not have access to toothbrushes and toothpaste. Slightly more than three-quarters (79.2%) of the children had dental caries. Of these, a higher proportion (40.3%) was in the βCLB group (Figure 12). This difference was however not significant (p>0.05).

90
80
70
60
50
40
30
20
10
0

Percentage of children

dental caries          normal
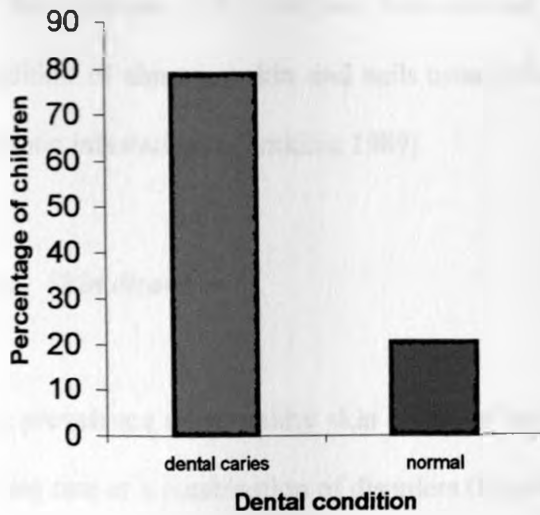
**Dental condition**

(N): number of children

*Figure 12: Dental condition of the children (N=77)*

Close to a quarter (20.8%) of the children had bleeding gums, slightly more than a quarter (27.3%) had oral abscess while throat pain was reported in slightly less than a quarter (15.6%). Painful gums were reported in three quarters (75.3%) of the study subjects. A higher proportion of these morbidity cases (: bleeding gums: 11.7%, oral abscess: 15.6%, throat pain: 10.4% and painful gums: 55.8% respectively) were in the βCRB although this did not present any significant difference than the βCLB group (p>0.05).

### 4.3.2. Nails and hair.

Brown hair was observed in close to three-quarters (71.4%) of the children while close to quarter (22.1%) had silky hair. The rest had normal hair. Abnormal hair color and texture may in most cases be a manifesting physical sign of malnutrition and a sick state of the body, which would probably mean that the body does not have enough vitamin A to boost the immunity to a level capable of warding off disease and infections (Latham, 1997).

A few children (7.8%) did not have normal nails although none were spoon shaped. The condition of abnormal skin and nails usually has a direct link to micronutrient deficiency and parasitic infestations (Tomkins, 1989).

### 4.3.3. Skin disorders

The prevalence of unhealthy skin observed was extremely high with 90% (70) of the children having one or a combination of disorders (Figure 13).
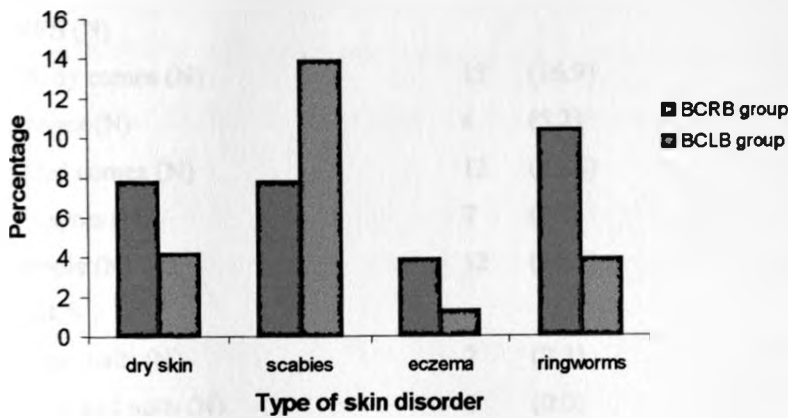


*Figure 13: Distribution of some skin disorders between the BCRB group and the BCLB group*

Slightly over a quarter of the children (28.6%) had wounds and sores while few children had dry skin (13%), scabies (11.7%), eczema (5.2%) and ringworms (14.3%). The βCRB group had these maladies in higher proportions of 7.8%, 7.8%, 3.9% and 10.4% respectively. There was however no overall significant difference between the two groups (p>0.05).

*Table 7: A summary of the clinical assessment done at recruitment*

| Clinical condition | Intervention Group βCRB (N=39) | | Non-Intervention Group βCLB (N=38) | |
|---|---|---|---|---|
| **1) EAR, NOSE, THROAT AND ORAL** | | | | |
| Discoloured teeth (N) | 24 | (31.2) | 25 | (32.5) |
| Dental caries (N) | 30 | (39) | 31 | (40.3) |
| Missing teeth (N) | 5 | (6.5) | 3 | (3.9) |
| Mottled teeth (N) | 1 | (1.3) | 3 | (3.9) |
| Oral pain (N) | 12 | (15.6) | 9 | (11.7) |
| Bleeding gums (N) | 9 | (11.7) | 7 | (9.1) |
| Oral abscess (N) | 4 | (5.2) | 4 | (5.2) |
| Ear pain (N) | 1 | (1.3) | 2 | (2.3) |
| Nose pain (N) | 3 | (3.9) | 3 | (3.9) |
| Throat pain (N) | 8 | (10.4) | 4 | (5.2) |
| **2) EYES (N)** | | | | |
| Non-shiny cornea (N) | 13 | (16.9) | 17 | (22.1) |
| Dry cornea (N) | 4 | (5.2) | 4 | (5.2) |
| Wrinkled cornea (N) | 12 | (15.6) | 10 | (13) |
| Bitot's spots (N) | 7 | (9.1) | 11 | (14.3) |
| Nyctalopia (N) | 12 | (15.6) | 14 | (18.2) |
| **3) NAILS** | | | | |
| Abnormal nails (N) | 2 | (2.3) | 4 | (5.2) |
| Spoon shaped nails (N) | 0 | (0.0) | 0 | (0.0) |
| **4) SKIN** | | | | |
| Unhealthy skin (N) | 36 | (46.8) | 34 | (44.2) |
| Dry skin (N) | 6 | (7.8) | 4 | (5.2) |
| Scabies (N) | 6 | (7.8) | 3 | (3.9) |
| Eczema (N) | 3 | (3.9) | 1 | (1.3) |
| Boils (N) | 0 | (0.0) | 0 | (0.0) |
| **5) HAIR** | | | | |
| Brown hair (N) | 31 | (40.3) | 24 | (31.2) |
| Silky hair (N) | 10 | (13) | 7 | (9.1) |
| **6) RESPIRATORY** | | | | |
| Flu with cough & headache (N) | 30 | (39) | 29 | (37.7) |
| Flu with fever (N) | 2 | (2.3) | 4 | (5.2) |

1. (N): number of children    2. %: N=77 – figures in parentheses are percentages

### 4.3.4. Muscle bulk

Wasted muscle is usually an indicator of severe protein-energy malnutrition. Muscle bulk appeared to be normal in all the children.

### 4.4. Dietary History and Dietary Intake

### 4.4.1. Dietary history

Before coming to the institution, the children had varied dietary exposure. Fruit consumption was reported in about three-quarters (74%) and vegetable consumption was slightly over a half (59.7%) of the children. This information, however, had no bearing to the findings of the study as the children were in the institution long enough for any residual effect to manifest in their nutritional status. Their nutritional status therefore, reflected the effect of their current dietary habits.

### 4.4.2. Dietary intake

The diet in the institution constituted mainly of maize meal, rice and beans. The diet was the same from Monday to Saturday varying slightly on Sunday. All meals were served in individual 500ml aluminum bowls and no cutlery was provided. The children took their breakfast at 07:00 hours, lunch at 13:00 hours and supper at 18:00 hours everyday. Maize meal porridge without was served every morning for breakfast. From Monday to Saturday, the children consumed boiled *githeri* for lunch while for Sunday lunch and supper:

Monday to Saturday, boiled rice and bean-stew were taken. *Ugali* and bean-stew were consumed for supper on Sunday. The average proximate composition and vitamin A content of the meals taken weekly for analysis is presented in table 8.

*Table 8:* *Proximate composition and vitamin A content in the school rations*

| COMPONENT WEIGHT OF FOOD | TYPE OF MEAL | | | |
|---|---|---|---|---|
| | UGALI AND BEANS | MAIZEMEAL PORRIDGE | *GITHERI* | RICE AND BEANS |
| WEIGHT (GRAMS) | 737.7 | 444.3 | 650.8 | 496.3 |
| WATER% | 75.33 | 86.29 | 69.13 | 72.83 |
| PROTEIN (%) | 2.72 | 1.10 | 4.35 | 3.25 |
| FAT (%) | 0.92 | 0.34 | 1.36 | 0.54 |
| FIBRE (%) | 1.41 | 0.77 | 3.16 | 1.68 |
| ASH (%) | 1.07 | 0.23 | 1.26 | 1.20 |
| CARBO. (%) | 18.55 | 11.27 | 20.74 | 20.49 |
| VITAMIN A (mg./100g) | 00.00 | 00.00 | 00.00 | 00.00 |

The above table presents the proximate analysis of meals eaten at the institute.

### 4.4.2.1. Daily average nutrient intake

Due to the limited number of dishes prepared for the children, the daily average intake was dictated by the routine portion-and-meals pattern.. There was slightly more protein, fat, fibre, and ash consumed during the week than on Sundays but this difference was not significant (Table 9). Carbohydrate consumption was however higher on Sunday and the food contained a higher moisture level on this day too.

*Table 9:  Daily average nutrient intake*

|  | MONDAY-SATURDAY | RDA | SUNDAY | TOTAL |
|---|---|---|---|---|
| WEIGHT (GRAMS) | 1,591.3 | Na | 1,673.3 | 11,221.1 |
| WATER% | 76.1 | Na | 78.2 | 76.3 |
| PROTEIN (grams) | 49.2 | 56 | 41.0 | 336.2 |
| FAT (grams) | 13.0 | 56 | 10.9 | 88.9 |
| FIBRE (grams) | 32.3 | Na | 22.2 | 216.0 |
| ASH (grams) | 15.2 | Na | 14.9 | 106.1 |
| CARBO (grams) | 298.2 | Na | 345.2 | 2,134.4 |
| VITAMIN A (µg retinol) | 00.0 | 500 | 00.0 | 00.0 |
| TOTAL CALORIES | 1,506.6 | 1,988 | 1,642.9 | 10,678.9 |

1. Na: not available

The daily individual, protein and fat and vitamin A intake are presented in figures 14 (a),

14 (b) and 14 (c), respectfully.
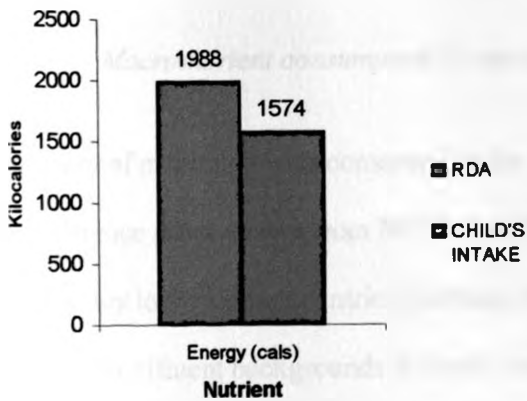


*Figure 14 (a): Daily individual energy intake*
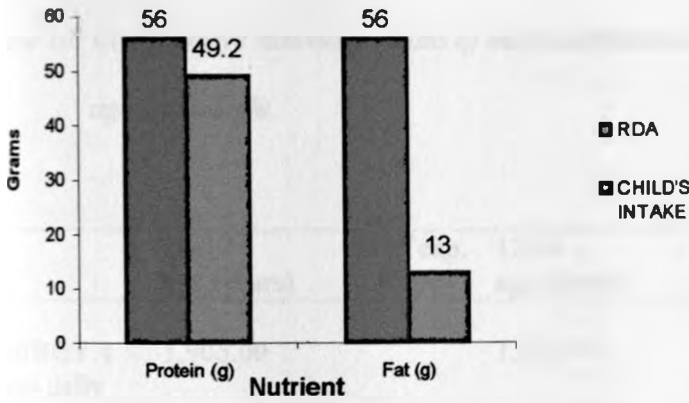
*Figure 14 (b): Daily individual protein and fat intake*
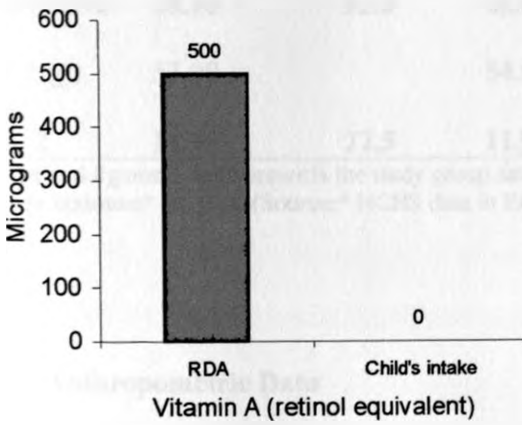


RDA: Recommended Daily Allowance

*Figure 14 (c): Daily individual vitamin A intake*

### 4.4.2.2. *Macronutrient consumption by age-group*

A summary of macronutrients consumed by the children was compiled by age and compared to the reference group drawn from NCHS data (Table 10). The reference children are similar to those in many low-income countries (Latham, 1997) whose nutritional status is different from children from affluent backgrounds in North America. It is clear from the table that the children's protein, energy and fat needs were not being met.

*Table 10: Comparative summary means of macronutrients consumed by the children by age and weight.*

|  | 10-12 age (years) | % of exp. RDA | 12-14 age (years) | % of exp. RDA | 14-16 age (years) | % of exp. RDA |
|---|---|---|---|---|---|---|
| ENERGY 1 (cals) daily | 1,905.00 |  | 1,955.00 |  | 2,030.00 |  |
| **ENERGY 2** | **1,574.75** | **80.7** | **1,574.75** | **80.5** | **1,574.75** | **77.6** |
| PROTEIN 1 (g) daily | 49.00 |  | 59.00 |  | 64.00 |  |
| **PROTEIN2** | **45.10** | **92.0** | **45.10** | **76.4** | **45.10** | **70.1** |
| FAT 1 (g) daily | 53.00 |  | 54.50 |  | 56.50 |  |
| **FAT 2** | **11.95** | **22.5** | **11.95** | **21.9** | **11.95** | **21.1** |

The text and figures in bold presents the study group data while the standard. Text and figures present the expected (exp.) or optimum* statistic. (Source:* NCHS data in FAO: Latham, 1997).These are nutrient provision from daily rations.

## 4.5. Anthropometric Data

### 4.5.1. Body Mass Index (BMI) of the children

In the base line study, children with a BMI <16 were about half (50.6%), and can thus be considered undernourished (Latham, 1997). Those who were moderately nourished (with a BMI of 16 to 18.5) accounted for slightly more than a third (35.1%) while a few; only 14.3% were considered normal (BMI of 18.5-25). The distribution of the children was almost similar and there was no significant difference between the two groups (p>0.05). The data is presented in table 11.

*Table 11: BMI data of the children between groups, at baseline*

| BMI | βCRB GROUP (N=39) | βCLB GROUP (N=38) | TOTAL (N=77) | $\chi^2$ | p-value |
|---|---|---|---|---|---|
| < 16 (Severely malnourished) | 20 (26%) | 19 (25%) | 39 (50.6%) | 0.00 | $1.00^{ns}$ |
| 16 – 18.5 (Moderately malnourished) | 15 (19.5%) | 12 (16%) | 27 (35.1%) | 0.18 | $0.67^{ns}$ |
| 18.5 – 25.0 (Normal) | 4 (5.2%) | 7 (9.1%) | 11 (14.3%) | 0.39 | $0.53^{ns}$ |

1. $^{ns}$: not significant    2. (N): number of children    3. $* \chi^2$ test: significant at 0.05 level

## 4.6. Parasitic Infestation

Stool examination was carried out on specimens collected before and after the intervention. The baseline parasitic infestation in the children was almost similar (Table 12) and no significant difference was found in worm load between the experimental group and the control group.

*Table12: Parasitic infestation of the children at baseline*

| | Intervention Group βCRB (N=39) | | Non-Intervention Group βCLB (N=38) | | Type of test | |
|---|---|---|---|---|---|---|
| | | | | | $\chi^2$ | p-value |
| *Hookworm* (N) | 3 | (3.9) | 2 | (2.3) | 0.00* | $1.0^{ns}$ |
| *S. mansoni* (N) | 2 | (2.3) | 0 | (0.0) | 0.51 | $0.48^{ns}$ |
| *A. lumbricoides* (N) | 6 | (7.8) | 8 | (10.4) | 0.08 | $0.78^{ns}$ |
| *T. trichiura* (N) | 8 | (10.4) | 8 | (10.4) | 0.07 | $0.79^{ns}$ |

1. *: significant at 0.01 level    2. ns: not significant    3. (N): number of children    4. %: N=77 – figures in parentheses are percentages

## 4.7. Baseline serum β-carotene and retinol analysis between the βCRB group and the βCLB group

The difference of baseline serum β-carotene and retinol means between the βCRB group

$(0.3270 \pm 0.024$ and $(0.257 \pm 0.065)$ and the βCLB group $(0.0527 \pm 0.052$ and $0.251 \pm 0.055)$

respectively, was not significantly different (p>0.5) as presented in table 13.

*Table 13: Baseline serum β-carotene and retinol analysis between the* βCRB *group and the* βCLB *group*

|  | Intervention Group βCRB (N=39) | | Non-Intervention Group βCFB (N=38) | |
| --- | --- | --- | --- | --- |
| 1) SERUM RETINOL (μg/100dl) | | | | |
| Deficient < 20 | 5 | (6.5) | 5 | (6.5) |
| Borderline deficient: 20-25 | 17 | (22.1) | 16 | (20.8) |
| Normal > 25 | 17 | (22.1) | 17 | (22.1) |
| MEANS (μg/100dl | | | | |
| 2) SERUM RETINOL: | 0.257 (0.065) | | 0.251 (0.055) | |
| 3) BETA CAROTENE | 0.03270 (0.024) | | 0.0527 (0.052) | |

1. (N): number of children     2. Figures in parentheses are percentages for serum retinol levels and standard deviations for the means

## 4.8.    Outcome Data

### 4.8.1.    *Nutrient content of the βCRB and the βCLB*

The βCRB baked for one hour while the βCLB baked for 30 minutes. The βCRB contained six times more β-carotene content ($1063\mu g/100g$) than the βCLB ($170\mu g/100g$) (Table 14). This difference was highly significant ($p=0.01$). The results of the βCRB and βCLB are presented below in figure 15.
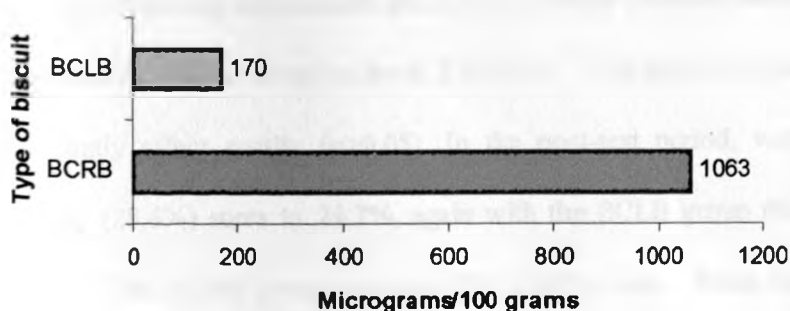


*Figure 15: β-carotene content in the β-carotene rich biscuit (βCRB) and the β-carotene low biscuit*

*Table 14: Nutrient content of the βCRB and the βCLB*

| NUTRIENT/COMPONENT | β-carotene rich biscuit (βCRB) | β-carotene free biscuit (βCLB) |
|---|---|---|
| KILOCALORIES | 478 | 468 |
| MOISTURE (%) | 4.15 | 5.33 |
| PROTEIN (%) | 4.99 | 6.22 |
| FAT (%) | 26.80 | 23.97 |
| CARBOHYDRATE (%) | 57.92 | 60.74 |
| CALCIUM (mg/100g) | 40.53 | Not determined |
| IRON (mg/100g) | 16.41 | Not determined |
| TOTAL ASH (%) | 1.46 | 1.14 |
| FIBRE (%) | 3.5 | 2.25 |

1. mg: milligrams

### 4.8.2. Post-test morbidity data

The morbidity increased during the study period. A typhoid outbreak at the institution affected 5.2% of the study subjects. Of these, 3.9% were in the βCRB group. The number of flu cases rose to nearly more than three quarter (76.6%) on the third week of supplementation, but dropped to 49% at post-test. Of those affected during the intervention period, 39% were in the βCRB group. There was a significant decrease ($p < 0.05$) in the number of children who had flu with cough and headache or flu with fever at post-test. The number of children with diarrhea was 9.1% (7) during intervention and 6.5% (5) in the post-test period, with a higher proportion of the cases (5.3%) occurring in the βCLB group. This figure was not large enough however, to significantly affect results ($p > 0.05$). In the post-test period, wounds reduced from 75.3% including (28.6%) sores to 24.7%, again with the βCLB group recording slightly more cases (39.0%). The βCRB group recorded 28 (36.4%) cases. There was however, no significant difference in morbidity between the two groups ($p > 0.05$).

*4.8.2.1.     Ear, nose, throat and oral conditions*

The number of children with discolored teeth was 63.6%, missing teeth 10.4% and mottled teeth 5.2%. This statistic remained the same throughout the study period. The number of children with dental caries, oral pain, bleeding gums and throat pain increased during the study period in the following percentages: 3.9% (from 61 to 64), 14.3% (from 21 to 32), 3.9% (from 16 to 19), and 5.2% (from 12 to 16) respectively. These changes however, were not significant (p>0.05) in all cases. There was a decrease at post-test in the number of children with oral abscess, ear pain and nose pain, but these changes were also not statistically significant.

## 4.8.3.     VADD PREVALENCE RATES

There was a general decrease on all ocular conditions observed. The number of children with dry cornea reduced from 10.4% to 2.3% (p>0.05), those with wrinkled cornea from 28.6% to 7.8% (p<0.05), those with Bitot's spot from 23.4% to 11.7% (p=0.09) while there was a highly significant decrease (p=0.000):from 33.8% to 5.2%, in those with nyctalopia.. In the experimental group, there was a significant reduction in nyctalopia (p<0.05) with 1.3% cases having the condition down from 15.6%, after supplementation. This change was however also noted in the control group (from 18.2% to 2.3%), which also recorded a significant reduction in the number of children with wrinkled cornea (p<0.05). This data is presented in table 15.

*Table 15:    Prevalence of ocular conditions in the pre and post intervention periods*

| OCULAR CONDITION | EXPERIMENTAL GROUP | PRE -TEST % | POST-TEST % | Test of significance | |
|---|---|---|---|---|---|
| | | | | $\chi^2$ | p-value |
| Dry cornea | *βCRB (39)* | *5.2* | *1.3* | 0.83 | 0.360 |
| | βCLB (38) | 5.2 | 1.3 | 0.83 | 0.360 |
| | **Total (77)** | **10.4 (8)** | 2.3 (2) | | |
| Nyctalopia | βCRB (39) | *15.6* | 2.3 | 8.40 | 0.004* |
| | βCLB (38) | 18.2 | 2.3 | 8.44 | 0.004* |
| | **Total (77)** | **33.8 (26)** | **5.2  (4)** | **20.00** | **0.000*** |
| Wrinkled cornea | *βCRB (39)* | *15.6* | 6.5 | 2.38 | 0.123 |
| | βCLB (38) | 13.0 | 1.3 | 6.27 | 0.012* |
| | **Total (77)** | **28.6  (22)** | **7.8  (6)** | **9.82** | **0.002*** |
| Bitot's spot | *βCRB (39)* | *9.1* | 6.5 | 0.09 | 0.760 |
| | βCLB (38) | 14.3 | 5.2 | 2.66 | 0.103 |
| | **Total (77)** | **23.4 (18)** | **11.7  (9)** | **2.80** | **0.09** |

1. In parentheses: the number of children in each group    2. * : test significant at 0.05 level

## 4.8.3.1.    Skin and hair conditions

The number of children with unhealthy skin (75.3%) increased during the intervention period peaking in the third week (80.5%) and then a sudden drop followed (39%) in the fourth week to levels of statistical significance (p=0.00) when compared with the baseline data.

The number of children with brown hair significantly (p<0.05) decreased from 55 (71.4%) to 37 (48.1%). The changes in morbidity were similar in both groups with no significant changes between the two groups observed. This data is presented in figure 16.
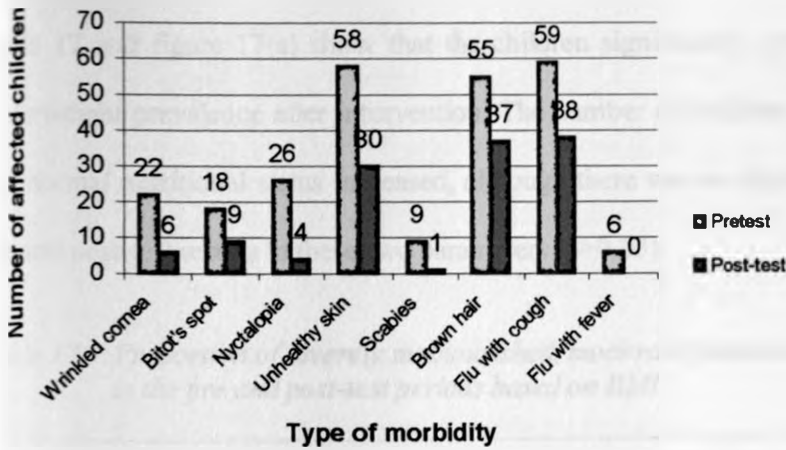
*Figure 16: Significant changes in morbidity post-test in the children*

## 4.9. Anthropometric Measurements

### 4.9.1. Weight Gain

There was a total average weight gain of 1.9 kilograms (Table 16) noted in the children with the *βCLB group* recording a slightly higher increase of (1.96kgs), 0.13kgs more than the *βCRB group* . There was, however, no significant difference (p>0.05) in weight gain between the two groups.

*Table 16: Weight gain after intervention between the βCRB and the βCLB groups*

| Weight gain/BMI | Experimental group | | Test of significance | |
|---|---|---|---|---|
| Difference | *βCRB group* | *βCLB group* | t-test | p-value |
| Weight gain (kgs) | 1.83 (0.91) | 1.96 (0.92) | -0.853 | $0.35^{ns}$ |
| BMI difference | 0.75 (0.45) | 0.83 (0.45) | -0.784 | $0.44^{ns}$ |

Figures in parentheses are standard deviations. $^{ns}$: not significant at 0.05 level

### 4.9.2 Post-Test Body Mass Index ( BMI)

Table 17 and figure 17(a) show that the children significantly (p<0.01) reduced their severe underweight prevalence after intervention. The number of children with moderate underweight and normal nutritional status increased, although there was no significant increase between the pre and post-test results in these two parameters (p>0.05).

*Table 17:* *Proportion of severely malnourished, moderately malnourished and normal children in the pre and post-test periods based on BMI.*

| BMI | PRE – TEST | POST – TEST | TOTAL | Test of $\chi^2$ | significance p-value |
|---|---|---|---|---|---|
| < 16 (Severely malnourished) | 39 (50.6) | 22 (28.6) | 61 | 6.95 | 0.008** |
| 16 – 18.5 (Moderately malnourished) | 27 (35.1) | 39 (50.6) | 66 | 3.21 | 0.051ns |
| 18.5 – 25.0 (Normal) | 11 (14.3) | 16 (20.8) | 27 | 0.72 | 0.397ns |

** significant at 0.01    ns: not significant
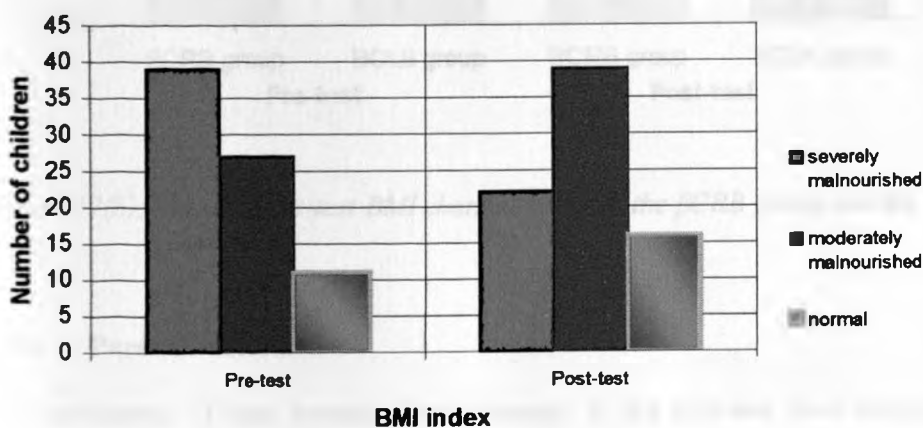


*Figure 17(a):* *Proportion of severely malnourished, moderately malnourished and normal children pre and post-test based on BMI*

*4.9.2.1.* *Changes in BMI in the children*

Presented in figure 17(b) is a comparative summary of the BMI changes within the two groups at baseline and in the post-intervention period. In the βCRB group, the number of children who were severely malnourished decreased from 26% (20) to 15.6% (12) while changes from 24% (19) to 13% (10) were observed in the βCLB group. Changes from 19.5% (15) to 28.6%(22) were observed in moderately malnourished children from the βCRB group while the changes from 15.6% (12) to 22.1% (17) were noted in the βCLB group. The differences in the normal category were 5.2% (4) to 6.5% (5) and 9.1% (7) to 14.3% (11) respectively.
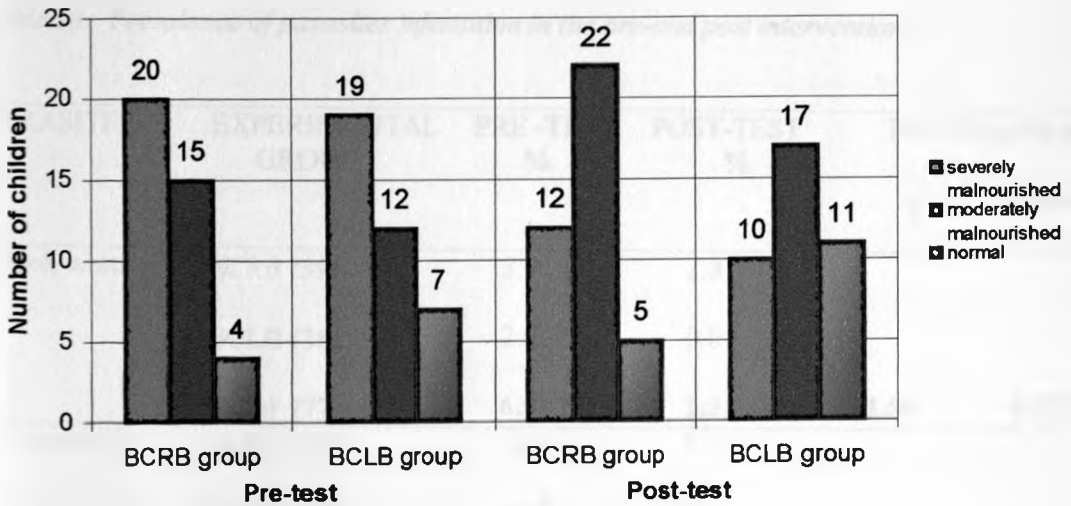


*Figure 17(b): Pre and post-test BMI changes between the βCRB group and the βCLB group: a Summary*

## 4.10. Parasitic Infestation

The prevalence of total parasite loads dropped in the post-test from slightly less than half (48.1%), to slightly more than a quarter (26%). This was probably because the children had been dewormed before intervention. Schistosoma mansoni doubled its prevalence from 1.3% (1), to

5.2% (4) but this proportion was too low to consider significant to the study (Table 18). At baseline the βCRB group had 2 cases of S. mansoni that still tested positive at posttest. There were no cases of S.mansoni in the βCLB group at recruitment, but at post-test, 2 cases emerged.

The prevalence of hookworm dropped from 6.5% (5 cases) in the pre-test to 1.3% (1 case) in the posttest but the number again, was too low to consider significant to the study.

There were no cases of A. lumbricoides recorded at posttest though they had been a prevalence of 18.2% (14) at baseline (Table 20). The number of *Trichuris trichiura* remained constant in both groups (10.4% average) at baseline and post-test (Figure 18).

*Table 18: Prevalence of parasites infestation in the pre-and post intervention*

| PARASITES | EXPERIMENTAL GROUP | PRE -TEST % | POST-TEST % | Test of significance $\chi^2$ | p-value |
|---|---|---|---|---|---|
| Hook worm | *βCRB (39)* | *3.9* | *1.3* | | |
| | βCLB (38) | 2.6 | 0.0 | | |
| | **Total (77)** | **6.5 (5)** | **1.3 (1)** | **1.56** | **0.212** |
| S. mansoni | βCRB (39) | *2.6* | *2.6* | | |
| | βCLB (38) | 0.0 | 2.6 | | |
| | **Total (77)** | **2.6 (2)** | **5.2 (4)** | **0.17** | **0.067** |
| A. Lumbricoides | *βCRB (39)* | *7.8* | *0.0* | | |
| | βCLB (38) | 10.4 | 0.0 | | |
| | **Total (77)** | **18.2 (14)** | **0.0 (0)** | | |
| T. trichiura | *βCRB (39)* | *10.4* | *9.1* | | |
| | βCLB (38) | 10.4 | 10.4 | | |
| | **Total (77)** | **20.8 (16)** | **19.5 (15)** | **0.04** | **0.841** |

1. In parentheses: the number of children in each group       2. – statistic not calculable
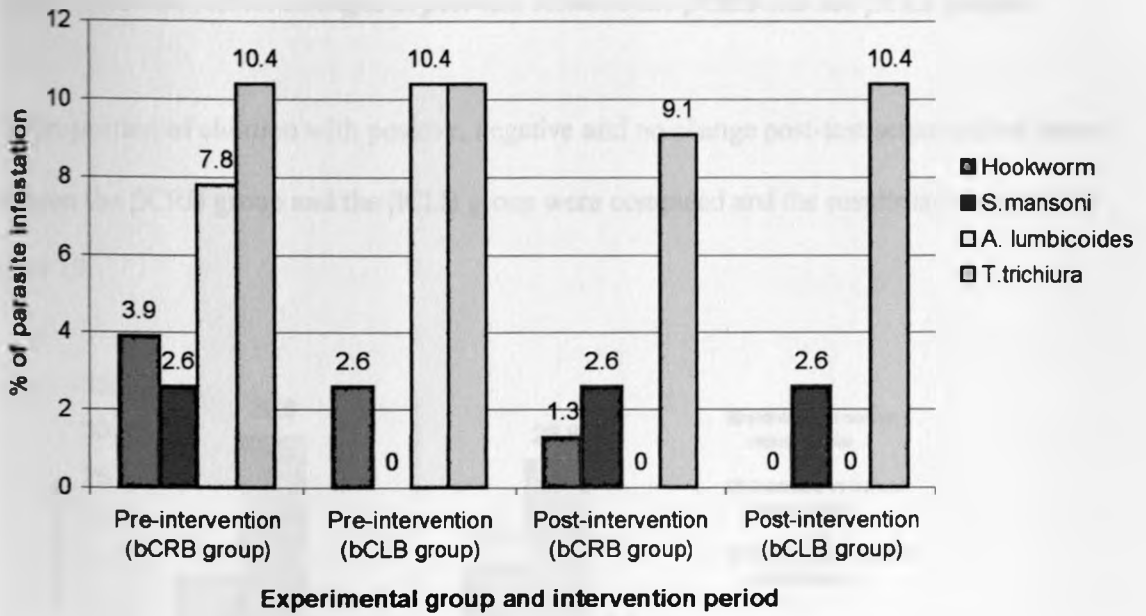
*Figure 18: Prevalence of parasitic infestations in the children pre and post-test*

## 4.11. Serum β-carotene and Retinol Levels in the Pre and Post-test Periods

### 4.11.1. Post-test retinol levels

There was no significant difference (p>0.05) in the prevalence of serum retinol deficiency after intervention (as presented in table 19) between the βCRB and the βCLB group. There were 6 destroyed samples for serum retinol and β-carotene, so the results presented in the table are for the remaining 71 valid samples.

*Table 19: Post-test distribution of children by serum retinol status*

| Retinol | Post-test | | Test of significance | |
|---|---|---|---|---|
| μg/100dl | βCRB N=37 | βCLB N=34 | $\chi^2$ | p-value |
| Deficient | | | | |
| < 20 | 11 (55) | 9 (45) | 0.06 | 0.81[ns] |
| Borderline deficient | | | | |
| 20-25 | 16 (50) | 16 (50) | 0.04 | 0.84[ns] |
| Normal | | | | |
| >25 | 10 (52.6) | 9 (47.4) | 0.00 | 1.00[ns] |

1.[ns]: not significant    2. $\chi^2$ test significant at 0.01 level    3. %: N=71 – figures in parentheses are percentages

### 4.11.2.    Serum retinol changes at post-test between the βCRB and the βCLB groups

The proportion of children with positive, negative and no-change post-test serum retinol status between the βCRB group and the βCLB group were computed and the results are presented in figure 19.
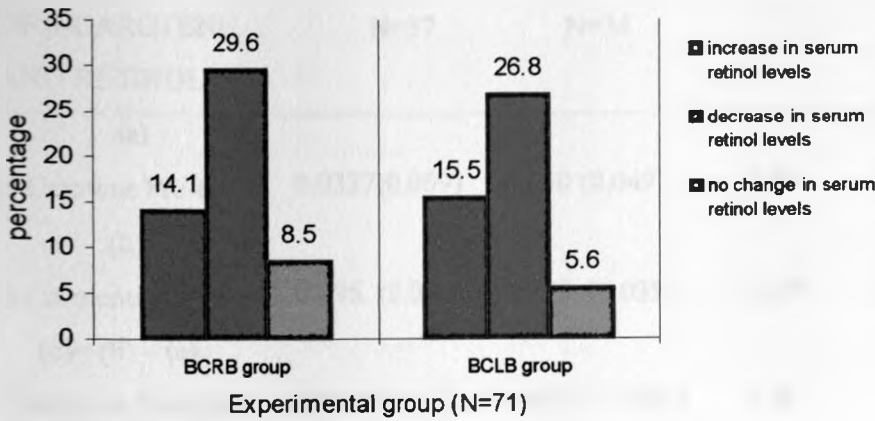


*Figure 19:  A comparison of serum retinol changes at post-test between the groups*

### 4.11.3.    Pre and posttest means of serum β-carotene and retinol

Table 20 shows that, before intervention, there was no significant difference ($p > 0.05$) in serum β-carotene and retinol levels between the two groups. However, after intervention, serum β-carotene significantly increased ($p < 0.05$) from $0.0327 \pm 0.069$ to $0.096 \pm 0.036$ (a difference of $0.0635 \pm 0.033$) in the βCRB group than it did in the βCLB group, which recorded an increase from $0.050 \pm 0.049$ to $0.076 \pm 0.035$ (a difference of $0.0262 \pm 0.051$). Despite this increase in β-carotene, serum retinol levels did not significantly change.

The table also shows that the changes in β-carotene levels after intervention were significantly ($p < 0.01$) higher (2.4 times) in the βCRB group than in the βCLB group (Figure 20{a}), but no significant difference was found in the serum retinol levels in both groups (Figure 20 {b}). Similar results were observed when the data was controlled for parasitic infestation.

*Table 20:* *Pre and posttest means of serum β-carotene and retinol between the βCRB and*

*the βCLB groups*

| μg/100dl | EXPERIMENTAL GROUP | | TEST OF SIGNIFICANCE | |
|---|---|---|---|---|
| SERUM LEVELS OF β-CAROTENE AND RETINOL | βCRB N=37 | βCLB N=34 | t-test | p-value |
| (a) β-Carotene Pre-test | 0.0327(0.069) | 0.050 (0.049) | -1.86 | 0.060 |
| (b) β-Carotene Post-test | 0.096 (0.036) | 0.076 (0.035) | 2.39 | 0.020 |
| (c)= (b) – (a) Change in β-carotene | 0.0635 (0.033) | 0.0262 (0.051) | 3.68 | 0.000* |
| (a) Retinol Pre-test | 0.257 (0.069) | 0.251 (0.055) | 0.43 | 0.672 |
| (b) Retinol Post-test | 0.235 (0.065) | 0.230 (0.055) | 0.38 | 0.709 |
| (c)= (b) – (a) Change in retinol | -0.022 (0.059) | -0.021 (0.053) | -0.529 | 0.958[ns] |

1. Figures in parentheses are standard deviations   2. *: Significance of $\chi^2$ test at 0.05   3. [ns]: not significant

The post-test serum retinol results showed an inverse relation (Figure 20 {c}) with the serum β-carotene levels. The βCRB group which recorded higher serum β-carotene levels posttest had slightly lower serum retinol than the βCLB group which had significantly lower posttest serum β-carotene levels. Both groups had lower post- test retinol levels when compared to the pretest period. There was no significant difference between the two groups (p>0.05)
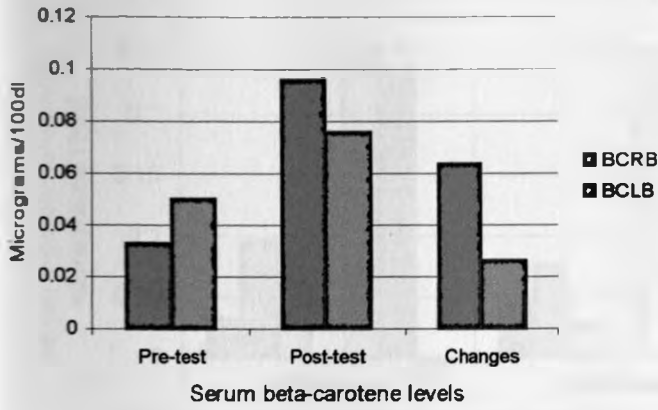
*Figure 20(a):* **Pre and post-test means and changes of serum β-carotene between the βCRB**
**group and the βCLB group**
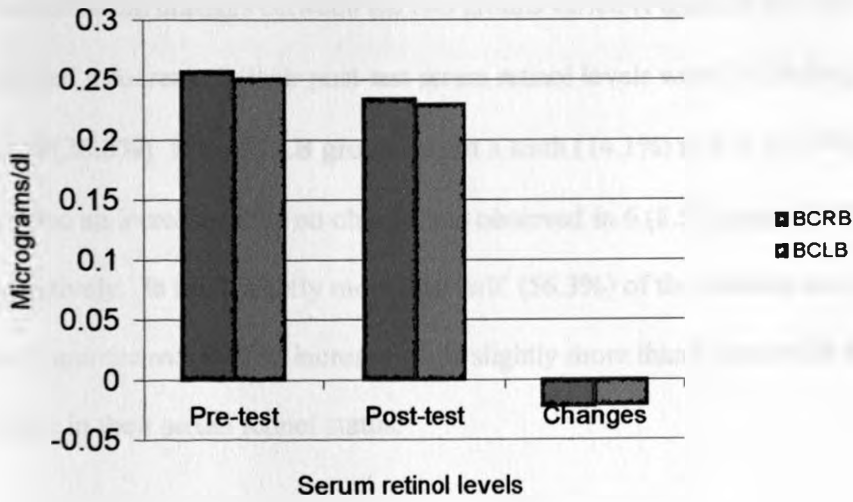


*Figure 20(b):* **Pre and post-test means and changes of serum retinol in the βCRB group and the**

*βCLB group*
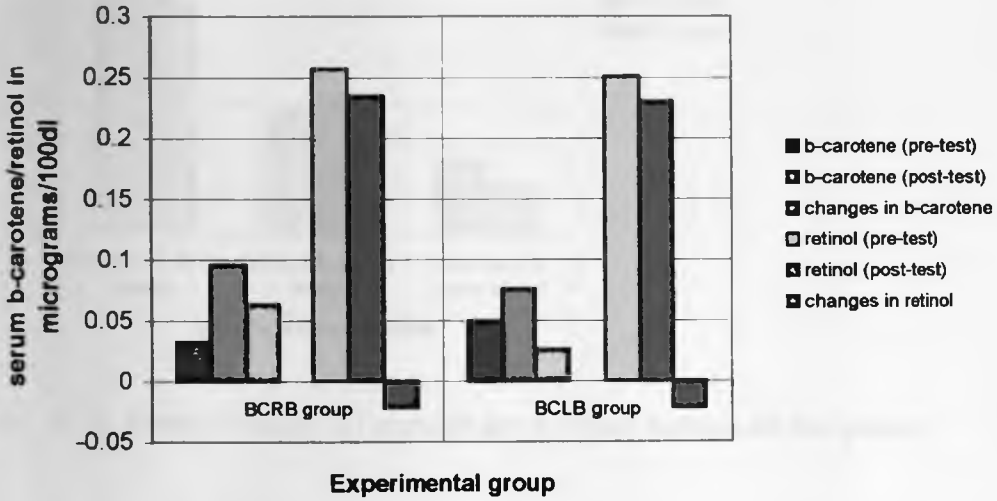
*Figure 20(c):* *A comparative summary of pre and post-test means and differences of serum*

*β- carotene and retinol between the experimental groups*

The individual changes between the two groups varied (Figure 20 (d)). The children who

recorded a decrease in their post-test serum retinol levels were 21 (29.6%) in the βCRB group

and 19(26.8%) in the βCLB group. About a tenth (14.1%) and 11 (15.5%) of the children

reported an increase while no change was observed in 6 (8.5%) and 4 (5.6%) children

respectively. In total slightly more than half (56.3%) of the children recorded a decrease, more

than a quarter recorded an increase while slightly more than a quarter(29.6%), recorded no

change in their serum retinol status.
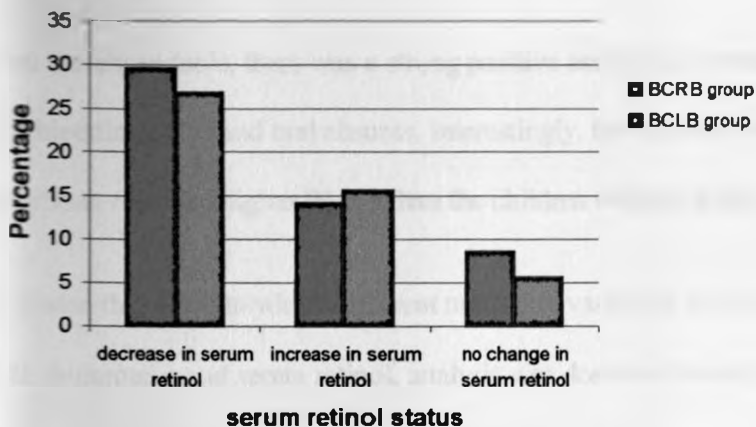
*Figure 20 (d) Detailed changes of post-test serum retinol between the two groups*

## 4.11.4 The association of morbidity variables with pre and post-test BMI, β-carotene and serum retinol

In order to assess the extent to which different morbidity variables were related to pre and post-

test BMI, analysis, using the Anova test, was done on data of all the 77 children at baseline
(Table 21).

*Table 21: The association of morbidity variables with pre and post-test BMI in the pre-test period (p-value)*

| Type of morbidity | 1 | BMI | 2 | BMI |
|---|---|---|---|---|
| Dental caries | 0.998 | | 0.906 | |
| Mottled teeth | 0.845 | | 0.926 | |
| Oral pain | 0.254 | | 0.527 | |
| Bleeding gums | 0.000** | | 0.001** | |
| Oral abscess | 0.01** | | 0.024* | |

Type of test: Anova
1. 1: pre-test      2. 2: post-test          3. * significant at 0.05    4.:** significant at 0.01

From the above table, there was a strong positive association between the BMI's of the children with bleeding gums and oral abscess. Interestingly, the children with these conditions recorded higher BMI's than the children without these conditions (p<0.05).

To assess the extent to which different morbidity variables were related to pre and post-test BMI, β-carotene and serum retinol, analysis was done on data at post-test, of 71 children with valid β-carotene and serum retinol data (Table 22).

Table 22:    The association of morbidity variables with pre and post-test BMI, serum β-carotene and retinol levels in the post-test period (p value).

| Type of morbidity | 1    BMI | 2   BMI | 1 Serum β-carotene | 2   Serum β-carotene | 1   Serum retinol | 2   Serum retinol |
|---|---|---|---|---|---|---|
| Dental caries | 0.868 | 0.914 | 0.601 | 0.438 | 0.915 | 0.803 |
| Oral pain | 0.167 | 0.183 | 0.580 | 0.439 | 0.04* | 0.071 |
| Bleeding gums | 0.001** | 0.010** | 0.082 | 0.155 | 0.088 | 0.420 |
| Oral abscess | 0.007** | 0.019* | 0.311 | 0.288 | 0.084 | 0.116 |
| Nyctalopia | 0.222 | 0.186 | 0.004** | 0.633 | 0.277 | 0.790 |
| Bitot's spot | 0.69 | 0.07 | 0.976 | 0.414 | 0.869 | 0.928 |

Type of test: Anova
1. 1: pre-test      2.  2: post-test      . 3. * significant at 0.05    4.:** significant at 0.01

There was also a strong positive association between β-carotene levels at baseline and nyctalopia (p<0.01). There was no significant association between morbidity and Bitot's spot (p>0.05)

# CHAPTER FIVE

## DISCUSSION

### 5.1. Background

The study looked at the potential of comfrey (*Symphytum periginum*) as a source of vitamin A for malnourished children aged 8-16 years in an approved school in Kiambu. All the study subjects were in lower primary.

Although this is the only correctional institute for girl juveniles in the country, it would appear that the location of the school in Central Province has biased the admissions with over half drawn from the home Province.

From the social and dietary history collected from the study subjects at recruitment, it was clear that there was no significant difference between the control and the experimental group. The baseline levels for serum β-carotene and serum retinol, the BMI, parasitic infestation, morbidity and VADD between the βCRB group and the βCLB were also not significantly different.

The protein and energy intake, though inadequate and the BMI's of 85.6% of the study subjects, which were unbelievably low, appear to have increased during the time of the study. From the data, the study subjects did not consume any vitamin A and when they did it was so negligible that it could not be detected on analysis of food samples. Such inadequate nutritional intake and the resultant low BMI would be expected to have a grave impact on the health status of the children especially when it is chronic (Latham, 1997). The children's immunity would be compromised resulting in high morbidity and low recovery rates, high re-infection risk and

eventually in latter years should the problem persist, mortality and difficulties during pregnancy and child birth (UNICEF 2000).

This problem is magnified when it is taken into consideration that these children will be mothers of underweight malnourished babies who the nation will depend on for it's continued survival economically, politically and socially. Such a society will not only regress, but will eventually not be able to sustain itself (Section 2.1.3.3). It is, therefore, crucial that nutritional adequacy be addressed to forestall these problems and even more so first address the four major critical nutritional deficiencies including and especially VAD.

Vitamin A deficiency is dubbed the silent killer (UNICEF, 2000). This is because VAD can persist without any clear symptoms presenting (or lack of knowledge to identify the VAD problems arising in the community) until the problem is well advanced especially in developing countries. Vitamin A is one of the most important micronutrients to humans as its deficiency as discussed not only leads to VADD but it threatens the very existence of communities (Mwaniki, 2002).

At the time of study, available data indicated that the prevalence of VAD in Kiambu in children of 6-72 months was 30%. Data released from the 1999 National Survey indicated that the prevalence of acute to moderate serum retinol deficiency for the same age was 13.13% (serum retinol<20µg/dL) and 84.4% (serum retinol<25 µg/dL) respectively (Mwaniki, 2002). The statistics for Kiambu for women was 18.95% and 50.7% respectively. These statistics are extremely alarming.

They indicate a deteriorating health status similar to the findings of this study where 13% of the children had acute serum retinol deficiency and 42% had moderate serum retinol deficiency making a total of 55% in the two groups in a district considered to have lower prevalence levels of nutritional problems (Mwaniki, 2002). Low serum retinol levels are considered a public health problem when the prevalence exceeds 5.0% (WHO Expert Group, 1982, using the threshold criteria). It is therefore clear that there was a vitamin A deficiency problem at the institution considering also that the children consumed no fruits or vegetables during the study period.

Using the threshold criteria developed by IVACG (1993) and WHO Expert Group (1982) for determining vitamin A as a problem of public health significance (as presented in chapter two) The prevalence of nyctalopia (33.8%) and Bitot's spot (23.4%) was high signifying that VAD is a serious problem at the institution.

The standard conversion rate for retinol/ β-carotene ratio is 1retinol equivalent (RE) = 6µg β-carotene. Using this ratio, the βCRB provided 177µg or 35.4% of retinol vitamin A while the βCLB provided 28µg or 5.6% of the RDA, set at 500µg retinol.

In both cases, this was not sufficient to meet the daily requirements of the children but the βCRB was considered appropriate in supplementing their diets.

Emerging and sometimes contradictory research data suggests that the bioefficacy of β-carotene in plant foods is much less than previously thought. The studies carried out in Indonesia in school children by De Pee in 1998 and in another study carried out by Khan in the same year in

Vietnam in breastfeeding women where both groups were fed on DGLVs, carrots and yellow and orange fruits yielded a β-carotene bioefficacy of 1:26 and 1:28 for DGLVs respectively. This meant that 1 retinol equivalent was equal to 26 and 28μg respectively. The bioeficacy for fruits was 1:12 in both studies. This was further confirmed by work done in 1999 by Tang et al whose findings yielded a 1:27 ratio. Although this information has not changed the carotene-retinol official ratios (West, 2001), based on this calculation, the βCRB provided only 7.9% of vitamin A RDA while the βCLB provided 1.3%.

Revised dietary RDAs only recently available, indicate that the vitamin A requirement for this group is 800μg. Calculations based on these two considerations would further reduce the amount of retinol the children were getting from the study as the βCRB would only provide 5% of RDA and the βCLB a dismal 0.78%. This then would mean that the study provided only negligible amounts of vitamin A, which would probably be one of the reasons why the β-carotene conversion to retinol was not observed.

This however, cannot be conclusive because the experimental group recorded significantly higher post-test serum β-carotene levels than the non-experimental group and a total of 21 (29.6%) children recorded increases in their serum retinol levels. Furthermore, in the Democratic Republic of Congo, a program designed to increase the fruit and vegetable intake of children in a kindergarten was implemented. For 5 days per week, for 10 weeks, 22 children were provided with 238g per day of green and yellow vegetables. This maintained serum retinol concentrations in the experimental group in a season of usually low intake, while those in the control group fell (Allen, 2001).

in this study, the βCRB group, however, recorded lower serum retinol levels for the same period creating an inverse relationship. These results presented a similar pattern observed in 1996 in work carried out by Chania on comfrey (Chania, 1998). Her results, however, reflected the total carotenoids effect on the children rather than β-carotene in relation to retinol vitamin A. It was, therefore, believed that other carotenoids may have had a negative effect on the bioconversion of β-carotene to retinol.

It is for this reason that this study was carried out as it was believed isolating the β-carotene for analysis might portray a different picture, which now does not seem to have been the case.

## 5.2.    Dietary factors

### 5.2.1.    Protein and energy

Adequate protein assists in maintaining desirable vitamin A status (McLaren, 2001). Carotenes are hydrolyzed by β-carotene 15-15'-dioxygenase of intestinal cells to release 2 moles of retinal, which is reduced to retinol (Satyanarayana, 1999). When a diet is deficient in protein and energy as was observed in the study, the absorption of β-carotene for conversion into active form of vitamin A is hampered (Allen, 2001) because retinol is transported to the extra-hepatic tissues by the plasma retinol binding protein (RBP) and is only released from the liver as free retinol as and when needed (Antia, 1973)

The diet in the institution constituted mainly of maize meal, rice and beans. The low protein intake would probably account for the inability of the children in the experimental group to convert the circulating β-carotene to serum retinol. Although some of the children received

visitors who brought them food tokens, the food was starchy in nature and only about 6.5% of the children received food from outside the institution. This did not have any significant impact on the serum retinol levels observed.

## 5.2.2.  *Vitamin A*

The children consumed a diet that was highly deficient in foods rich in vitamin A. Food sources rich in vitamin A such as meat, eggs or milk were not consumed by the children during the entire study period. These foods were not also included in the standard menu of the institution. No fruits were consumed during the study period and the only vegetable consumed in the same period: spinach was consumed only once. When the analysis of the food was done, no β-carotene was detected. This was probably because the amounts were negligible as the spinach had been mixed with the beans and were hardly visible in the meal and also because of loss during cooking. The dry beans and spinach were cooked for the same duration. Since beans require a much longer cooking period than spinach, the nutrient content of the spinach may have been destroyed by excessive cooking.

## 5.2.3.  *Zinc*

It is believed that zinc plays an important role in retinol mobilization. The RDA for zinc is 15mg. The absorption of zinc is inhibited by food constituents such as phytates, oxalates and tannins. Less than half of the zinc in the diet is absorbed and absorption is also further reduced if large amounts of whole cereals rich in fibre and phytic acid are eaten (Osendarp, 2001).

It is therefore possible that the high ingestion of beans by the children may have suppressed retinol mobilization, which may explain the lack of significance in posttest retinol levels.

## 5.2.4.   *Fat*

The low amounts of fat in the diet may explain the lack of conversion of β-carotene to vitamin A, which takes place in the walls of the intestines. This is because carotene is poorly utilized when the diet has a low fat content (Latham, 1997).

## 5.3.   Morbidity

Clinical assessment including morbidity data was taken because although a great deal of research has been done, very little is known about the precise contribution that VAD makes to morbidity in infectious diseases (McLaren, 2001).

The high rate of morbidity may have had a negative effect on serum retinol availability as there were strong associations between morbidity, BMI, VAD and pre-test carotene levels. When there is morbidity especially VAD, flu, skin infections and parasitic infestation, thedemands on the immune system is great and it is clear from the study that the morbidity cases dropped significantly which probably means that whatever vitamin A was available may have been utilized in fighting off morbidity.

The dental health of the children deteriorated mainly because there was no oral hygiene being practiced which made the children vulnerable to increased poor dental health due to the high sugar content of the experimental biscuits. The remedy offered by the institution for dental caries was tooth extraction. Poor dental health reduces the efficiency of mastication due to pain

and sensitivity, which in turn affects total nutrient intake (Buss, 1978). Surprisingly, children with poor oral health recorded higher BMI's than the children without these conditions.

It would appear that the majority of the children ignored their discomfort (only 3 children lost weight due to poor dental health) and consumed the biscuits probably due to their organoleptic properties or due to an inherent need to meet deficiencies in their diet.

Infections of the upper and lower respiratory tracts, part of the gut, and the genito-urinary tract cause a depression of serum retinol (McLaren, 2001). Diarrhea, fever, cough, bronchitis and pneumonia have such a negative effect on serum retinol that even when vitamin A is administered, there is no impact on vitamin A status but there might be a reduction in severity if not duration, of the respiratory symptoms (Fawzi, 1996). Cases were not medically handled efficiently and the drugs mainly dispensed for morbidity in the institution were pain relievers, anti-malarial tablets, skin creams and ear and nose drops. It was not possible to verify availability as there were none in stock in the institution. The explanation given for this was that all medicines were purchased as needed. Only 7.8% of the children confirmed receiving treatment of some kind during the entire study period and of these 6, it is the researcher who identified a severe case of syphilis. The child was immediately taken to the Kenyatta National Hospital where she was started on a course of antibiotics. The lack of appropriate and timely medical response to the children's morbidity may have made their nutritional condition worse.

Although usually there is a blood serum-drug interaction phase, the number of children involved did not make any significant difference to the study. It can, therefore, be assumed that available stored amounts of vitamin A, no matter how negligible, were taken up and used to maintain or strengthen the immunity of the children. This can be supported by the fact that the children

added weight at a rate higher than usual for the period of study (1.9kgs). The normal average weight gain for a reference child of this age group is 0.8kg. in one month (Latham 1997).

The considerable drop in morbidity also supports this because it is clear the majority of the children had recovered although this may have been perpetuated by the treatment of intestinal parasites. What is not clear from this study is whether any β-carotene was converted to serum retinol and if any was converted, whether the utilization demands were far greater than the conversion rate.

### 5.3.1    HIV/AIDS

Although the HIV status of the children was not determined, the national HIV/AIDS prevalence for this group is 14% (Nduati, 2002). It is believed that some of the children may have been affected given their social orientation before they were institutionalized.

After the study period on a follow up visit, two of the children from the study group had run away from the institution. Although they came back, information received suggested that they had run away in search of sexual fulfillment.

HIV depresses serum retinol levels and in mortality is higher in those with lower serum retinol levels (McLaren, 2001). Vitamin A supplementation of a group of HIV-positive injection users had no effect on HIV load or CD4 lympocyte count (Semba, 1995). Maternal vitamin A deficiency during HIV infection is reported to predispose to growth failure and increased infant mortality. This has a grave significance to the study because the study group, who manifested an already depressed immune system due to malnutrition and VAD, were adolescents whom the

ation would look up to for population development, maintenance and growth. Vitamin A supplementation of HIV-infected infants significantly reduced morbidity (Coutsoudis, 1994) and reduced mortality among both HIV-infected and non-infected malnourished children (Fawzi, 1999).

## 5.3.2   *Intestinal parasites*

The prevalence of intestinal parasites in the study group post-test was 26%. Intestinal parasites lower concentrations of RBP in plasma hence reducing the ability of the body to convert carotene to vitamin A   (McLaren, 2001). *Giardia lamblia, Ascaris lumbricoides*, and *Ankylostoma duodenale* (hookworm) have all been shown to reduce vitamin A absorption with the impact being greater on carotenoids than on preformed vitamin A (Curtale, 1995).

The presence of intestinal parasites in the children, even after de-worming, may have affected the bioconversion of serum β-carotene to serum retinol. The *Trichuris trichiura* and *Schistosoma mansoni* appeared to be resistant to the Albendazole dose administered at recruitment.

## 5.4.   Anthropometric Measurements

The average weight gain observed in children during the period of study was 1.9 kilograms. This is more than double that expected for the same period of time (0.8Kgs). This can be explained by the treatment of intestinal parasites, making the body more efficient in utilizing nutrients and the extra (average) 473 calories the study group was consuming daily. It created a daily excess of 142 calories from the recommended 1,574 calories (Nduati, 2002).

When there is an increase in body weight, the nutritional demands increase as well. The rapid increase in weight observed in the children may have placed a great demand on nutrients consumed and since the diet was deficient, the negative serum retinol results may have arisen from this factor.

## 5.5. Confounding Factors

The deficient and borderline deficiency of serum retinol in the pre (53.5%) and posttest (73.2%) periods, indicate low levels of retinol in the liver in the groups. When levels are low, no increases in serum retinol are to be expected (Latham, 1997). Apart from dietary factors, it is possible that this condition may have been occasioned by other factors like the duration of β-carotene supplementation, which is a significant predictor of β-carotene response (McLaren, 2001). More recent studies done in India reported statistically significant increases in serum retinol levels after six weeks and further increases at eight weeks of spinach supplementation. (Metha, 2001). The supplementation period may have, therefore, have been too short to get results. Although the study period was increased to 30 days from the 14-day- period covered by Chania, in a similar study, it would appear that the bioconversion of serum β-carotene to serum retinol requires a much longer period although to date, there is no data available on the exact length of this period (Burri, 2001).

## 5.6. Potential of Comfrey in Alleviation of VAD

Comfrey has a higher β-carotene content than other DGLVs that are high in this provitamin such as amaranths and pumpkin leaves. This can be processed without significant loss of the

provitamin by using unblanched comfrey powder (Chania, 1998). The significant increase of serum β-carotene in the experimental group taking into consideration that the comfrey had been macerated before baking indicates it's ability to remain stable during processing. This factor is critical in vegetable processing as most vegetables loose their nutrient content at this stage due to chopping and washing and over-cooking.

The comfrey-enriched biscuit (βCRB), was very well received by the children both visually and organoleptically. This strengthens the case for comfrey use as a source of vitamin A because not only was the biscuit presentable and easy to consume, but it left no astringent taste in the mouth as is commonly the case in DGLVs. Further more, the biscuit, having a moisture content of only 4.15% can store well especially if this moisture level is further reduced (but >2.4%) to facilitate longer shelf life and environmental stability. The matrix in which protein, carbohydrates, vitamins and minerals are embedded is easily broken down in this process thus making these nutrients more available.

The use of comfrey as a source of vitamin A should not be discouraged and further research needs to be carried out to elucidate the reasons why the serum β-carotene was not converted to serum retinol and if it was, why the amounts converted had a negative impact on the serum retinol status of the children.

# CHAPTER SIX

## CONCLUSION AND RECOMMENDATIONS

### I. Conclusions

1. Vitamin A deficiency is still a problem of public health significance among children aged 8 - 16 at the Kirigiti Girls Approved School in Kiambu. Key negative factors such as morbidity and under-nutrition may be responsible for the low serum retinol levels in the post-test period and this has to be taken into consideration.

It is therefore difficult to conclude that the study had a negative effect on the children because the β-carotene levels increased in the experimental group although there is no evidence that it was converted to serum retinol. The BMI's of the children also increased and morbidity decreased significantly.

2. The study has investigated the potential of comfrey as a source of vitamin A for malnourished children aged 8-16 years and the hypotheses: there is no difference in the post-intervention serum retinol A levels of children ( aged 8 – 16 years) fed on comfrey containing biscuit and comfrey free biscuit is accepted. However the hypotheses stating that there is no difference in the post-intervention serum b-carotene levels of children (aged 8 – 16 years) fed on comfrey containing biscuit and comfrey free biscuit has been rejected.

3. The visual and organoleptic features of the baked β-CRB as well as it's ease in handling and consuming and storage properties makes it an easily acceptable and widely distributable product of nutritional value.

## 6.2. Recommendations

1. Controlled intervention studies with baked products incorporating DGLVs should be carried out which would also investigate confounding factors that affect bioconversion of serum β-carotene to serum retinol (section 2.2.5.). The intervention period of such a study should be made longer to facilitate positive serum retinol changes as some studies with DGLVs have shown improved vitamin A status in participating communities. The amount of sugar in such a product should be considered to reduce the incidence of oral health problems. The benefits should be weighed against the cost.

2. The potential of reconstituting the βCRB or similar products with milk or water into a gruel for consumption by infants should be investigated.

3. Communities should be encouraged to grow DGLVs and more so comfrey because of it's high nutritional value and low fibre content and the fact that comfrey grows easily with minimum watering and does not require much nurturing or expensive farm inputs.

4. The macerating of vegetables for baking purposes after washing the leaf without chopping should be encouraged as there is a minimal nutrient loss.

The health status of the children should be improved by;

1. Increasing the daily consumption of fruits and vegetables to improve the children's micro-nutrient status. Some of these can be grown in the institute because there is enough land and water and the soil is fertile and suitable for this purpose.

2. Providing timely and effective treatment for morbidity cases and facilitating and promoting the practice of proper hygiene including oral hygiene.

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**Appendix 1:** **Informed Consent Form**

## 1. STUDY

**ASSESSMENT OF THE POTENTIAL OF COMFREY (SYMPHYTUM PEREGINUM) AS A SOURCE OF VITAMIN A FOR MALNOURISHED CHILDREN (8-16 YEARS) AT THE KIRIGITI GIRLS APPROVED SCHOOL, KIAMBU, KENYA.**

## 2. PURPOSE

The purpose of the study is to assess the potential of comfrey (*Sympytum pereginum*) as a source of vitamin A.

## 3. CONDUCT OF STUDY AND INVOLVEMENT OF PARTICIPANTS

A stool sample will be required from each participant and each participant will be de-wormed before the commencement of the feeding on either a comfrey-rich biscuit or a comfrey-free biscuit.

Participants will be fed on two biscuits everyday for four weeks after which 5 milliliters of blood will be drawn from each participant for analysis. Qualified medical personnel will carry out all clinical procedures.

Any abnormal reactions arising from any procedure of the study will be referred to the nearest government clinic or examined/treated by the qualified medical doctor on the team.

## **INFORMED CONSENT**

I have read and understood the purpose and the procedures involved in the study and what is required of me.

I,_____, hereby consent to participate and adhere to the

protocol of the interventional and experimental feeding study

as outlined in page 1 of this document this _____(date) day of

_____(month) , _____ (year).


NAME                      SIGNATURE                      DATE


_____    _____    _____
( PARENT/GAURDIAN /
INSTITUTION  MANAGER)


_____    _____    _____
 (INVESTIGATOR)


_____    _____    _____
(DIRECTOR OF CHILDRENS
   SERVICES)


_____    _____    _____
( DISTRICT OFFICER
     KIAMBU )

Questionnaire number_____ Date of interview _____

Name of interviewer _____

Study center_____

**Demographic and background information:**

1.  Name of respondent_____ 2.  Sex _____

3.  Age ____ years ____ months.    4.   Education level _____

5.  Name of Father _____ Occupation _____

6.  Name of mother _____ Occupation _____

7.  Number of sisters _____ Number of brothers _____

8.  Parity _____         9.Ethnicity _____

10. Religion _____

11. Name of guardian _____ 12. Number of children in HH _____

**12 . Date of admission to the Kirigiti Girls Approved School?**

_____/_____/_____

**Day       Month      Year**

| Sex | Education | Parity | Ethnicity | Religion |
|-----|-----------|--------|-----------|----------|
| 1=male | 1=illiterate | 1=first born | 1=Kikuyu | 1=Catholic |
| 2=female | 99=preschool | 2=second born | 2=Luo | 2=Protestant |
| | 2=primary | 3=third born | 3=Luhya | 3=Muslim |
| | 3=secondary | 4=fourth born | 4=Kamba | 4=Other _____ |
| | | 5=fifth born | 5=Kisii | |
| | | 6=other (specify) | 6=other (specify) | |

**Social background**

1. Where is your home _____.

2. Where were you staying before you came to the Kirigiti Girls Approved School? _____.

3. For how long were you there? _____

4. Where were you before then _____.

5. Whom were you staying with at that time _____.


## 2. CLINICAL EXAMINATION

### (2.a) Medical history

1. Have you had any disease in the last 7 days?
       1 = yes         2 = No

2. If yes which ones:
   1=diarrhea 2=fever 3=cough 4=vomiting 5=scabies 6= worms
   7= other (specify)

3. Have you had any treatment?     1 =Yes        2 = No

                Specify treatment_____

### (2.b) Physical examination

1. Dental status: Normal                1=Yes    2=No
                Discolouration          1=Yes    2=No
                Dental Caries           1=Yes    2=No
                Missing teeth           1=Yes    2=No
                Mottled                1=Yes    2=No

2. Oral mucosa: Normal        1 = Yes     2 = No
                Pain              1 = Yes     2 = No   Part _____
                Bleeding gums   1 = Yes     2 = No
                Abscess        1 = Yes     2 = No

3. Physical handicap       1 = Yes      2 = No

4. Pallor                 1 = Yes      2 = No

5. Jaundice                         1 = Yes        2= No


6. Do you bump into things at night?
                                    1 = Yes        2 = No

7. Cornea:    Shiny and moist       1 = Yes        2 = No
              Dry                   1 = Yes        2 = No
              Wrinkling             1 = Yes        2 = No
              Bitot Spots           1 = Yes        2 = No
              Keratomalacia         1 = Yes        2 = No


8. Nails      Normal                1 = Yes        2 = No
              Spoony                1 = Yes        2 = No

9. Pain in ear _____ , nose_____ and throat_____.
                                    1=Yes          2=No

10. Skin abnormalities    Healthy   1 = Yes        2 = No
                  Dry and Scaling   1 = Yes        2 = No
                          Scabies   1 = Yes        2 = No
            Generalized dermatitis  1 = Yes        2 = No
                           Eczema   1 = Yes        2 = No
                            Boils   1 = Yes        2 = No
                       Ring worms   1 = Yes        2 = No

11. Hair:             Normal        1 = Yes        2 = No
                      Brown         1 = Yes        2 = No
                      Silky         1 = Yes        2 = No

12. Oedema                          1 = Yes        2 = No

13. Muscle Bulk    Normal           1 = Yes        2 = No
                   Wasted           1 = Yes        2 = No


3      **Dietary  history**


A

       What foods did you eat at home?  Mention them
       1.  Maize and beans
       2.  Maize, beans and vegetables
       3.  Ugali and  green vegetables
       4.  Ugali and cabbage
       5.  Ugali and beans
       6.  Ugali and fish
       7.  Rice and beans

8. Rice and green vegetables
9. Porridge
10. Fruits (specify)_____
11. Others _____

**B** Which vegetables have you consumed in the last 7 days

|        | Vegetables | Amount | Source | Freq./Week |
|--------|------------|--------|--------|------------|
| (i)    |            |        |        |            |
| (ii)   |            |        |        |            |
| (iii)  |            |        |        |            |
| (iv)   |            |        |        |            |
| (v)    |            |        |        |            |
| (vi)   |            |        |        |            |

**Freq.=frequency**

**C.** Do you consume any fruits?

<div align="center">1= Yes      2 = No</div>

**D** Which fruits have consumed in the last 7 days?

|        | Fruits | Amount | Source | Frequency/Week |
|--------|--------|--------|--------|----------------|
| (i)    |        |        |        |                |
| (ii)   |        |        |        |                |
| (iii)  |        |        |        |                |
| (iv)   |        |        |        |                |
| (v)    |        |        |        |                |
| (vi)   |        |        |        |                |

## 4. FOOD INTAKE: 24 HOUR RECALL

| Time | Dish | Amount cooked (grams) | Name of ingredient | Source of ingredient | Amount consumed by the child (g) | Amount left over (grams) |
|---|---|---|---|---|---|---|
| Break-Fast | | | | | | |
| | | | | | | |
| | | | | | | |
| Lunch | | | | | | |
| | | | | | | |
| | | | | | | |
| Dinner | | | | | | |
| | | | | | | |
| | | | | | | |

## 5. Blood sample analysis

Date of specimen collection (Pre-test)_____ (Post-test)_____

| Test | serum/plasma level in um/l | | normal 1 | | low 2 | | acceptable 3 | | high 4 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| | | | | | | | | | | |
| Vit A | | | | | | | | | | |
| | | | | | | | | | | |
| B-carotene | | | | | | | | | | |
| | | | | | | | | | | |
| Hemoglobin | | | | | | | | | | |
| | | | | | | | | | | |
| Albumin | | | | | | | | | | |

1= pre-test
2=post-test

## 6. Anthropometric measurements

Take the following anthropometric measurements and fill in the table below.

| MEASUREMENT | FIRST | | SECOND | | AVERAGE | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 1 | 2 | 1 | 2 |
| Weight (kg) 0.1kg | | | | | | |
| Height (cm) 0.5cm | | | | | | |
| MUAC (mm) | | | | | | |

1= Pre-test
2= Post-test

## STOOL SPECIMEN SHEET

7.    Name of child _____ Age_____

Container dispensed?        **Pre-test**   1=Yes 2=No    Date_____
                           **Post-test**   1=Yes 2=No    Date_____

Stool specimen received ?   **Pre-test**   1=Yes 2=No   Date_____

                           **Post-test**   1=Yes 2=No    Date_____

Stool specimen examined ?   **Pre-test**  1=Yes 2=No   Date_____
                           **Post-test**  1=Yes 2=No     Date_____

Results : Total Number **(Pre-test)**_____ **(Post-test)**_____

Number of parasites **(Pre-test)**_____ **(Post-test)**_____

Parasites present Pre-test_____.

Parasites present Post-test_____.

**Parasite prevalence based on microscopic stool examinations**

| Parasite type | Egg count per gram of stool | |
| --- | --- | --- |
| | **Pre-test** | **Post-test** |
| Ascaris | | |
| Hookworm | | |
| Trichuris | | |
| S. mansoni | | |
| Others | | |