SERUM VITAMIN A AND NUTRITIONAL STATUS OF CHILDREN FED GARI AND BEANS STEW

BY

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DECLARATION

I, Alatiah Gabriel Ajediwe, author of this thesis "Serum vitamin A and nutritional status of children fed gari and beans stew" do hereby declare that this thesis is the result of my own work carried out at the Department of Nutrition and Food Science, Faculty of Science, University of Ghana, under the supervision of Prof. Matilda Steiner-Asiedu and Dr. Kwesi Firibu Saalia. All references to other works have been duly acknowledged.

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DEDICATION

This work is dedicated to my wife, Philomina and our three children, Awepiah, Aweniah

and Achanah.

ACKNOWLEDGEMENT

ABSTRACT

Introduction: Vitamin A deficiency (VAD) is endemic among children in low income countries. Food-based strategies have been identified globally as more sustainable in addressing micronutrient deficiencies in populations. However, there is paucity of knowledge on the effect of food based interventions in combating vitamin A deficiency in Ghana. The objective of the present study was to determine the serum vitamin A and nutritional status of children fed gari and beans stew prepared with red palm oil.

Methodology: The study was a 6 month one-group longitudinal pre - and - post - test design. A convenient sample of 142 school children (6 to 12 years) randomly selected at Kodzobi Basic School (Primary-JHS) in the Volta Region of Ghana participated in the study. A survey was conducted to determine the vitamin-rich foods in the community. A 24 hour dietary recall and food frequency questionnaire instruments were employed to obtain information on the diet of the participants. The nutritional composition of the intervention diet was determined using a standard method. The β-carotene content of the diet and the serum retinol level of the participants were determined using High Performance Liquid Chromatographic (HPLC). The weight and height of the children were measured to determine weight-for-age z-scores, height-for-age z-scores and body mass index-for-age z-scores. Differences in means before and after the intervention period were determined using the paired t-test. Chi-squared test was used to examine the association between serum retinol and some categorical variables. Logistic regression models were used to explore the relationship between serum retinol and the background characteristics of study participants.

Results: The survey revealed seasonal availability and accessibility of vitamin A-rich foods in the community. HPLC analysis of the intervention diet showed that it contained high vitamin A (823.56µg RE). Based on the 24 hour dietary recall the macronutrient

intakes between the pre and post interventions were significantly different. Carbohydrate intake pre-intervention was 357.76±56.50 g while the post intervention intake was 411.36±58.99 g; pre-intervention protein intake was 31.86±8.43 g whereas the post intervention intake was 45.64±9.20 g. Fat intake pre-intervention was 33.52±7.20 g as against post intervention intake of 35.04±7.44 g. Median vitamin A intake increased from 3.84 (0.0, 1392.14) µg RE to 4.61 (0.0, 1681.79) µg RE. None of the reported socio-demographic variable showed a significant association with vitamin A status. The mean serum retinol level (12.13 µg/dL) of participants at pre-intervention was significantly different from the mean serum retinol of (16.11 µg/dL) at the post-intervention period, p <0.001. The prevalence of vitamin A deficiency (serum retinol concentrations < 20 µg/dL) among the participants decreased from 95.1% in pre-intervention to 77.5% in post-intervention. Comparisons between the pre and post-intervention nutritional status showed a significant difference in height-for-age z-scores (p<0.001). However, there were no statistical differences between the pre and post intervention weight-for-age and BMI-for-age z-scores (-2SD). Mean malaria parasite density for the pre intervention was 2923.42±5667.09/µL and this was significantly different from the post intervention parasite density of 865.16±1312.12/µL.

Conclusion: VAD is a public health challenge among the participants. The intake of vitamin A rich foods was poor due to seasonality and accessibility. There was high level of wasting and underweight among the participants. The intervention diet, gari and beans stew made with red palm oil served three times per week did improve serum retinol levels of the children and this may be a sustainable strategy in combating VAD.

LIST OF ACRONYMS AND ABBREVIATIONS

µg	Microgram
µg/dL	Microgram per dicilitre
µg/g	Microgram per gram
AED	Academy for Educational Development
ALRI	Lower Respiratory Illnesses
BAZ	Body Mass Index-for-age z-scores
Cm	Centimeter
FAO	Food and Agricultural Organization
FFQ	Food Frequency Questionnaire
g	Gram
GDHS	Ghana Demographic and Health Survey
GH¢	Ghana Cedis
GHS	Ghana Health Service
GSS	Ghana Statistical Service
H/A	Height-for-age z-scores
HPLC	High Performance Liquid Chromatography
IOM	Institute of Medicine
IRB	Institutional Review Board
IU	International Unit
IVACG	International Vitamin A Control Group
JSS	Junior Secondary School
Kcal	Kilocalorie
mg	Milligram
ml	Millilitre

MOH	Ministry of Health
NMIMR	Noguchi Memorial Institute for Medical Research
P-value	Probability value
RDA	Recommended Daily Allowance
RDI	Recommended Dietary Intake
RE	Retinol Equivalent
RPO	Red Palm Oil
RI	Recommended Intake
SD	Standard Deviation
SSS	Senior Secondary School
UNICEF	United Nations Children's Fund
VAD	Vitamin A deficiency
W/A	Weight-for-age z-scores
WHO	World Health Organization

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Micronutrient malnutrition, hidden hunger remains a key public health challenge for individuals, households, governments, non-governmental organisations and the international community (Jones *et al.*, 2005). Paramount among these micronutrient challenges is Vitamin A deficiency (VAD). VAD is particularly endemic among children in low income countries, especially in sub-Saharan Africa (Arlappa *et al*., 2008). Globally, WHO (1995) estimated that 28 million children under age five years had clinical VAD whereas 251 million children suffered from sub clinical VAD. Subsequently, Mason *et al.* (2001) appraised the situation and reported that 3.3 million children had clinical VAD and 75-140 million children suffered sub-clinical VAD. Despite the differences in these estimates which may be attributable to the lack of reliable and representative data, VAD remains a major challenge worldwide. Africa is the second largest host of these children after Southeast Asia with nearly 50% of children affected. In 1993, the Ghana Vitamin A Supplementation Trial Team reported a 65 % of severe vitamin A deficiency in children in northern Ghana. In 1997, the Ministry of Health Vitamin A prevalence survey revealed that 37.2% of children in southern Ghana had low serum levels of vitamin A. Subsequently, a cross-sectional study conducted among school children 2 – 10 years in Okwenya community of the Eastern Region of Ghana (Egbi, 2012) indicated that 36% of them were vitamin A deficient (serum retinol concentration < 20 ug/dL).

Inadequate food intake (yellow flesh potato, mangoes, green leafy vegetables, liver, and milk), poor dietary quality, poor bioavailability (due to the presence of inhibitors and mode of preparation) and infection are among the major contributors to VAD

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(Ramakrishnan, 2002). The vital role vitamin A plays in proper immune function makes its deficiency deleterious. Severe vitamin A deficiency can result in xeropthalmia, cornea ulceration, and permanent blindness. Some studies have associated night blindness and xerophthalmia with infrequent consumption of foods containing vitamin A (Mele *et al*., 1991; Hussain *et al*., 1993 and Shanker *et al*., 1998). Vitamin A deficiency can also increase the severity of infections such as measles and diarrhoea and increase the risk of mortality (GSS, 2008).

In view of the devastating consequences of VAD, several interventions tailored at redress have been proposed. These include: 1) high-dose vitamin A capsule supplementation, 2) food fortification, and 3) food diversification and nutrition education. Research suggests that high-dose vitamin A capsule supplementation to vulnerable young children is the most cost-effective short term intervention. However, food-based strategies have been identified globally as more viable and sustainable alternatives for addressing micronutrient deficiencies in populations (Benadé, 2003). In spite of this, many international VAD interventions have been more focused on supplementation.

In Ghana, attention has been focused on vitamin A supplementation of infants and young children using immunisation as a key contact point. Industrial fortification, mainly voluntary is also another area that is being encouraged. Although dietary diversification is mentioned in the Ghana VAD control strategy (West Africa Conference on Vitamin A Deficiency, 1993), it is not being accorded the deserved attention. Dietary diversification strategies have mainly focused on nutrition education and behaviour change communication. In some instances, nutrition education may emphasise vitamin A rich

foods that are expensive and unaffordable by low income households who rely exclusively on staple crops like cereals, roots and legumes for their energy and nutrient needs.

There is therefore a paucity of knowledge on the potential efficacy and effectiveness of food-based approaches that rely on plant sources to improve vitamin A status. Hence, there is need for evidence-based research to generate the required information to improve the content of health education messages for enhanced public health practice.

Cowpea, a legume rich in energy, protein, iron and other nutrients, for instance is extensively consumed in West Africa. Cowpea-based foods are widely consumed in Ghana and hence constitute a major source of protein in the diet. Red Palm Oil (RPO) is carotenoid rich food which can be utilized as a fortificant in cowpea based diets to improve the vitamin A status and nutritional status of children. In spite of its availability throughout the year, children rarely consume RPO, and less than 40% of households adequately consume RPO (Amoaful, 2001). Providing at least one meal a day made from cowpeas and red palm oil may be a long term strategy to improving the nutrition situation of children. It is against this backdrop that the current study sought to test the use of cowpea and palm oil in improving the vitamin A status as well as the nutritional status of children in Kodzobi in the Volta Region of Ghana.

1.2 Problem statement

The very serious functional consequences of VAD among children continue to attract public health concern worldwide. In Ghana, it is estimated that VAD affects 26% of the country's under five population. Analysis using the PROFILES computer software show that VAD accounts for 1 out of 6 of all child deaths between the ages of 6 and 59 months.

Between 1997 and 2001 alone the number of child deaths due to VAD was estimated to be 49,000. Elimination of VAD by the year 2001 could save the country substantial amounts. For instance, families could save about 11 million US dollars in terms of the care of sick children whilst savings to the nation would be about 58 million US dollars (Academy for Educational Development, 2003).

Evidence from the GSS (2008) indicate that 57% of children under five in the study region-Volta Region of Ghana received vitamin A supplements 6 months prior to the survey. There is however a dearth of literature on the efficacy/effectiveness of plant-based food strategies in curtailing the VAD menace. Furthermore, notwithstanding the fact that VAD in children have strong ties with poor dietary practices the necessary steps are rarely made to reverse the situation. It is therefore important to assess the extent to which food-based strategies can help to improve the vitamin A and nutritional status of children.

1.3 Objectives

1.3.1 Main objective

To determine the serum vitamin A and nutritional status of children fed gari and beans stew.

1.3.2 Specific objectives

1. To identify the availability and accessibility of vitamin A/carotenoid rich foods in the community.

2. To determine the carotenoid content of the intervention diet.

3. To evaluate the dietary carotenoid intake of the children pre and post intervention in relation to RDI.

4. To determine the serum retinol level of the children pre and post intervention.

5. To quantify the effect of vitamin A on the occurrence of infection among the children.

6. To assess the nutritional status of the school children pre and post intervention.

1.3.3 Study hypotheses

1. The consumption of cowpea-based foods will improve the nutritional status of the children.

2. The consumption of RPO will improve the vitamin A status of the children.

CHAPTER TWO

2.0 LITERATURE REVIEW

Globally, undernutrition is one of the principal issues of public health significance that continues to engage the attention of the international community. Micronutrient undernutrition or hidden hunger is particularly of greater concern as its deleterious effects pervade the biological, physical, socioeconomic and cultural aspects of humanity (WHO, 2000; and UNICEF, 2012). Vitamin A is of huge relevance to human existence as this is palpable through the consequences of its deficiency (Kapil and Bhavna, 2002).

Several interventions have been implemented over the years towards the mitigation of vitamin A deficiency. The universal standard interventions for vitamin A deficiency (VAD) control include; supplementation, fortification and dietary diversification (Butt *et al*., 2007). It is the latter of these interventions that is the theme of this thesis. The review is therefore focused on the existing knowledge and gaps on vitamin A, and its deficiency control with emphasis on food based approaches in relation to vulnerable Ghanaian children fed gari and beans stew.

2.1 Vitamin A physiology and importance

Vitamin A comprises retinoids and precursor varieties, provitamin A or carotenoids. Retinoids (retinol, retinal, and retinoic acid) are the biological functional forms of vitamin A. The interrelationship between these forms of vitamin A is shown diagrammatically in figure 1.

Retinol ⇄ Retinal → Retinoic acid
Retinyl esters (storage)
Rhodopsin
Excretion products
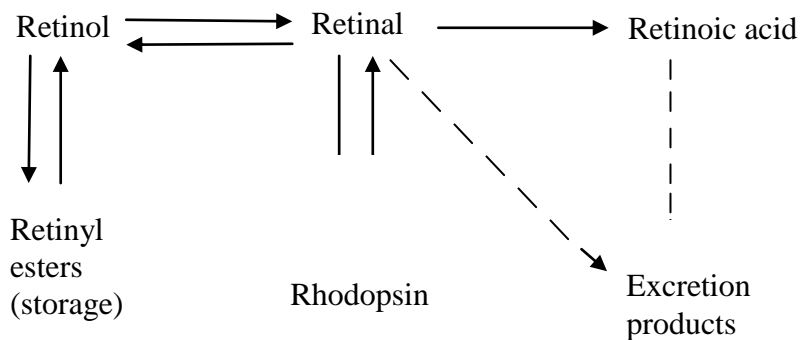
(Nelson and Cox, 2005)

Figure 1: Interrelationship between retinol, retinal, and retinoic acid

Retinol, the most reduced form of the vitamin, satisfies the requirements for all known functions of vitamin A. Although, retinol is not the active form for all the metabolic processes in the body that require vitamin A; it can be converted to retinal and retinoic acid, other active forms of the vitamin A family (Bowman and Russell, 2001). Vitamin A is present in food as retinol esters. Upon food consumption, there is hydrolysis of retinol esters in the intestinal mucosa, yielding retinol and free fatty acids. Retinol yielded from either esters or carotenes must undergo re-esterification to long chain fatty acids in the mucosal lining of the intestines. It is then secreted as a constituent of chylomicrons into the lymphatic system where its absorption and storage takes place in the liver (Awan, 2005). Figure 2 shows a schematic diagram of vitamin A metabolism.

DIETARY
SOURCE

RETINOL   RETINYL PALMITATE   BETA-CAROTENE

Hydrolase
(pancreatic juice)

Dioxygenase
(intestinal mucosa)

RETINAL

**Intestine
(Metabolism
& Absorption)**

RETINOL

Emulsification and absorption
Re-esterification with palmitic acid

**Liver
(Storage)**

(RETINOL + ESTER)

**Blood
(Transport)**

RBP   RBP   RETINOL
          RETINOL   TRANSTHYRETIN

**Periphery
(Utilization)**   Epithelial cells   Other target cells   Photoreceptors

(Shils *et al*., 2006)

Figure 2: Schematic diagram of vitamin A metabolism

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During periods of low serum retinol levels, retinol is released from the liver into the blood by retinol-binding protein (RBP). In the blood, the retinol-RBP then binds to a bulkier protein, transthyretin. The transthyretin-RBP-retinol compound then circulates in the blood, distributing retinol to the tissues; while resisting renal excretion as a result of its large size (Blomhoff *et al*., 1991). The uptake of the retinol-RBP compound by the cells of peripheral tissues is facilitated by specific receptors on the surfaces of the cells. Cellular RBP facilitates the transport of retinol to the nucleus where it performs functions similar to those of steroid hormones (Ong, 1994).

Inadequate dietary intake of protein, energy and some micronutrients such as zinc can militate against the hepatic synthesis of certain proteins that are crucial to the mobilization and transport of vitamin A. Zinc for example is required for the synthesis of RBP (Mann and Truswell, 2000). As a result, zinc deficiency decreases the amount of serum retinol in circulation which causes a functional deficit in vitamin A even when hepatic stores are adequate (WHO, 2011). Respiratory infections (Stephensen *et al.*, 1994), diarrhoea and abnormal kidney performance (Alvarez *et al.*, 1995) are among the factors that can result in vitamin A loss via urine. Fortunately, retinol-RBP can re-extract retinol in urine thereby resulting in its conservation (Champe *et al*., 2005; and Green and Green, 1994). This process ensures the prevention of urinary losses of vitamin A. As such vitamin A status cannot be assessed using urine (Green and Green, 1994).

The role of vitamin A as a constituent of rhodopsin which is the visual pigment in the rod cells of the retina required for vision has long been established (Arlappa *et al*., 2008). Other functions of vitamin A include; its critical role in cellular differentiation and proliferation, growth, reproduction, proper performance of epithelium mucosa, and it is an

integral part of immunity (Stephensen, 2001). Specifically, the oxidized form of retinol, retinoic acid is regarded very relevant in the differentiation of white blood cells (Gaines and Berliner, 2003). The vitamin is particularly required for proper brain, kidney and liver development during the early stages of life (Ghoshal *et al.*, 2003). During childhood, vitamin A facilitates tooth and bone development (Butt *et al*., 2007). When dietary consumption of vitamin A is sufficient, the risk of the occurrence of cardiovascular diseases and cancer among others is minimal (Weber, 1994; and Verma, 2003). In an in vitro study, Hamzah *et al.* (2003) demonstrated that vitamin A inhibits the proliferation of Plasmodium falciparum, the bacterium that causes malaria. This suggests that the vitamin may be protective against malaria.

Studies show that vitamin A and protein nutriture are directly related. Children who are protein-energy deficient have been reported to exhibit low concentrations of total proteins, albumin and retinol-binding proteins (Burri *et al*., 1990 and FAO/WHO, 2001). VAD may also compromise immune function in humans. This is because goblet cell numbers decrease in epithelial tissues during VAD. As a result, mucous secretions which contain antimicrobial components also diminish. This situation leads to a failure of the regeneration and differentiation of cells lining the surfaces of protective tissue resulting in the flattening and keratinizing of these surface cells. These factors, that is: the decline in mucous secretions and the loss of cellular integrity weaken resistance to invading pathogenic organisms (Ross and Stephensen, 1996; and Stephensen, 2001). In an earlier study, Vobecky *et al.* (1986) found low concentrations of immunoglobulin IgG and IgM in children with low vitamin A consumption.

In view of the physiological roles of vitamin A, it is necessary for the levels of the vitamin to be kept at normal levels in the body. The normal serum status of vitamin A is 30 to 95 g/dL although this may be a bit lower in children. Serum levels of carotene and retinol esters are usually 50 to 200 g/dL and 3–5 µg/dL respectively (David *et al.*, 1996). In order to maintain these values, it has been suggested that a 700 RE Recommended Daily Allowance (RDA) for adults and for 1 to 13 year-old children the RDA is 300 to 600 RE or 60.00 IU/Kg of body weight (Trumbo *et al.*, 2001). Attempts to meet the RDA figures for vitamin A must be carefully done since toxicity can result from overdose of vitamin A. For example, when children consume up to 330,000 IU of vitamin A, toxicity will result. In adults, the intake of 660,000 IU of vitamin A can lead to toxic effects such as dry, rough skin, hepatomegaly, joint pains, growth retardation, and hemorrhage. However, it has long been pointed out that no amount of carotenoids intake can result in toxicity since the bioconversion of these precursor forms is inversely proportional to a person's vitamin A status (Wang *et al.*, 1991).

2.2 Dietary sources of vitamin A, pro- vitamin A and their bioavailability

The dietary sources of vitamin A are primarily retinoids from animal tissue and carotenoid from plants. Retinol is the form of vitamin A that satisfies all known functions of the vitamin. Retinol, mainly found in foods of animal origin is readily used by the human body. The foods that are abundant in vitamin A (retinol) include; fish oils, liver, other organ meats, full-cream milk, and butter. Fortified foods such as fortified cereals and margarine also provide vitamin A (Guthrie and Picciano, 1995; and Trumbo *et al.*, 2001). On the other hand, pro-vitamin A carotenoids are found in plant diets and they are convertible to vitamin A. The major contributors of provitamin A carotenoids are leafy vegetables, palm fruits, yellow fruits, carrots, cantaloupes, sweet potatoes, and spinach

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(Harrison, 2005). Red palm oil (RPO) has a high content of carotenoids and is one of the abundant sources of β-carotene. Manorama and Rukmini (1991) revealed that RPO contains approximately 550 µg/g of total carotenes and 375 µg/g of β-carotene. A reasonable part of vitamin A is provided by provitamin A carotenoids (Trumbo *et al.*, 2001). Common carotenoids found in foods are β-carotene, α-carotene, lutein, zeaxanthin, lycopene, and cryptoxanthin (de Pee and West, 1996). Of the 563 identified carotenoids, less than 10% are precursors for vitamin A (Bendich and Olson, 1989). Among these, β-carotene is the most efficiently converted to retinol. Alpha-carotene and β-cryptoxanthin are also converted to vitamin A, but only half as efficiently as β-carotene. Lycopene, lutein, and zeaxanthin are carotenoids that do not have vitamin A activity but have other health promoting properties (Olson and Kobayashi, 1992; Olson, 1996; and Paiva and Russell, 1999). These carotenoids have been shown to function as antioxidants in laboratory tests. Antioxidants protect cells from free radicals, which are potentially damaging by-products of oxygen metabolism and may contribute to the development of some chronic diseases (Butt *et al.*, 2007).

Animal sources of vitamin A are well absorbed and used efficiently by the body. Plant sources of vitamin A are not as well absorbed as are animal sources (Olson, 1996; and Futoryan and Gilchrest, 1994). In developed nations, animal source foods (dairy products, eggs, liver) and fortified foods are the leading sources of vitamin A (West, 2000). However, plant sources; fruits, roots, tubers and leafy vegetables are the main contributors of provitamin A (carotenoids); representing more than 80% of total food intake of vitamin A in sub-Saharan Africa and this is because of their low-cost, high availability and diversity (Codjia, 2001). Although widely consumed by poor populations, plant provitamin A activity has been proven to be less (12µg of β-carotene for 1µg of retinol),

(Institute of Medicine, 2001) than previously assumed (6μg of β-carotene for 1μg of retinol) (FAO/WHO, 1976). These findings may partially account for the reason why VAD still persists in low income countries such as Ghana.

2.3 Factors affecting bioavailability

Vitamin A is a fat-soluble vitamin and as such it must be incorporated into micelles in a bile dependent reaction before it can be absorbed (Butt *et al*., 2007). The addition of oils or other fats to carrots, greens or other carotenoid-rich foods during cooking will therefore optimize the absorption of vitamin A. Accordingly, low (<15% of total energy) fat consumption reduces the absorption of carotenoids and vitamin A (Vijayaraghavan *et al*., 1997). About 70 to 90% of Vitamin A is retained after food preparation across varying temperatures. For example, the results of a study by Manorama and Rukmini (1991) in India found that cooking losses of β-carotene were between 22 to 30% in most meals prepared with RPO. This stability of vitamin A and carotenoids to high temperature is beneficial for their bioavailability. In 2007, Butt *et al* suggested that the bioavailability of β-carotene and other carotenoids increases several-fold during cooking because heat releases these substances from proteins to which they are bound in foods.

2.4 Vitamin A deficiency and its prevalence

The total body stores of vitamin A range from 300 to 900 mg, 80% being in the liver and 20% in peripheral organs and tissues. In normal individuals, the liver concentration of vitamin A should be 450 mg (Leo and Lieber, 1999). According to the WHO (2011) VAD assumes severe public health significance requiring intervention when $\geq$ 20% of a population exhibits serum retinol concentration $\leq$ 20 μg/dL. A well-nourished child will usually have adequate liver stores of vitamin A to maintain serum retinol levels of 10 to 14

µmol/1itre (WHO, 1995). Therefore, in values below these, normal levels of serum retinol may be difficult to attain or certain roles may be impaired (WHO, 1995).

VAD methodological estimates date back to the 1960s. Using clinical records, anecdotal observations and personal interviews; 20,000 to 100,000 children were projected to be affected worldwide (Underwood, 2004). Subsequent estimates, recorded that 13 million children globally were xerophthalmic (Underwood, 2004). In 2000, the Administrative Committee on Coordination—Subcommittee on Nutrition (ACC/SCN) estimated that 140–250 million preschool age children were at risk of vitamin A deficiency disorders (VADD, clinically deficient and subclinically vulnerable), including 3 million who have clinical signs every year. According to West (2002), 127.2 million preschoolers were vitamin A–deficient worldwide. Of this number, 25% of them live mostly in developing countries. Additionally, 26% of these vitamin A–deficient children are hosted by Africa, with the largest number in Ethiopia (6.7million). In more recent global population-based surveys WHO (2009) puts VAD among preschool children at 190 million. Considering these statistics on the burden of VAD, Africa comes second to South-east Asia. However, VAD is almost non-existent in the developed world.

Maziya-Dixon *et al.* (2006) reported that in neighbouring Nigeria, 33% of children under five years are affected by VAD. In Ghana, the vitamin A supplementation trial in 1993 (VAST, 1993) reported 65 % severe vitamin A deficiency in children in the northern part of the country. In 1997, four years later, the Ministry of Health Vitamin A prevalence survey revealed that 37.2% of children in southern Ghana had low serum levels of vitamin A. Subsequently, a cross-sectional study conducted among school children 2 to 10 years in

Okwenya community of the Eastern Region of Ghana (Egbi, 2012) indicated that 36% of them were vitamin A deficient.

2.5 Causes of VAD

Having underscored the global significance of the VAD menace, it is relevant to identify some of the contributory factors to this preventable condition especially among women and children. Generally, the occurrence of VAD is dependent on age, sex and season (WHO, 1995). Miller *et al.* (2002) noted that children suffer VAD as a result of inadequate secretion of the nutrient in breast milk; low consumption of vitamin A-rich foods, and infection. As have been categorized in other previous sections, the causes of VAD will be viewed bearing in mind the situation in developed countries on the one hand; and developing countries on the other hand.

Mothers in low income countries have low vitamin A concentration in their breast milk because they consume diets low in vitamin A. For example, the National Health and Nutrition Examination Survey (NHANES III, 2000) results revealed that women in high income countries such as the United States meet 75–90% of the recommended Daily Allowance (RDA) whereas their counterparts in low income economies like Bangladesh meet only 31 to 57% of RDA (Zeitlin *et al.*, 1992).

Low consumption of vitamin A-rich foods has been identified as another cause of VAD in childhood. For instance West *et al.* (2002) stated that children in developed economies obtain most of their vitamin A from animal foods, while underprivileged children in developing nations eat most of their vitamin A from affordable plant sources which may have low vitamin A activity. For example, among 1 to 3-year-old children, the median

consumption of vitamin A from animal sources NHANES III (2000) was 404 μg, which exceeded their RDA of 300 μg by as much as 35%. However studies of preschool children in some developing countries; India (Ramakrishnan *et al.*, 1999), Kenya, Mexico, and Egypt (Calloway *et al.*, 1993) revealed that intakes of animal sources of vitamin A were 33, 50, 119, and 174 μg respectively, supplying only 11 to 58% of the RDA. In a similar study in Bangladeshi children, the only source of preformed vitamin A consumed was breast milk with children hardly obtaining vitamin A from animal sources (Zeitlin *et al.*, 1992). These low intakes of preformed vitamin A coupled with the low bioavailability of provitamin A carotenoids help explain why children in developing countries continue to suffer from VAD.

A third major contributor to VAD among children in developing countries is infection. For example diarrhoea and helminthes infestation undermine the integrity, morphology and the performance of the intestinal absorptive mucosa all of which may result in malabsorption of vitamin A (Stephensen, 2001). Several studies have estimated that the prevalence of diarrhoea among children in developing countries vary from 10 to 19% (Molbak *et al.*, 1994; and McLaren and Frigg, 2001). Consequently, the average child in a developing country may suffer diarrhoea about 36 to 70 days annually. According to Black *et al.,* (1982) the prevalence of acute respiratory illness suffered by children in developing countries is staggering, 25 to 60%. Illness aggravates vitamin A status mainly by decreasing the quantity of food consumed due to anorexia and malabsorption and increasing food utilization through higher catabolism and urinary loss. Anorexia is a leading contributor to low dietary consumption during periods of diarrhoea among children, a situation under which dietary intake is best studied (Bentley *et al.*, 1991). Reduction in food intake appears to be more profound among weaned children suffering

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from diarrhoea compared to children still breastfeeding. For instance, diarrhoea resulted in 20% reduction in energy intake among weaned Guatemalan children not receiving breast milk (Martorell *et al.*, 1980).

Beyond reductions in food intake due to diarrhoea, malabsorption of vitamin A may arise during diarrhoeal disease and lower respiratory infection. Sivakumar and Reddy (1972) demonstrated virtually complete absorption of oily retinol by healthy children whereas there was a 30% decrease in the absorption of the same oily retinol among children with respiratory infection and gastroenteritis. In a related study, the absorption of a 100,000 IU of supplemental vitamin A was reduced by 22 and 17% when added to oral rehydration salt solution and water, respectively in diarrhoeal children (Reddy *et al*., 1986). Apart from malabsorption, higher catabolism of vitamin A during infection may as yet be another factor leading to VAD. In a study by Beisel (1972), it was revealed that higher catabolism is a consequence of the acute-phase response to infection, such as fever and metabolic degradation of muscle and adipose tissue. In healthy adults fed an adequate diet free of vitamin A, there was a 0.5% of total body lost of vitamin A per day (Sauberlich *et al*., 1974).

2.6 Consequences of VAD

This section is focused on the adverse effects that VAD has on the overall health and well-being of the human body. VAD negatively impacts many physiological systems in the human body in several ways. In VAD, normal mucus secreting epithelium is replaced by keratinized epithelium resulting in a decline in cellular immunity (Butt *et al*., 2007). There is evidence to suggest a relationship between low serum retinol and birth weight. Gazala *et al*. (2003) found that lower serum retinol concentration in cord and maternal serum at delivery from healthy mothers and healthy mature newborns related positively to

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lower birth weight. VAD also contributes to growth retardation (Mwanri *et al*., 2000). It is well established that VAD results in xerophthalmia, night blindness, damages respiratory, genitourinary and gastrointestinal tracts, impairs growth and bone formation and leads to anaemia as well as increased susceptibility to infections (Sommer, 1989). Prolonged VAD leads to corneal ulceration and eventually partial or total blindness (keratomalacia). VAD appears to influence morbidity patterns especially in developing countries. For example, there is a two-fold and a three-fold risk of ARIs and diarrhoea respectively for children with mild vitamin A deficiency. Such children have a six to nine times higher risk of dying. Furthermore, when intervention is non-existent during VAD (keratomalacia), the mortality rate is as high as 60% (WHO, 1995).

2.7 Vitamin A deficiency control strategies

In view of the undesirable consequences of vitamin A which are largely preventable, the control of its deficiency has been a major subject of scientific exploration for the last two decades. Three strategies: vitamin A supplementation, fortification and dietary diversification are generally recognized as pillars in the combat of VAD (Bloem *et al*., 1998; Filteau and Tomkins, 1999; and Chakarvarty, 2000). Of these three strategies, dietary diversification has been observed to be the most effective and sustainable approach in dealing with VAD. Each of these strategies will be examined in the remaining part of this section of the review.

2.7.1 Vitamin A supplementation

One of the earliest scientific investigations with daily dose vitamin A supplements was conducted among measles patients in London in the 1930s and it demonstrated that supplementation could reduce measles case-fatality by 50% (Sommer, 2008). Some other randomized controlled trials found nearly the same result (Hussey and Klein, 1990; and

Sommer and West, 1995). By the early 1990s, there was global publicity by high level international organizations such as United Nations Children's Fund (UNICEF) and United States Agency for International Development (USAID) of the effectiveness of vitamin A supplementation on overall mortality especially from measles based on ample evidence. This publicity stimulated the institution of national programmes in more than 70 countries in which about 500,000 vitamin A supplements are distributed every year saving 350, 000 deaths among children (Sommer, 2008).

 High dose vitamin A supplementation is recognized as the quickest response to improve the vitamin A status in deficient population among vulnerable groups (children, refugees, women, elderly) (Food and Agriculture Organization, FAO, 1992). High dose vitamin A supplementation programmes adopt three ways of reaching affected populations: Xerophthalmia Treatment, Universal Distribution, and Targeted Distribution to vulnerable children (WHO, 1997). Most countries including Ghana, have adopted WHO/UNICEF/IVACG (1997) recommendations for the operations of their vitamin A supplementation programmes. These recommendations stipulate that in VAD endemic communities, children 6 to 12 months should receive a 100,000 IU dose, while children $\geq$ 12 and mothers should be given 200,000 IU of vitamin A once every 4 to 6 months.

Albeit supplementation programmes have been appraised to be cost-effective and acceptable, they are not sustainable in the long term because they fail to tackle adequate dietary vitamin A intake to meet the body's physiological requirements. Vitamin A supplementation programmes are also coverage dependent and have challenges with compliance (Butt *et al*., 2007). In a recent commentary published at the launch of the World Public Health Nutrition Association Journal, Latham (2010) also challenged with

several reasons the conventional practice of giving children massive doses of synthetic vitamin A.

In contrast to the claim by the Beaton report *et al*.(1993) that vitamin A supplementation had a 23% reduction on overall mortality; some studies suggest adverse effects of vitamin A on morbidity. For example, a randomized placebo-controlled double masked trial in Indonesia among 1407 young children concluded that high dose vitamin A supplements amplified the incidence of Acute Respiratory Illnesses (ARI) by 8%, and Acute Lower Respiratory Illnesses (ALRI) by 39%. The study also found that adverse effects on acute lower respiratory illnesses were more profound among well-nourished children (Dibley *et al.*, 1996). In a Meta-analysis of 9 randomised controlled trials Grotto *et al.* (2003) concluded that high medicinal doses of vitamin A increased the incidence of respiratory tract infections and it is not defensive against diarrhoea among children. Furthermore, there is no reliable data in the entirety of countries earmarked for vitamin A supplementation (UNICEF, 2005 and 2007). High dose vitamin A supplements have also been found to antagonize the action of vitamin D which results in bone fragility (Rohde *et al.*, 1999; and Rohde and DeLuca, 2005). Another difficulty with the vitamin A capsule initiative is the economics associated with it. Estimates from Ghana, Nepal and Zambia showed that the annual cost for an average national vitamin A supplementation programme is a colossal $US 3 million (UNICEF, 2007), which would be a huge burden for many developing countries, as asserted by Latham (2010). In 2009, a conclusion was drawn by the Micronutrient Initiative to the effect that: 'Supplementation remains largely a push-driven rather than a demand-driven intervention'.

In the light of the above concerns, many scientists (Latham, 2010; and Gopalan *et al*., 2010) have called for a paradigm shift which emphasizes food-based strategies in the solution to the VAD challenge. These critics of the vitamin A capsule programme however, concede that supplementation should only be used in VAD endemic areas.

2.7.2 Food fortification

Food fortification is the process of adding a desirable/essential nutrient to a commonly consumed food product in order to enhance the content of the nutrient in the food for the purposes of avoiding and/or controlling a deficiency in a population (FAO/WHO, 1994). This method has been part of the success story in the elimination of vitamin A deficiency in developed countries (Osei *et al*., 2010). There have been several studies to suggest that this method is a successful way of improving vitamin A status. Lotfi *et al.* (1996) showed that several foods have been vitamin A fortified to help in the elimination of VAD. Among these foods include; rice, wheat, sugar, tea, monosodium glutamate, fats and oils, and biscuits. In view of this, several countries are fortifying common food products with vitamin A (UNICEF, 1996).

Fortified vegetable oil used in the preparation of a rice-based diet has been successful in significantly improving the concentration of plasma retinol and liver stores of vitamin A (Favaro, 1992). In a similar instance, consumption of 40 g of bread prepared from wheat flour fortified with 4.5 g/kg vitamin A has also been found to significantly result in higher liver stores of vitamin A among school children in the Philippines (Solon, 1997). Vitamin A fortified monosodium glutamate has also been proven efficacious in the Philippines (Solon *et al*., 1985) and Indonesia (Muhilal *et al*., 1988). Van Stuijvenberg *et al.* (1999) studied 6 to 11-year-old rural children in KwaZulu-Natal, South Africa consuming biscuits

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fortified with synthetic β-carotene and indicated that 50% of their RDA for retinol was provided.

For food fortification to have the desired impact on vitamin A status it is important for fortification to be mandatory and centrally processed (Dary and Mora, 2002). This may be the limiting factor in the developing world. In Ghana, it is the fortification of salt with iodide that has been mandatory and even then there may be challenges with the enforcement of the law (Ghana News Agency, GNA, 2009). The high cost and slow progressive nature of fortified food products may also be another key challenge which limits food fortification as a strategy in curtailing VAD among poor populations (Shiundu *et al*., 2007).

2.7.3 Dietary diversification

Dietary diversification is the consumption of food from the various food groups in order to meet nutrient needs. In the case of vitamin A, this implies consuming animal source foods such as milk, dairy products, liver, eggs, poultry, fish and a variety of plant based foods which contain an abundance of carotenoids, β carotene (green leafy vegetables, carrots, mangoes, sweet potatoes, red palm oil). Although animal sources are richer in retinol compared to plants, they are often expensive and unaffordable in developing countries. On the contrary, plant source foods are locally available and as such are more affordable, acceptable and culturally appropriate and therefore easy to be incorporated in the diet of poor households (Latham, 2010; and Codjia, 2001). There are some studies that indicate that plant based diets are cost-effective in meeting the vitamin A and other nutrients of populations where attention is paid to their consumption.

In a three-year project, it was shown that frequent consumption of vegetables among under five year-olds resulted in an annual per capita cost of US $0.13 (Greiner and Mitra, 1999). Ncube *et al.* (2001) also indicated in a randomised trial that consumption of tropical fruits improved the serum retinol of women as effectively as dosing with β-carotene. Studies suggest that if 35 to 50% of the RDA of vitamin A is met by the consumption of RPO, it will be sufficient to prevent VAD (Benadé, 2003). In a pre-post test study design, RPO used in the preparation of school lunch meals was found to significantly improve the serum retinol concentration of school children in Burkina Faso (Zeba *et al.*, 2006).

A cross examination of the literature reviewed so far reveals that dietary diversification is the most appropriate strategy for combating VAD in the developing world including Ghana.

2.8 Role of palm oil in nutrition and health

Red Palm Oil (RPO) is a product of the oil palm fruit (*Elaesis guineensis)* that has been part of civilization for over 5000 years. Palm oil originated from Africa but in contemporary times it is widely consumed in other continents such as South-east Asia, and South America. In 2007, it was estimated that the world production of RPO had reach 40 million metric tons of which Malaysia alone accounted for nearly 50% (Sarmidi *et al.*, 2009). Although, RPO originated from Africa; Asia has become the leading producer of RPO in the world. For instance, Malaysia and Indonesia alone account for 90% of palm oil export trade in the world (Rupilius and Ahmad, 2007). In Ghana, palm oil is the preferred cooking oil among rural households except in the three northern regions where shea butter is the oil of choice due to the inadequate availability and accessibility of red palm oil in

that part of the country (GSS, 2008). As a result, red palm oil is much more expensive in those regions due to the additional transportation and other handling costs.

RPO has many components such as carotene (the dominant one being β-carotene), vitamin E, sterols, squalines, enzymes and phenolic compounds that have several nutritional and other applications in the food industry (Choo *et al.*, 1996; Lau *et al.*, 2008). The quantity of carotenes contained in RPO is nearly 15 times higher than that contained in an equal weight of carrots, and 44 times that found in leafy vegetables (Scrimshaw, 2000). This makes RPO the richest known source of provitamin A (α and β carotenes). RPO can therefore be appropriately harnessed to fight VAD which is wide spread throughout the developing world (Scrimshaw, 2000). According to the Malayasian Palm Oil Council (2006), the RDA (350 to 400 RE) for vitamin A can be provided for children by one teaspoon (6 grams) whereas two teaspoon (12 grams) will meet the adult RDA (800 RE)). Several interventions have shown that RPO improves serum concentrations of vitamin A. In a two month snack trial among school children 7 to 9 years, it was revealed that serum retinol was significantly improved. Further assessment of vitamin A using the Modified Relative Dose Response Test also showed a saturation of liver stores of retinol (Jozwiak and Jasnowska, 1985). Van Stuijvenberg *et al.* (2001) investigated the effect of β-carotene among 5 to 11-year-old children in KwaZulu-Natal, South Africa and it was shown that biscuits fortified with RPO met 30% of the RDA for the children. Apart from its vitamin A nutritive value, RPO also has other benefits for the human body especially in cardiovascular health. The antioxidants contained in RPO have cancer and hypercholesterolemic protective effects (Pearce *et al.*, 1992; Tomeo *et al.*, 1995; Slattery *et al.*, 2000). In a recent study, Ong and Goh (2002) indicated that palm oil increases high-density lipoprotein (HDL) cholesterol whereas it decreases blood cholesterol, low-density

lipoprotein (LDL) cholesterol, and triglycerides; the overall effect being improved cardiovascular health. It is also known that Co-enzyme Q, a component of palm oil is defensive against artherosclerosis and heart disease (Kontush *et al*., 1995).

Despite the numerous health benefits of red palm oil, some concerns have been raised suggesting that red palm oil could be a risk factor in the development of cardiovascular diseases (CVDs) because of its saturated fatty acid (oleic, palmitic, linoleic acids among others) content which have been reported to be hypercholesterolemic (Mensink and Katan 1990; and Lichtenstein *et al.*, 1999). In a recent study among 15 adults in Boston in the United States of America, Vega-Lo´pez *et al.* (2006) found that intake of palm oil enriched diets resulted in high plasma concentrations of LDL cholesterol and apolipoprotein which are a risk factor for CVDs.

In spite of this limitation, red palm oil like all fats also provides energy (Fasina *et al*., 2006) in addition to its other health benefits discussed earlier and as such red palm oil is a useful energy source and rich in pro-vitamin A, and relatively cheap; hence an excellent food resource which must be included in the diet of growing children, especially of developing countries. Accordingly, the advantages of red palm oil outweigh its disadvantages especially when consumed in moderation.

2.9 Role of cowpea in nutrition and health

Cowpea (*Vigna unguiculata L. Walp*) is one of the most extensively consumed legumes in the developing world (Phillips *et al*., 2003). In the Indian subcontinent and Africa, it constitutes a major part of the diet of vegetarians (Salunkhe, 1982). Cowpea is a common household food item in Ghana, Nigeria and other West African countries. Cowpeas are a

drought and heat resistant crop that requires as little as 300 mm^3 of rain to mature (Apata and Ologhobo, 1997). For cowpeas ability to tolerate drought conditions, they can be grown at least once in the two major climatic seasons (rainy and dry season) across the vegetation zones of Ghana. Some cowpea varieties found in Ghana are Bengpla, Ayiyi, Asontem and Soronko (Osei and Sefa-Dedeh, 1993). Chemical analysis done on cowpea varieties showed the following composition: protein 20.7-25.4%; fat 1.38-1.99%; crude fibre 1.06-3.98%; ash 2.82-3.55% (Osei and Sefa Dedeh, 1993; Preet and Punia, 2000). Tshovhote *et al.* (2003) reported a protein concentration of 253.5 to 264.3 g/kg in some varieties of cowpea. The amino acid profile of cowpeas shows they have low sulphur content but high in lysine (Coertze and Venter, 1996). Although, cowpea is high in lysine, the digestibility of the lysine in cowpea is low compared to that of methionine (Tshovhote *et al.*, 2003). Overall, the nutrient content of cowpea is similar to soybeans and canola. Comparable to other legumes, cowpea contains non-digestible and anti-nutrient factors (Igbasan and Guenter, 1997) such as protease inhibitors, non-starch polysaccharides (NSP), pectins and phenolic compounds (Arora, 1995). These substances undermine the protein quality and nutrient digestibility of cowpea. Protease inhibitors particularly hinder the functionality of pancreatic enzymes like chymotrypsin and trypsin.

In Ghana cowpea is processed into many traditional foods. Many households in Ghana consume at least one form of cowpea based-food. In the southern part of Ghana available cowpea based foods include; apapransa, borboe, akara, koose, red-red, cowpea stew, cowpea vegetable soup and waakye. In the northern part of the country can be found indigenous traditional cowpea based foods like tubani, nyombeka, gablee and koose. Cowpea flour is also used as condiment in preparing soups and stews in northern Ghana. Most often cowpea is eaten in combination with other food groups (cereals, roots, tubers

and vegetables). Cowpea based diets such as waakye, koose and red-red are served by restaurants and hotels in urban areas. Despite their wide range of use, cowpea requires a long time to soak or cook. The long processing duration therefore makes the use of cowpea in the diet too expensive (Tshovhote *et al.*, 2003). In spite of some of the challenges associated with cowpea mentioned already, the current study still employed cowpea because of its availability, affordability, and cultural acceptability in the study setting. Another reason for the choice of cowpea is its cholesterol-lowering effects in humans (Frota *et al*., 2008). This ability of cowpea may offset any hypercholesterolimic effects that may be posed by the red palm oil in the intervention diet.

2.10 Anthropometric methods/measurements

Anthropometry is the measurement of the human body parts. This can be a relatively rapid way of describing the growth and nutritional status of a person or population (Gorstein *et al*., 1994). This method is particularly used to determine protein energy malnutrition in children, and overweight and obesity in all age categories. The measurements commonly taken are weight and height. Anthropometry is quite objective and the technology for its application is simple (Duggan, 2010). The anthropometric indicators commonly applicable to child growth monitoring and development from 5 to 19 years are weight-for-age, length/height-for-age, and BMI-for-age (WHO, 2009).

2.10.1 Weight-for-age

Weight-for-age measures underweight: weight for age < -2 standard deviations (SD) of the WHO Child Growth Standards median. Low weight for age (underweight) can be an indication for 'wasting' (low weight-for-height), representing acute weight loss, 'stunting',

or both. This makes weight-for-age a complex indicator and therefore presents interpretative challenges (WHO, 2009).

2.10.2 Length/height-for-age

This indicator measures stunting: length/height-for-age < -2 SD of the WHO Child Growth Standards median. Height-for-age reflects cumulative linear growth and it is usually influenced by long-term food shortages, chronic and frequent recurring illnesses, inadequate feeding practices, and poverty (WHO, 2009).

2.10.3 BMI-for-age

This replaces weight-for-age for children ten years and above. BMI-for-age is interpreted within -3 SD, -2 SD, -1 SD, +1 SD, +2 SD and +3 SD. The BMI values for children 5 to 19 years are interpreted as those of adults in the WHO reference (overweight occurs at +1 SD (BMI =25 kg/m^2); the cut-off for obesity is +2 SD (BMI=30 (kg/m^2), whereas morbid obesity occurs at the +3 SD (BMI of above 35 kg/m^2). Thinness and severe thinness occur at the -2 and -3 SD cut-offs respectively (WHO, 2009).

2.10.4 Biochemical methods

Preformed vitamin A, retinol is the most abundant form of vitamin A in the blood. The vitamin is usually stored in the liver. When blood levels of vitamin A fall, liver stores of the vitamin are used to boost the circulating levels in order to meet the requirements of body tissues. The transport of retinol to body tissues is facilitated by its carrier protein, retinol-binding protein (Gibson, 2005). Retinol-binding protein forms a complex with transthyretin in the blood. Transthyretin is responsive to certain receptors on target cells thereby regulating critical roles in the body. Retinol levels in serum do not adequately correlate with liver stores of the vitamin unless a person's liver stores are exhausted (<

0.07 μmol/g liver) or too high (> 1.05 μmol/g liver) (Gibson, 2005). Within this range, retinol levels in blood are regulated through homeostasis and do not always reflect vitamin A intake or clinical signs of deficiency. As a result, serum retinol is not a good indicator for evaluating the vitamin A status of individuals and may not be responsive to intervention programmes. However, serum retinol levels and inflammation/infection can provide population specific data on vitamin A status and serve as the basis for public health intervention (WHO, 1996). In many instances, serum retinol values are measured in young children, and vulnerable groups (WHO, 1996).

There are several ways of assessing serum retinol. Serum retinol can be measured by High-pressure Liquid Chromatography (HPLC), by fluorescence, or by ultraviolet (UV) spectrophotometry. In spite of its higher cost, HPLC is the appropriate and preferred method for serum retinol determination because of its relatively high sensitivity and specificity (WHO, 1996). Serum retinol can be determined in a venous or free-flowing capillary blood sample. Samples to be analysed should not be exposed to light and must be kept chilled prior to centrifugation. Analysis on samples should be carried out within a 12 hour period of sample collection (WHO, 1996). Serum retinol may also be determined using dried blood spots (Gibson, 2005).

2.10.5 Dietary methods

There are different methods for collecting dietary intakes and the choice is influenced by time, cost and the purpose of the data. In addition to the mentioned factors, each method has its own pros and cons and all these together dictate the method. In this study, time and cost were the greatest influence in the selection of the 24-hour recall and food frequency questionnaire. The food frequency questionnaire was used to buttress the 24 hour recall.

2.10.5.1 24-hour recall

In the 24-hour recall method of dietary assessment, the participant answers a series of questions aimed at eliciting information on all the drinks and foods as well as their portion sizes that have been consumed the previous day. This method can be very flexible in that the tool can be self-administered, through face-to-face interview or via telephone (Buzzard *et al*., 1996; and Casey *et al*., 1999). Probing is very essential to the success of a 24-hour recall exercise and as such it requires interviewers to be properly trained in the administration of the tools. To ensure maximum success, dieticians, nutritionists or interviewers should have adequate knowledge on the food distribution as well as some of the food processing techniques within the study area. The 24-hour recall presents several advantages to the researcher. It does not require literacy on the part of participants since all the questions are asked in a language they understand and the recording of information is done by the interviewer. There is less burden placed on participants and recall bias is minimized since a 24-hour period is relatively short. It is also easier to get people to agree to participate in a study using a 24-hour recall than recruiting people to participate in a study employing food record (Thompson and Subar, 2001).

2.10.5.2 Food frequency questionnaire (FFQ)

This method requires study participants to provide information on foods they commonly consume from a list of usually consumed foods in their locality (Willett, 1998; and Zulkifli and Yu, 1992). Some FFQs also include questions on portion sizes for the purpose of approximating relative or absolute nutrient consumption. However, there is some disagreement as to whether or not the estimation of portion sizes should be included in the food frequency questionnaire. Whereas some advocate the inclusion of portion sizes in FFQs, others prefer the use of FFQs without the inclusion of portion sizes (Willett, 1998).

In a typical FFQ, the total nutrient consumption is obtained by the summation of all foods, the product of the frequency of each food item by the quantity of the nutrient in a particular serving of the food to yield an average nutrient intake, dietary components and food groups per day. Depending on the list of items included in a FFQ, the administration may be between 30 to 60 minutes. In such instances, participant burden should be considered. In spite of this concern some studies have revealed that participant burden is not critical in the use of FFQ (Subar *et al*., 2001; Eaker *et al*., 1998; Morris *et al*., 1998; and Johansson *et al*., 1997).

CHAPTER THREE

3.0 METHODOLOGY

3.1 Study design and setting

The study was a 6 month one-group longitudinal pre - and - post - test design. The study was in 3 parts:

Part 1 was a survey to collect information on socio-demographics and vitamin A-rich foods in the community.

Part 2 was an assessment of the chemical composition of the intervention diet.

Part 3 was the intervention where data were collected at pre, and post intervention.

The study was conducted in Kodzobi in the Adaklu-Anyigbe district in the Volta Region. This district is one of the newly created districts that shares boundaries with the Ho Municipal Assembly, and the north and south Tong districts. The vegetation is mainly savanna with patches of forest. The inhabitants are mainly subsistence farmers engaged in cowpea, maize, groundnut and cassava cultivation.

3.2 Study participants, sample size and sampling

The study participants were school children (6 to 12 years) at Kodzobi Basic School (Primary-JHS). A sampling frame was constructed by pooling together the class registers from class 1 to Junior High School 3 (JHS). All children 6-12 years in the sampling frame qualified for the study. A convenient sample of 142 children were selected after using simple random sampling where every third child was selected to participate in the study. The selection took place among children whose parents consented and the children also agreed to participate in the study. Prior to and during the study, all children who reported to be allergic to beans, palm oil or gari as well as those who were found to be using

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vitamin and mineral supplements or any dietary supplements were excluded from the study.

3.3 Data collection

3.3.1 Background data

A pretested semi-structured questionnaire made up of open and close ended questions was employed in the data collection. The instrument was in five parts (Appendix I): Part A elicited socio-demographic data on the participants, Part B retrieved information on participants' health/nutrition; Part C was used to assess the water and sanitation situation of participants; Part D assessed the cultural practices pertaining in the community; and Part E, a food frequency questionnaire which collected information on the frequency of food consumption especially vitamin A rich foods among the participants.

3.3.2 Proximate analysis of intervention diet

The nutritional composition of the intervention diet (gari and beans stew) was also analysed. Moisture content, energy, carbohydrate, crude protein, crude fat, and crude fibre were estimated by employing the standard method of analysis (AOAC, 1995).

3.3.3 Determination of total carotenoids content

Carotenoids were analyzed as described by Rundhaug *et al*. (1988) with slight modifications. Briefly, triplicates (0.7825g, 0.6185g and 0.6346g) of the diet was each transferred into a mortar and a small amount of anhydrous sodium sulphate added as a desiccant to allow for effective grinding and extraction with 50ml cold acetone. The mixture was filtered and the residue re-extracted under the same conditions until the extraction solvents became colourless. The filtrate was then partitioned on 20ml petroleum ether in a 500ml separating funnel. About 1L of distilled water was used to wash away the acetone by draining away the lower aqueous layer. The upper petroleum ether layer was

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dried by passing it through anhydrous sodium sulphate sited on a cotton wool at the base of a funnel and the extract collected into a labeled centrifuging tube. The extract volume was recorded and then stored at -14°C to prevent oxidative damage until further analysis. Absorbance of the petroleum ether extract of the diet were measured at 450nm using a Shimadzu UV-120-02 spectrophotometer and total carotenoids content (expressed as β-carotene equivalents) estimated using the equation below;

$$\text{Total carotenoids content (µg/g)} = \frac{\text{Absorbance} \times \text{Total volume of extract (ml)} \times 10^4}{\text{Sample weight (g)} \times \text{Absorbance coefficient of β-carotene (2592)}}$$

$$\text{Total carotenoids content (µg/100g)} = \text{Total carotenoids content (µg/g)} \times 100$$

3.3.4 Determination of β-carotene content

A standard β-carotene solution was prepared and the absorbance read on a Shimadzu UV-120-02 spectrophotometer. Then, 20µl was injected into an HPLC and the areas were obtained. The concentration of the standards was calculated using the formula;

$$\text{Conc. of β-carotene standard (ng/µl)} = \frac{\text{Absorbance} \times 10000}{\text{Coefficient of extinction of standard}}$$

The mobile phase was n-hexane/benzene (6:1) at a flow rate of 0.8 ml/min and UV detection at 450 nm. Petroleum ether extract (400µl) of each triplicate stored was measured with a pipette into an eppendorf tube and evaporated to dryness under a steam of nitrogen gas. It was then reconstituted with a known volume (200µl) of the mobile phase and vortexed for thirty seconds. Subsequently, 20µl was injected into the HPLC and their corresponding areas obtained. Using the areas obtained, the concentration of β-carotene content of the sample was calculated using the following formulae:

$$\text{Conc. of β-carotene (ng/µl) in each extract} = \frac{\text{Area of each extract} \times \text{Conc. of standard (ng/20µl)}}{\text{Area of standard}}$$

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Conc. of β-carotene (ng/ml) in each extract = $\dfrac{\text{Conc. β-carotene (ng/μl) in extract} \times \text{Recons. Vol}}{\text{Volume of extract injected into HPLC}}$

Conc. of β-carotene (ng/ml) in each extract = Conc. of β-carotene (ng/ml) in extract × Total Vol.

Conc. of β-carotene (ng/g) = $\dfrac{\text{Conc. of β-carotene (ng/ml) in each extract}}{\text{Sample weight (g)}}$

Conc. of β-carotene (μg/g) = Conc. of β-carotene (ng/g) × 0.001

Conc. of β-carotene (μg/100g) = Conc. of β-carotene (ng/g) × 100

3.3.5 Anthropometric measures

3.3.5.1 Body weight

The weights of the participants were measured using the electronic bathroom scale (Precision Health Scale UC-300). The weight measurement was taken in accordance with WHO (2006) protocol for anthropometry. The weight of each participant was taken three times and recorded to the nearest 0.1kg. The mean of the three readings was then taken as the actual weight of the participants. To ensure accuracy, weight measurements were done an hour prior to lunch with the participants wearing light clothing; the scale was also standardized using an object with a known weight.

3.3.5.2 Height

The height of the participants was taken with a stadiometer. Participants stood straight with the head in a position such that the Frankfurt plane was horizontal, feet together, knees straight and heels, buttocks and shoulder blades in contact with the vertical surface of the stadiometer. Furthermore, the arms were hanged loosely by the sides with the palms facing the thighs (WHO, 2006). Height measurements were also taken in triplicates and to

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the nearest 0.1cm. The mean of the three readings was considered as the actual height of the participant.

3.3.6 Dietary assessment

3.3.6.1 Food frequency questionnaire (FFQ)

The FFQ was used to assess the frequency of food consumption of participants with emphasis on cowpea-based foods and carotenoid-rich foods. Handy measures were used to help determine the quantities of cowpea-based foods and carotenoid-rich foods consumed. The information generated by the FFQ facilitated the determination of the frequency of consumption of the particular food and its implications on the vitamin A and nutritional status of the children.

3.3.6.2 The 24-hour diet recall

This was done for two week days and one weekend to help determine the 24-hour dietary intake of the participants. The 24-hour dietary recall consisted of a listing of foods and beverages consumed the previous day or the 24 hours prior to the recall interview. Foods and amounts were recalled from memory with the aid of interviewers who were trained in methods for soliciting dietary information. The interview was conducted face-to-face and whenever it was appropriate interviewers enquired from participants their activity history to facilitate probing for foods and beverages consumed.

3.3.7 Biochemical data

The present study concentrated on serum retinol to determine the vitamin A status of the participants. Blood (5 ml) was obtained from each participant by venipuncture by a well-trained phlebotomist. The blood samples were then allowed to clot before centrifugation at 2500rpm for ten minutes to obtain serum. The serum was labeled legibly and stored at -80 ^{o}C and analysed within two weeks. For each subject, duplicate aliquots of serum were

analysed. Frozen serum samples were put into racks and covered with aluminum foil to prevent contact with light as retinol is very sensitive to light. Serum retinol was determined with the High Performance Liquid Chromatographic (HPLC) method (Noguchi Memorial Institute for Medical Research, NMIMR, 1997).

3.4 Baseline study

During the baseline study, the anthropometric measurements were conducted to assess the nutritional status of the children (6-12 years). Their dietary vitamin A intake from cowpea-based foods and red palm oil (RPO) and other vitamin A rich foods were also assessed using a food frequency questionnaire. The vitamin A status of the children was assessed by determining their serum retinol concentration. Blood samples were collected by a phlebotomist under standard laboratory protocols. The participants were screened for some infections using self-reporting and laboratory techniques. The giemasa staining technique was used to screen the participants for malaria whereas the kato-catz technique was employed in screening participants for helminthes (hookworm) infestation. The participants who were found to have malaria were encouraged to go to the hospital for treatment whereas all the participants were dewormed with 400 mg of albendazole prior to the intervention.

3.5 Intervention study

During the intervention study, the participants (6-12 years) were fed 300g (246.5g beans, 40g gari and 13.5g RPO) gari and beans stew prepared with RPO for lunch thrice a week for a period of six months. The rate of infection (malaria) during the intervention was also recorded in order to help to determine the frequency of infection among the participants. This was done in order to quantify the possible effect of vitamin A on infection among the participants. The diet was prepared under hygienic conditions to prevent contamination

and loss of nutrients. The participants were provided with feeding cups and spoons. They were also served clean drinking water during the meals. To prevent the participants from sharing their meals with their classmates, the feeding was closely supervised. The participants were apparently healthy and resident in the community throughout the study period.

The research assistants were adequately trained to ensure appropriate data collection. All the tools for the data were pre-tested to ensure that the necessary corrections were made to aid in the collection of the appropriate data. To ensure efficiency and accuracy during the data collection, research assistants were trained and provided with a comprehensive protocol on the data collection procedure.

3.6 Data capture and analysis

The data were entered, cleaned and analysed using the Statistical Package for Social Sciences for Windows (version 16.0, SPSS Incorporated, Chicago, USA). Frequencies were used to describe background characteristics of participants that were categorical in nature. Nutrient contents of portion sizes were calculated using the Food Processor PLUS Nutrition and Fitness Software with Cooking and Preparation Yields (version 6.02, ESHA Research, USA). For dietary data obtained from food frequency, the frequency of consumption of the various food groups was calculated for each participant. Percent RDA met for each nutrient for both pre and post-test was reported as nutrient adequacy ratios.

Weight-for-age (W/A), Height-for-age and BMI-for-age z-Scores were calculated for each participant. Means and standard deviations were calculated for continuous variables such as age, weight, height, and serum retinol that were reasonably normally distributed.

Differences in means of intervention variables such as retinol concentration, W/A, H/A and vitamin A status before and after the intervention period were determined using the paired t-test.

Vitamin A status was categorised as a binary variable (deficient or normal) using serum retinol as indicator. Chi-squared test was used to examine the association between serum retinol and some categorical variables. Logistic regression models were used to explore the relationship between the main outcome variable (serum retinol) and background characteristics of study participants. Treating the main outcome of this study as a continuous variable, univariate and multivariate linear regression models were used to explore the relationship between serum retinol and socio-demographic characteristics of participants. All variables that were significantly associated with serum retinol from the univariate linear regression were adjusted for in the multivariate linear regression.

3.6.1 Anthropometric data

Anthropometric indices; weight-for-age and height-for-age, weight-for-height and BMI-for-age were computed using the WHO anthroplus software, the latest growth standard proposed by the World Health Organization (WHO, 2007). The means and standard deviations of the z-scores for weight-for-age, height-for-age and BMI-for-age were determined.

3.6.2 Food consumption data (nutrient intakes)

The actual amount of foods consume by the children was estimated using representative food samples. The total amounts of the various nutrients: Protein, fat, carotene, and energy was calculated based on 100 grams portion of food with the help of Food Processor Plus Software (FPRO), a nutrient database and the Food Research Institute's food composition table.

The total amount of protein, fat, carotenoids, carbohydrate, and energy calculated was compared to the Recommended Dietary Allowances (RDAs). Nutrient Adequacy Ratio (NAR) was then calculated based on the information above as follows:

$$\text{NAR (Nutrient Adequacy Ratio)} = \frac{\text{Actual nutrient in the diet}}{\text{Recommended Dietary Allowance for nutrient}}$$

3.7 Ethical consideration

Ethical approval was sought from the Institutional Review Board (IRB) of Noguchi Memorial Institute for Medical Research, Accra, Ghana, before the commencement of the study. The reference for the ethical approval is: Federalwide Assurance FWA 00001824; IRB 0001276; NMIMR-IRB CPN 016/09-10 and IORG 0000908).

Permission to conduct the study was obtained from the parents/caregivers and headmaster and teachers in the school. Informed consent (written and/or oral) was sought from selected participants who could express themselves whereas parental assent was sought for those participants who could not consent. Participation in the study was absolutely voluntary. The purpose of the study as well as the assurance of confidentiality was explained to the participants.

3.8 Dissemination of findings of the study

The findings of the study were presented at the postgraduate seminar to the Department of Nutrition and Food Science, University of Ghana, Accra. The findings of the study will also be made available to stakeholders throughout the world through journal publications and conferences. The findings were communicated to parents/caregivers and the authorities of the study school through a community durbar.

CHAPTER FOUR

4.0 RESULTS

This chapter describes the outcome of the study under the following headings: Socio-demographic characteristics, availability and accessibility of vitamin A/carotenoid rich foods in the study community, carotenoid content of the intervention diet; serum retinol level of the participants and nutritional status of the participants.

4.1 Socio-demographic characteristics of participants

The participants were school children aged 6-12 years; comprised of 54.2% males and 45.8% females (Table 1). In general the children were from intact homes as demonstrated by 85.9% of their mothers who revealed that they were married. The fathers of these children had higher educational attainments as compared to their mothers. Farming was the predominant occupation for both mothers and fathers (Table 1). Although formal employment in the households of these participants was relatively low, the majority (67.6%) had national health insurance (Table 1).

Table 1: Socio-demographic characteristics of participants (N=142)

Variable	No. of participants N	Percentage (%)
Sex		
Male	77	54.2
Female	65	45.8
Age		
6-9 years	102	71.8
10-12 years	40	28.2
Marital Status (Mother)		
Married	122	85.9
Single	8	5.6
Widowed	8	5.6
Separated	4	2.8
Educational Level (Mother)		
No formal education	14	10.0
Primary/adult education	55	39.3
JSS/Middle school	65	46.4
SSS/Technical	6	4.3
Educational Level (Father)		
No formal education	5	3.7
Primary/adult education	17	12.5
JSS/Middle	103	75.7
SSS/Technical	11	8.1

Table 1 Continued

Variable	No. of participants n	Percentage (%)
Occupation (Mother)		
Farming	117	84.2
Artisan	6	4.3
Salaried worker in government/company	2	1.4
Trading	9	6.5
Labourer	5	3.6
Occupation (Father)		
Farming	76	55.9
Artisan	21	15.4
Salaried worker in government/company	24	17.6
Labourer	15	11.0
Mother's monthly income		
< minimum wage (GH¢121)	142	100
≥ Minimum wage (GH¢121)	0	0
Father's monthly income		
< Minimum wage (GH¢121)	108	76.1
≥ Minimum wage (GH¢121)	29	20.4
Household Monthly Income		
< Minimum wage (GH¢121)	76	55.5
≥ Minimum wage (GH¢121)	61	44.5
Household National Health Insurance		
Registered	96	67.6
Unregistered	46	32.4

The total sample size (N=142) but it changes for some variables because of missing values.

4.2 Availability and accessibility of vitamin A/carotenoid rich foods in the community

The first part of the study was a survey on the availability and accessibility of vitamin A-rich foods in the community. The survey revealed seasonal availability and accessibility of vitamin A-rich foods in the community. The study showed that there were no mangoes and pawpaw fruits in the community at the time of the survey. There were only a few mango and pawpaw trees scattered in the community while palm fruits were either bought or gathered from the wild. There was also a scarcity of red palm oil in the community. There were however, a number of green leafy vegetables present in the community which were mostly seasonal. Hence, green leafy vegetables were available during the raining season

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while they were non-existent in the dry season. There were generally no food taboos in the community which forbade children from eating vitamin A-rich foods.

4.3 Carotenoid content of the intervention diet

This was an intervention study using cooked cowpea, gari and palm oil to explore the effect of the diet on the vitamin A and nutritional statuses of beneficiaries. HPLC analysis of the intervention diet showed that it contained high β-carotene content. Consequently, the adequacy of the vitamin A content of the diet in retinol equivalents was also 100% (Table 2).

Table 2: Proximate composition, caratenoid and vitamin A content of the intervention diet

Nutrient	Nutrient composition/300g	1/3 DRI (g/day)	NAR* (% DRI)
Moisture (g)	201.60	-	
Energy (kcal)	436.20	ND**	
Carbohydrate (g)	15.33	43.33[a]	35.38
Protein (g)	63.36	6.33-11.33[b]	100
Fat (g)	13.50	ND	-
Total carotenoids (µg)	7892.25	-	-
β-carotene (µg)	1935.00	-	-
Vitamin A (µg RE)	823.56	150-200[c]	100

*NAR= nutrient adequacy ratio
**ND = Not determine
[a,b] based on Subcommittee on the Tenth Edition of the Recommended Dietary Allowances, Food and Nutrition Board (1989).
[c] is the range of recommended safe intakes for age groups similar to those of the participants (6-12 years) (FAO/WHO, 2001).
DRI =Dietary reference intake

The macronutrient content of the diet was also assessed in relation to WHO/FAO recommended intakes. The macronutrient content of the diet showed that the percent

contribution of its protein content was higher than the recommended intake whereas its carbohydrate content was below the recommended intake. However, the percent contribution of fat to total energy was within recommended intake (Table 3).

Table 3: Energy contribution of macronutrients of the intervention diet relative to recommended intakes

Nutrient	% Energy	*Recommended intakes (%)
Carbohydrates	14.06	55-75
Protein	58.09	10-15
Fat	27.85	15-30

*Based on WHO/FAO recommended nutrient intakes (2002).

4.4 Nutrient intake of participants

The usual dietary intakes as revealed by 24-hour diet recall showed that the intake of all nutrients assessed were significantly different for the pre and post interventions. Total energy consumed in the pre intervention was significantly lower than the total energy consumed in post intervention, $p < 0.001$ (Table 4). The mean intake of provitamin A was significantly higher in the post intervention period compared to pre intervention $p = 0.029$ (Table 4).

Table 4: Nutrient intakes among participants pre-intervention and intervention relative to recommended intakes

Nutrient	Pre-intervention			Post-intervention		P-value
	RDI/day	(Mean ± SD)	NAR (% RDI met)	(Mean ± SD)	NAR (%RDI met)	
Total Energy (kcal)	1800-2500[a]	1860.10± 224.02	100.00	2130.10± 294.89	100.00	<0.001
% Energy from macronutrients[b]						
Carbohydrates (%)	55-75	76.64± 4.83	-	76.20± 6.60	-	0.447
Protein (%)	10-15	6.94±1.97	-	8.68±2.39	-	<0.001
Fat (%)	15-30	16.45± 4.06	-	15.11±5.11	-	0.001
Macronutrient (g)[c]						
Carbohydrate (g)	130	357.76±56.50	100.00	411.36±58.99	100.00	<0.001
Protein (g)	19-34	31.86±8.43	93.71-100.00	45.64±9.20	100.00	<0.001
Fat (g)	ND	33.52±7.20	-	35.04±7.44	-	<0.001
Micronutrient (g)[d]						
β-carotene (μg)	-	46.05 (45.75, 16999.20)	-	55.26 (54.90, 21154.56)	-	<0.029
Vitamin A from β-carotene (μg RE)	450-600	3.84 (0.0, 1392.14)	23.44-31.25	4.61 (0.0, 1681.79)	31.71-42.28	0.029

Statistical significance was taken at p = 0.05

[a, and c] is the range of recommended dietary allowance for age groups similar to those of the participants (6-12 years) (Food and Nutrition Board, 1989).

[b]Based on WHO/FAO recommended nutrient intakes (2002).

[d] Based on FAO/WHO recommended nutrient intakes (2001).

4.5 Determinants of Vitamin A status among participants

The vitamin A status of study participants was categorised as a binary variable, thus deficient Vitamin A status or normal. Relationships between socio-demographic characteristics of study participants and the outcome variable (vitamin A status) were explored using univariate logistic regression models. National health insurance was identified as one of the variable that was significantly associated with Vitamin A deficiency. The likelihood of vitamin A deficiency among children without health insurance was about three folds compared to those with valid national health insurance, Odds ratio = 2.67, [95% CI=(1.19, 5.99), P=0.02]. The results also show that children in households with income below the minimum monthly wage were 73% more likely to be

vitamin A deficient compared to children in households with income above the minimum

wage (Table 5).

Table 5: Predictors of Vitamin A status among the participants

Variables	Normal n (%)	Deficient n (%)	Odds ratio	95% CI	P-value
Gender					
Female	15 (23.1)	50 (76.9)	1		
Male	17 (22.1)	60 (77.9)	1.06	(0.48, 2.33)	0.89
Age (years)					
6-9	16 (21.9)	57 (78.1)	1		
10-12	16 (23.2)	53 (76.8)	1.08	(0.49, 2.36)	0.86
Malaria status					
Positive	19 (20.0)	77 (80.2)	1		
Negative	13 (28.3)	33 (71.7)	0. 63	(0.28, 1.41)	0.26
Mother's education					
Some formal education	29 (23.0)	97 (77.0)	1		
No formal education	3 (21.4)	11 (78.6)	0.91	(0.24, 3.49)	0.89
Marital status					
Married	27 (22. 1)	95 (77.9)	1		
Other	5 (25.0)	15 (75.0)	0.85	(0.28, 2.56)	0.78
National Health Insurance					
Yes	16 (16.7)	80 (83.3)	1	(1.19, 5.99)	0.02
No	16 (34.8)	30 (65.2)	2.67		
Household Income					
≥ Minimum wage (GH¢121)	12 (19.7)	49 (80.3)	1		
< Minimum wage (GH¢121)	19 (25.0)	57 (75.0)	0.73	(0.32, 1.66)	0.46
HAZ					
Normal	27 (22. 1)	95 (77.9)	1		
Stunted	5 (25.0)	15 (75.0)	0.85	(0.28, 2.56)	0.78
WAZ					
Normal	31 (23.3)	102 (76.7)	1		
Underweight	1 (11. 1)	8 (88.9)	2.43	(0.29, 20.20)	0.41
BAZ					
Normal	29 (22.7)	99 (77.3)	1		
Severe	3 (21.4)	11 (78.6)	1.07	(0.28, 4.11)	0.92

4.6 Food consumption pattern among participants

Data was also collected on the weekly food consumption of the participants. The results

from the food frequency questionnaire as shown in Table 6 indicated that cereal and grain

products constituted the major source of energy for the participants. Over 80% of the

participants consumed diets prepared from cereal/grain on a daily basis. The consumption

of roots and tubers was not common with only 22.5% of the participants consuming them on a daily basis. The results also showed a low fruit intake among the participants. Nearly 50% of the participants had not consumed fruits in the week preceding the interview; 38% of them consumed fruits 1-3 times a week. Only 2.8% of the participants consumed fruits on a daily basis. Vegetable consumption per week was however higher compared to fruit intake with 43.7% of the participants consuming vegetables daily. Poultry, meat and fish group was consumed on a regular basis. Twenty percent of participants consumed foods 1-3 times per week from this group whereas 31.7% of them consumed foods from this group on a daily basis. Fats and oils had not been consumed by 43% of the participants in the week preceding the interview. Furthermore, less than one percent of participants consumed diets prepared with items from the fats and oils group daily. The participants rarely consumed dairy and dairy products. Over 95% of the participants did not consume foods from this group on a weekly basis (Table 6).

Table 6: Weekly food consumption frequency of participants

Food Group	Weekly Consumption			
	Never	**1-3 Times**	**4-6 Times**	**≥7 Times**
	n (%)	**n (%)**	**n (%)**	**n (%)**
Cereals & grains	-	-	25 (17.6)	117 (82.4)
Roots & Tubers	11(7.7)	50 (35.2)	48 (33.8)	32 (22.5)
Legumes & Nuts	44(31.0)	76 (53.5)	19 (13.4)	3 (2.1)
Fruits	68 (47.9)	54 (38.0)	16 (11.3)	4 (2.8)
Vegetables	8 (5.6)	25 (17.6)	47 (33.1)	62 (43.7)
Poultry, Meat & Fish	-	29 (20.4)	68 (47.9)	45 (31.7)
Fats & Oils	61(43.0)	56 (39.4)	24 (16.9)	1 (0.7)
Dairy & Dairy products	140 (98.6)	2 (1.4)	-	-

4.7 Serum retinol level of the participants

The mean serum retinol level (12.13 µg/dL) of the participants at pre-intervention was significantly different from the mean serum retinol of (16.11 µg/dL) at the post-

intervention period, p <0.001. The results however showed that the mean serum retinol of males did not differ from females in the pre intervention, p = 0.608 and in the post intervention, p = 0.975 (Table 7).

Table 7: Serum retinol levels of participants pre and post interventions

Variable	Pre-intervention (Mean ± SD) (μg/dL)	Post-intervention (Mean ± SD) (μg/dL)	P-value
Serum retinol	12.13±6.60	16.11±6.41	<0.001
Sex			
Male	11.87±5.45	16.13±6.00	<0.001
Female	12.44±7.78	16.09±6.92	0.007

The association between socio-demographic characteristics of the participants and serum retinol was explored in a univariate linear regression model. It was observed that none of the reported socio-demographic characteristics was significantly associated with the serum retinol of the participants (Table 8).

Table 8: Association between serum retinol and socio-demographic characteristics of participants

Variables	Regression coefficient	R-squared	P-value
Mother's education	1.041	0.010	0.34
Mother's income	1.002	0.011	0.21
NHI	1.124	0.022	0.08
Family size	0.995	0.001	0.69

The study established the prevalence of vitamin A deficiency (VAD) among the participants. VAD was defined using the WHO cut-off of serum retinol concentration < 20 µg/dL. Therefore, participants with serum retinol concentrations < 20 µg/dL were considered deficient while those with serum retinol concentrations ≥ 20 µg/dL were considered to be normal. The prevalence of vitamin A deficiency among the participants reduced from 95.1% in pre-intervention to 77.5% in post-intervention (Figure 3).
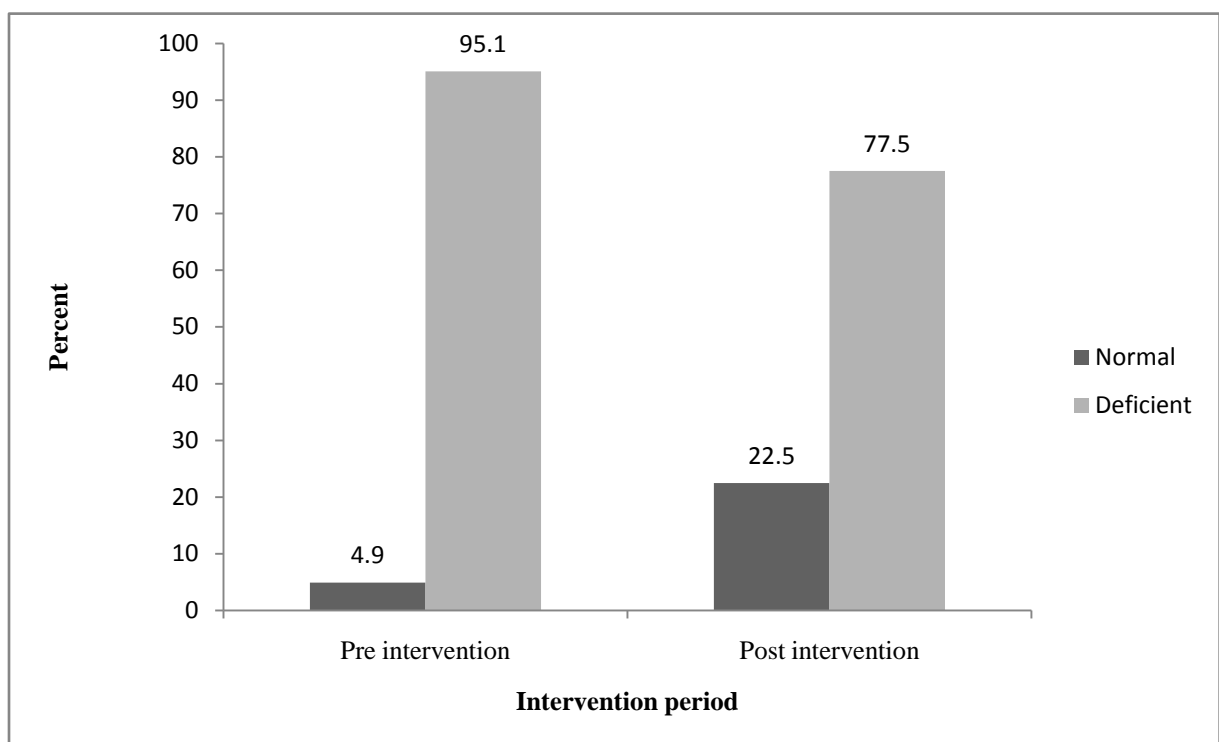
Figure 3: Prevalence of vitamin A deficiency among participants

The results also showed the prevalence of VAD among female and male participants in the study. There was a seventeen percent reduction in VAD among female participants while there was an eighteen percent reduction in VAD among males (Figure 4).
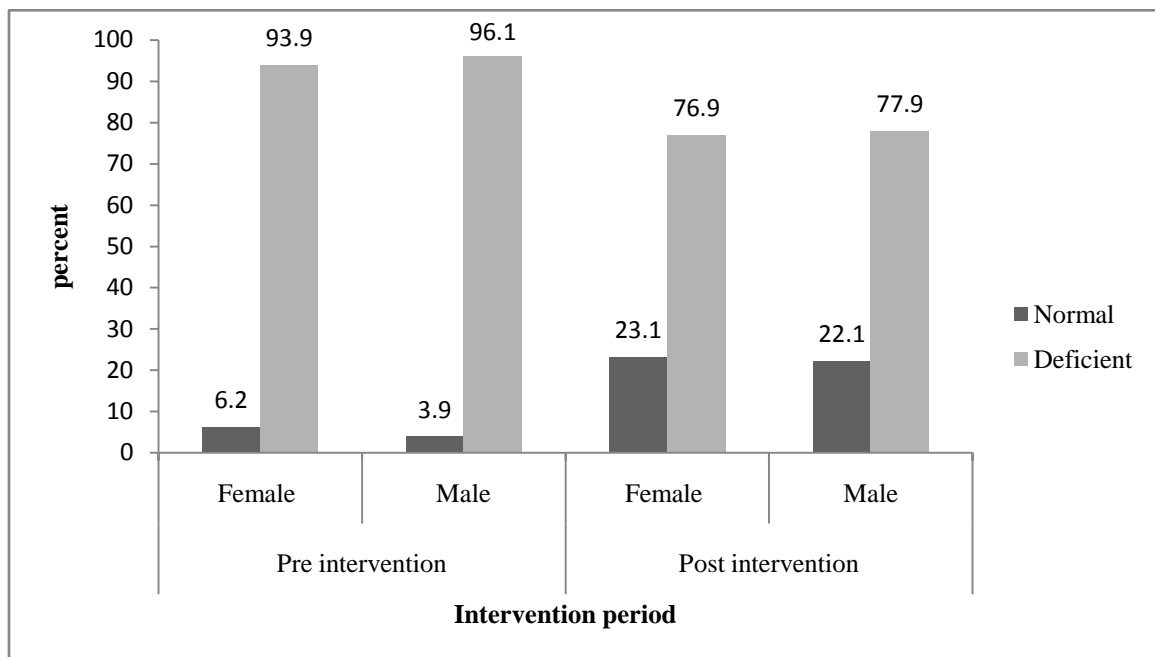
Figure 4: Prevalence of VAD among participants according to gender

Results from a multi linear regression model explored the joint effect of some indicators of nutritional status on the differences in serum retinol observed at the end of the intervention. These variables accounted for about 17% of the variability in serum retinol (Table 9). WAZ, β-carotene as RE* and BAZ were significantly associated with serum retinol with p-values 0.04, 0.05 and 0.03 respectively (Table 9).

Table 9: Association between serum retinol and nutritional status indicators

Variables	Regression coefficient	R-squared	P-value
WAZ	0.892	0.17	0.04
NHI	0.999	0.17	0.06
BAZ	1.204	0.17	0.03
β-carotene as RE*	1.138	0.17	0.05

*RE = retinol equivalent

4.8 Effect of vitamin A on the occurrence of infection among the participants

The mean malaria parasite density for the pre intervention was 2923.42±5667.09/µL and this was significantly different from the post intervention parasite density of 865.16±1312.12/µL, p = 0.008 (Table 10).

Table 10: Malaria parasite density of participants pre and post interventions

Variable	Pre-intervention (Mean ± SD) (µL)	Post-intervention (Mean ± SD) (µL)	P-value
Malaria parasite density	2923.42±5667.09	865.16±1312.12	0.008
Sex			
Male	2594.84±3351.81	1090.32±1419.29	0.036
Female	3252.00±7337.88	640.00±1175.39	0.062

The pre and post intervention malaria status did not differ significantly between males and females (p = 0.792 and 0.455 respectively) (Table 11).

Table 11: Malaria status of participants pre and post interventions

Variable	Malaria status					
	Pre-intervention			Post-intervention		
	Positive	Negative	P-value	Positive	Negative	P-value
Sex						
Male n (%)	53 (37.3)	24 (16.9)	0.734	46 (33.1)	30 (21.6)	0.345
Female n(%)	43 (30.3)	22 (15.5)		43 (30.9)	20 (14.4)	

4.9 Nutritional status of the participants

The study also explored the potential effect of the intervention diet on the nutritional status of the participants. Comparisons between the pre and post-intervention nutritional status showed a significant difference in height-for-age z-scores (p<0.001). However, there was no statistical difference between the pre and post intervention weight-for-age z-scores, p=0.941 (Table 12).

Table 12: Nutritional status of participants' pre and post interventions

Variable	Pre-intervention	Post-intervention	P-value
Weight (kg)	26.86±7.95	28.59±8.60	<0.001
Height (cm)	128.46±15.34	133.15±14.26	<0.001
Weight-for-age z-score (WAZ)	-1.05±0.82	-1.04±0.94	0.941
Height-for-age z-score (HAZ)	-1.24±1.10	-0.871±1.16	<0.001
Body mass index-for-age z-score (BAZ)	-0.59±0.92	-0.74±0.98	0.054

The nutritional status of the participants was measured in terms of WAZ (underweight), HAZ (stunting) and BAZ (thinness). Undernutrition was defined using z-scores < -2 S.D. There was an improvement in the prevalence of stunting. The prevalence of stunting reduced from 24.7 in pre-intervention to 14.1 in post intervention (Figure 5).
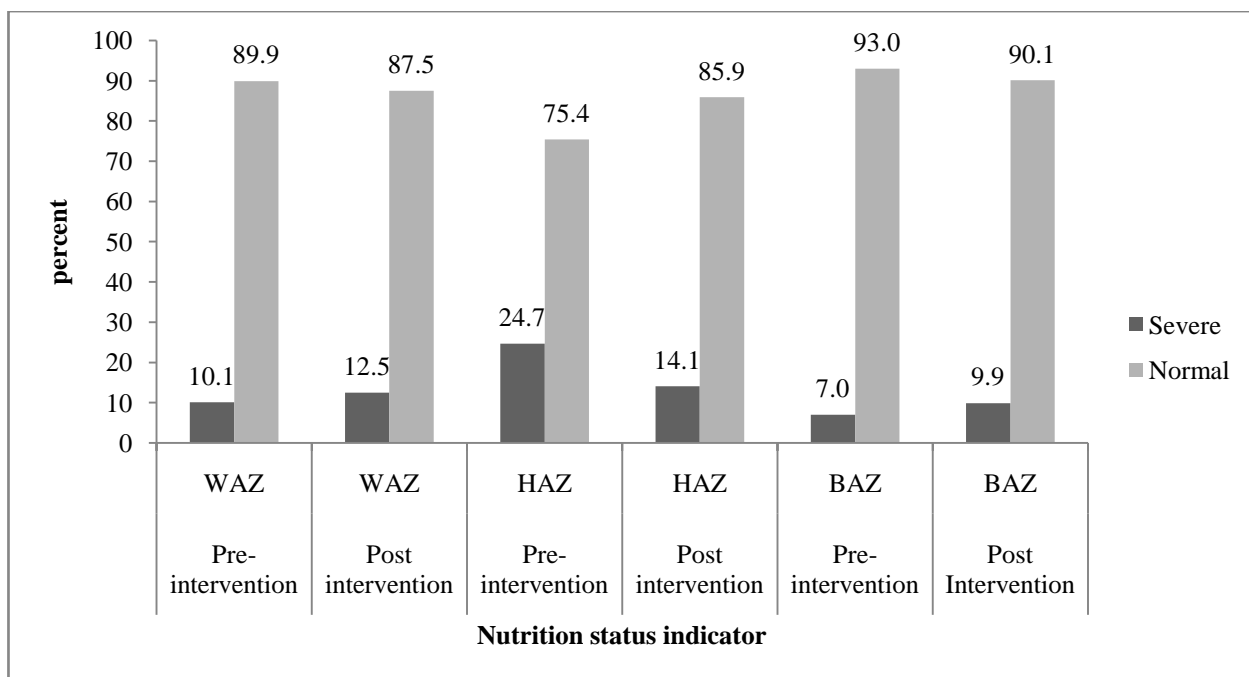
Figure 5: Nutritional status of participants pre and post interventions

CHAPTER FIVE

5.0 DISCUSSION

This was a pre-test-post-test intervention study conducted to explore the effect of gari and beans stew prepared with red palm oil on the serum vitamin A and nutritional status of school children (6-12 years) in Kodzobi community in the Adaklu-Ayigbe district of the Volta Region of Ghana. The study also included a dietary assessment and the effect of the intervention diet on infection episodes among the participants. This study was necessitated by the long search for a sustainable solution to vitamin A deficiency (VAD) especially among children in low income rural households.

5.1 Vitamin A nutrition and background characteristics of participants

Demographics and socio-economic characteristics are known to impact on nutritional status negatively or positively (Hesketh *et al*., 2003: Ajao *et al.,* 2010: and Babar *et al*., 2010). It is well documented in the literature that children and women are among the most vulnerable in society (Nahar *et al.,* 2010*:* Ajao *et al.,* 2010 and UNICEF, 2012); and large family size increases vulnerability (UNICEF, 2012). Poverty has been identified as the single most influential factor on nutritional status (Ajao *et al.,* 2010*)*. However, studies on income, level of education and nutritional knowledge as predictors of nutritional status have produced conflicting results (Rolland-Cachera *et al*., 2000; Jackson *et al*., 2002 and Shi *et al*., 2005).

In this study none of the socio-demographic and the economic variables (maternal education and income, household income and family size) was significantly associated with vitamin A nutritional status. This finding is similar to the results of a study exploring the effect of socio-demographic and health related risk factors for subclinical vitamin A

deficiency conducted in Ethiopia among children 6-72 months (Demissie *et al*., 2009). In that study it was found that maternal education categorised as "illiterate" and "literate" was not associated with vitamin A nutrition. Similarly, Kidala *et al*., 2000 found no association either between maternal nutrition education and vitamin A or serum retinol levels. The present finding which is consistent with literature may suggest that having education or being literate does not mean one understands and applies nutrition in everyday living. Maternal nutrition education has been found elsewhere to increase the consumption of carotene and improve serum retinol concentrations among teenagers (Kuhnlein and Burgess, 1997). On the contrary, Vijayaraghavan *et al*. (1997) documented that despite improved knowledge, production and consumption of vitamin A-rich foods among children participating in a nutrition education and horticultural controlled trial in India, mean serum retinol levels did not improve significantly compared to those of non-participants. This may imply that there are other factors that are influencing vitamin A nutrition.

It is generally known that when income improves, nutrition also improves and this is well documented in the UNICEF (1990) malnutrition framework. In the present study, the setting indicates an impoverished area, which automatically skews the study population into a low socio-economic status which is usually plagued with multifaceted nutritional challenges. It is therefore not surprising that in this study there was no significant positive association between household income and vitamin A intake and serum retinol levels among the participants in univariate logistic regression. Nevertheless, it is noteworthy that children in households with incomes below the minimum daily wage (GH¢4.48) in Ghana (Ghana News Agency, 2012) were 73% more likely to be vitamin A deficient compared to their counterparts in households with income above the minimum wage.

In contrast, some studies in developing countries have exposed a clear significant positive association between high household income and the intake of vitamin A-rich foods as well as serum retinol levels among children (West and Mehra, 2010; and West and Darnton-Hill, 2008). In spite of the above evidence, it must be reiterated that having high/good income does not necessarily translate into consumption of vitamin A rich foods but it must be backed with the nutritional knowledge in food choice, selection and nutrient content of foods.

Another socio-demographic characteristic that influences nutritional status is family size (Babar *et al*., 2010). Having large family size is something ingrained in the Ghanaian culture. The average family size in Ghana according to GSS (2008) is 3.7 with rural families consisting of a higher number (4) of persons per household. In the present study, family size on the average was 6.1 which is typical of rural Ghana. Having a large family size is linked with poverty (Orbeta, 2005; Omonona, 2010) because there are more mouths to feed which is a nutritional challenge for many in Ghana and other poor regions across the globe. In this study, family size did not significantly affect the intake of vitamin A or the serum retinol levels of the children. This finding corroborates the finding of an earlier study by Demissie *et al.* (2009) which established that family size was not associated with vitamin A deficiency. From the literature reviewed and the findings of this study it may be said that selecting foods rich in vitamin A to feed the family may be a key to improving vitamin A nutrition.

5.2 Dietary intake and nutritional status of participants

In many developing countries where animal source foods are prohibitively expensive or non-existent (West, 2000), plant source foods constitutes a significant proportion to the overall nutrient intake (Codjia, 2001). In this study, it was found that the nutrient intake of the participants was largely obtained from plant sources of food. Analysis of the 24 hour dietary recall showed that total energy, carbohydrate and protein needs of the participants were adequate for both pre and end interventions. There was also a significant difference between the pre and end line intervention intakes of these nutrients. This finding is in contrast to the finding of an earlier study by Takyi (1999). In that study, the author reported that the total caloric intake of school children in Saboba, a rural setting in northern Ghana was inadequate (only 26.5% of RDA) whilst the protein intake among the children was adequate with respect to RDA. Another contradiction to the present study was documented in a much more recent study in neighbouring Nigeria where it was found that the energy and protein intake of children of low income earners were inadequate, meeting only 72% and 10.42% RDA respectively (Amosu *et al*., 2011).

A possible reason which could be credited for the observed difference between the pre and post intervention in this study is the evidence that the intervention diet was fortified with palm oil which contained an adequate level of fat, a higher energy yielding nutrient (Fasina *et al*., 2006). The other constituents of the intervention diet, cowpea and gari could have also contributed to the protein and the carbohydrate intakes of the participants.

Based on the 24 hour dietary recall that was conducted, the vitamin A intake of the participants was far lower than the RDI (FAO/WHO, 2001). This was probably because the children obtained their vitamin A mainly from green leafy vegetables which contain provitamin A (carotenoids) as compared to animal source foods which are richer in

vitamin A. This revelation is a reflection of the situation in many countries in sub-Saharan Africa as has been reported by some earlier studies (Codjia *et al.*, 2001; Faber *et al.*, 2002). Codjia and colleagues in 2001 reported that 80% of the overall intake of vitamin A in sub-Sahara Africa is provided by plant sources of food. Subsequently, Faber and co-workers in 2007 also found that nearly 70% of the vitamin A intake of children in KwaZulu Natal, South Africa was obtained from dark green leafy vegetables. These studies attributed the high consumption of provitamin A carotenoids to their affordability, high availability and diversity.

In the present study setting, major provitamin A rich foods such as mango, pawpaw and palm fruit are not widely grown in the community. This made accessibility to these foods expensive to buy in the community even when they were in season in the country since they had to be transported from other places. As a result, community members relied more on dark green vegetables which are not as high or rich a source of vitamin A (Institute of Medicine, 2001) for their vitamin A needs. It may therefore be held that the difference observed between the pre and post intake of vitamin A was as a result of the red palm oil which was a major constituent of the intervention diet.

As described above, the dietary intake of the participants based on the 24 hour and weekly recalls demonstrated a general adequacy of macronutrient intake. However, the vitamin A intake of the participants was generally lower than the recommended intake. Additionally, green leafy vegetables were the major contributor of provitamin A carotenoids. Low dietary fat also characterised the diet of the participants, a situation which militates against vitamin A absorption (Vijayaraghavan *et al.*, 1997). In the present study, the food

frequency results also showed that the daily consumption of fats/oils among the participants was low (less than one percent).

Consequently, the recorded difference in serum retinol levels between the pre and post interventions is most likely the effect of the intervention diet. This is because β-carotene intake based on the 24 hour dietary recall was not found to be significantly associated with the serum retinol of the children. Red palm oil is a rich source of β-carotene which is readily convertible to retinol in a fat medium. It could therefore be stated that the fat content of the intervention diet provided the appropriate fatty milieu for the absorption of the retinol (Vijayaraghavan *et al*., 1997).

The consumption of animal protein was not routine. For example, dairy and dairy products were never consumed by 98.6% of the children in the week preceding the recall. Dry fish was the only animal protein which was consumed by only 32% of the participants on a daily basis. This may not be enough to account for the differences observed in the serum retinol level of the participants. Viewed in another way, the protein that was provided by the cowpea also ensured the appropriate levels of amino acids which may be necessary for the formation of retinol binding protein which is required in the transport of retinol within the body.

The overall significant improvement in the weight and height of the children suggests that the intervention diet with respect to the total energy, carbohydrate and fat content contributed to the differences observed since the 24 hour dietary recall suggests that the children had higher intakes of these nutrients at the end of the intervention compared to the pre-intervention intakes. The weight could also be the result of a gain in fat. The high

level of provitamin A provided by the RPO in the diet probably also contributed to the growth of the children. This observation is consistent with an observation made by West *et al*. (1997) that vitamin A supplementation was found to improve linear growth among a cohort of 3377 rural Nepalese children.

The improvement in stunting among the participants may be attributed largely to the intervention. The combination of gari and beans and fat from the RPO was a potent tool in generating the required nutrients for growth. This finding is consistent with the finding of Sedgh *et al. (*2000) who demonstrated in a longitudinal study that dietary intake of vitamin A improved stunting rates and recovery among 8174 Sudanese children. In a much more recent study, Kimani-Murage *et al. (*2012) showed that vitamin A supplementation improved stunting among children in Kenya.

The lack of effect of the intervention on weight-for-age could be related to infection-diarrhoea and malaria which was high among the participants throughout the study. This finding is consistent with earlier studies (Griffiths *et al*., 2004 and Alasfoor *et al*., 2007) which also indicated that in low income settings, low weight-for-age z-scores are associated with recent morbidity from diarrhoea. Infections cause anorexia, and consequently decreasing nutrient intake. For instance, children with acute respiratory infections or diarrhoea in Guatemala consumed 8% and 18% fewer calories per day, respectively, than did asymptomatic children (Stephensen, 2001).

5.3 Vitamin A status and infection rates among the participants

The nexus between vitamin A nutrition and health has been well established. The influence of vitamin A nutrition on health is observed in both communicable and non-communicable diseases (Slattery *et al*., 2000; Stephensen, 2001). For instance, diarrhoea,

pneumonia, and measles are frequently observed prior to or with xerophthalmia (Sommer and West, 1996). The link between vitamin A deficiency and infection has been observed in retrospective, case-control studies among pregnant women and also in longitudinal, observational studies conducted among children (Stephensen, 2001). These medicinal properties of vitamin A are occasioned by its role in immune function (Stephensen, 2001).

In the present study, the association between vitamin A and malaria was explored. Vitamin A has been identified to be an anti malarial agent due to its inhibition of the growth of the Plasmodium falciparum in vitro (Hamzah *et al.*, 2003). The mechanism of this influence is not well understood. However, the Plasmodium falciparum, appears to gather vitamin A from its host and concentrate it in the cytoplasm of late trophozoites for use as an antioxidant. This may partially account for the hypovitaminosis A observed in malaria patients (Mizuno *et al.*, 2003). In this study a 30% reduction in malaria parasite density between pre and post intervention was recorded. Accordingly, the reduction in the malaria parasite density between pre and post intervention levels may be attributed to the adequacy of the vitamin A provided by the intervention diet. This assertion is supported by some studies in other parts of the developing world. In Papua New Guinea, Vitamin A supplementation among children reduced the frequency of malaria attacks by 30% and cut down the malaria parasite density by as much as 36% (Shankar *et al.*, 1999). Similarly Zeba *et al.* (2008) reported a 30% reduction in malaria among supplemented children from neighbouring Burkina Faso. In the Congo, a case control study among pre-schoolers revealed that 37.5% of the children suffering from malaria had lower than 10 μg/dl (0.3 μmol/L) plasma retinol levels (Galan *et al.*, 1990). Another factor that could account for this difference was health and nutritional conditions in the community which did not improve during the intervention.

The only factor which significantly translated into improvements in vitamin A status in this study was accessibility to healthcare (for which access to National Health Insurance was used as a proxy). This was probably because access to health insurance resulted in affordable and early attendance to health facility and hence timely treatment of infections such as malaria and diarrhoea which tend to undermine vitamin A nutrition. Children from households without health insurance were three times more likely to be vitamin A deficient compared to children from households with health insurance. There is a dearth of information on the direct effect of having health insurance on vitamin A status. However, population based studies suggest that access to healthcare can improve nutrition status of children (WHO, 2001; Babar *et al*., 2010; Srivastava *et al*., 2012).

5.4 Implications of the study for nutrition and health

The findings of this study have several implications for clinical as well as public health practice within the study location and beyond. The study showed that the intervention diet was effective in lowering stunting among the participants. This knowledge can be harnessed in designing appropriate dietary regimen for school children at risk of chronic malnutrition. The study also demonstrated the potential of red palm oil in reducing malaria parasite density. Therefore, in addition to routine malaria medication health workers should also emphasise the need for adequate dietary intake of vitamin A. In this light, community members should be advised to improve the cultivation of vitamin A rich foods such as mangoes, "kontomire", and palm oil among others. The study has also revealed that red palm oil can be used as a means of combating vitamin A deficiency (VAD) among vulnerable rural children. Hence, the study will help to enhance the quality of nutrition education messages among health workers. In a broader national perspective, this study

can boost the quality of interventions such as the Ghana School Feeding Programme (GSFP).

In all of these considerations, it is very important to note that vitamin A does not work in isolation. Accordingly, in addressing vitamin A deficiency related nutrition challenges, other issues such as family size, nutrition education, income and infections must all be tackled in a holistic manner.

5.5 Limitations of the study

Although the study was done with caution within the limits of both human and experimental errors, it is important to reiterate unavoidable limitations that may have implications on the findings. The results show that the vitamin A status and nutritional status of children improved between pre and end intervention. It must however be borne in mind that without the benefit of a control group, it was difficult to determine the independent contribution of the intervention. Setting up a control group is not simple (De Pee *et al*., 2000; Habicht *et al*., 1999). In this respect, it is hard to completely attribute the successes found to the intervention alone. The use of a convenient sample size might have introduced bias in the findings since the sample might not have been representative of the population. By extension, this also limits the generalisability of the findings of the study.

Recall bias might have influenced the process of recall of foods eaten during the past 7 days. Also, the use of the 24-hour recall might not be representative of usual daily nutrient intake of the participants. Hence, measurement bias in measuring nutritional intake could have contributed to some amount of bias in this study. Recall bias was especially likely in this study since the participants were children. As a result, the estimated nutrient intake

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may have been higher or lower than the usual intakes of the participants. Perhaps a better method for the dietary assessment may have been weighed food intake (Trabulsi and Schoeller, 2001).

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusion

From the current study, the following inferences may be made:

- Dietary intake of vitamin A rich foods among the participants in the study was poor and this was due to seasonality and accessibility of pro-vitamin A rich foods such as mangoes, pawpaw, palm oil and green leafy vegetables in the community. However, their intake of macronutrients (carbohydrate, fats and proteins) seemed adequate from the dietary recall but did not reflect in the anthropometric indicators which showed otherwise with high levels of wasting and underweight.

- The prevalence of Vitamin A Deficiency (VAD) among the participants was as high as 95.1% in the pre-intervention. At the end the intervention, the prevalence of VAD remained as high as 77.5% clearly indicating that vitamin A deficiency is a public health challenge in the study population. The prevalence of VAD was higher among the male participants than it was among the female participants.

- The 300g intervention diet contained 823.56 µg RE and this contributed significantly to the mean serum retinol level of 16.11±6.41 µg/dL at the end of the intervention.

- In this study the 30% reduction in malaria parasite density between pre and post intervention could be attributed to the consumption of vitamin A rich palm oil.

- Clearly then, feeding children with gari and beans stew prepared with red palm oil three times a week may be a long term sustainable food based strategy in the battle against vitamin A deficiency.

6.2 Recommendations

Further research

- There is the need for further research using a control group and a larger sample size to explore the effect of red palm oil and cowpea based diets on the vitamin A and nutritional status among children. Such studies should also be conducted over a longer period (at least one year).

- Future studies should also be community based in order to test the effectiveness of red palm oil in addressing vitamin A deficiency.

Policy for public health practice

- In the short term, there is the need for horticultural practices such as home gardening among residents to increase the production of vitamin A-rich fruits and vegetables.

- In view of the low consumption of vitamin A-rich foods in the study community, health education and promotion activities should be intensified by the Ghana Health Service (health workers) to ensure higher consumption of especially vitamin A rich fruits and vegetables as well as red palm oil among children in the community.

- In the long term, the participation of agricultural extension officers may also be required to ensure the production of vitamin A food trees such mangoes and oil palm in the community.

- The consumption of gari and beans stew prepared with red palm oil three times per week is recommended among children to improve vitamin A status. This may be applied in the Ghana School Feeding Programme and similar programmes.

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APPENDICES

APPENDIX I: QUESTIONNAIRE

QUESTIONNAIRE FOR CHILD AND PARENTAL BACKGROUND FOR THE STUDY - SERUM VITAMIN A AND NUTRITIONAL STATUS OF CHILDREN FED GARI AND BEANS STEW

Questionnaire number…………………..   Date of interview……………..

A. Socio-demographic data

Child data

1.   Name of Child……………………….…..   2. Sex …………………………

3.   Age of child…………………………………   4. Date of birth (DOB)…………

5. Source of verification of DOB (e.g. birth certificate, growth monitoring card etc)

…………………………………………………………………………………..……

6. Name of School……………..………..

7. Class…………………………………   8. Number of siblings under five……….

Parental data

Father

9.   Age (in years) a. less than 20 b. 20-25 [ ] c. 25-30 [ ] d. 31-35 [ ] e. 36-40 [ ] f. 41-45 [ ] g. 46-50 [ ] h. 51-55 [ ] i. 56-60 [ ] j. 61-70 [ ] k. 71+ [ ]

10. Marital status a. single [ ] b. married [ ] c. Separated [ ] d. divorced [ ]

e. Widowed [ ] f. Other (specify)…………………..…….

11. How many wives or partners do you have?...

12. Educational status a. No formal education [ ] b. Primary [ ] c. JHS/Middle School [ ] d. SSS [ ] e. Training college [ ] f. Vocational/Technical [ ] g. University [ ]

13. Primary Occupation a. Farmer [ ] b. Trader [ ] c. Teacher [ ] d. Fisherman[ ]

    d. Other (specify)……….

14. Secondary Occupation Farmer [ ] b. Trader [ ] c. Teacher [ ] d. Fisherman [ ]

    e. Other (specify)……….

15. Religion a. Christian [ ] b. Traditionalist [ ] c. Islam [ ] d. Buddhist [ ] d. Other
    (specify).

    Ethnicity a. Ewe b. Akan   c. Ga   d. Fante e. Other (specify)……………………

16. Monthly income

    Mother

17. Age (in years) a. less than 20 b. 20-25 [ ] c. 25-30 [ ] d. 31-35 [ ] e. 36-40 [ ] f. 41-

    45 [ ] g. 46-50 [ ] h. 51-55 [ ] i. 56-60 [ ] j. 61-70 [ ] k. 71+ [ ]

18. Marital status a. single [ ] b. married [ ] c. Separated [ ] d. divorced [ ]

    e. Widowed [ ] other (specify)…………………………….

19. How many partners do you have? …………………………

20. Educational status
    a. No formal education[ ] b. Primary [ ] c. JHS/Middle School [ ] d. SSS [ ]
    e. Training college [ ] f. Vocational/Technical [ ] g. University [ ]

21. Primary Occupation a. Farmer [ ] b. Trader [ ] c. Teacher [ ] d. Fish monger

    e. Other (specify)……………………………………….…….

22. Secondary Occupation Farmer [ ] b. Trader [ ] c. Teacher [ ] d. Fish monger
    e. Other (specify)……………………………………..…….

23. Religion a. Christian [ ] b. Traditionalist [ ] c. Islam [ ] d. Buddhist [ ] d. Other
    (specify).

24. Ethnicity a. Ewe b. Akan   c. Ga   d. Fante e. Other (specify)……………………

25. Monthly income…………………………….

26. National health Incurance status of child
    a.   Registered   b. Unregistered

B. Child Health/Nutrition

27. Have you fallen sick in the past two weeks? Yes [ ] No[ ] *(please consult parents to confirm)*

28. If you answered 'yes' to question, what disease was it? A. malaria b. diarrhea c. dysentery d. catarrh ....

29. Have you received Vitamin A capsule before?   Yes [ ] No [ ]

30. What was the colour of the capsule you received? a. Blue [ ]   b. Red [ ] c. Other (specify)……

31. If yes for vitamin A supplementation how many times?.....................

32. Iron supplementation status   Yes [ ] No [ ]

33. If you answered 'yes' for iron supplementation how many times have you received it?.....................

34. Have you received a dewormer in the last 3 months? Yes [ ] No [ ] (please check with parent/teacher to confirm)

35. What type of salt do you/your parents use to prepare your meals at home? a. rocky salt[ ] b. Annapurna [ ] c. Other (specify)…………………………

C. Water and Sanitation

36. What is your source of drinking water? a. Bore hole [ ] b. Well [ ] c. Dam [ ] d. Tap [ ] e. Other (specify)………………………………..…..

37. How do you dispose your refuse a. refuse dump [ ] b. Dust bin [ ] c. Refuse tank [ ] d. dugout Pit [ ] e. other (specify)………

38. Where do you ease yourself? a. home [ ] KVIP b. [ ] Water closet [ ] c. free range pit latrine [ ] d. home bucket latrine [ ] e. public toilet[ ]

D. Cultural Practices

39. Do you have any food taboos in your community? Yes [ ] No [ ]

40. If your answered 'yes' to question 36, what are these foods?

………………………………………………………………………………………

………………………………………………………………………………………

………………………………………………………………………………………

41. Why are children forbidden from eating these foods?...

………………………………………………………………………………………

………………………………………………………………………………………

………………………………………………………………………………………

E. Food Frequency Questionnaire

Food	More than once/day	Once/day	No. of times/week	Seldom	Never
Animal and Animal Products					
Fish					
Milk					
Eggs					
Poultry					
Meat					
Wole/ayikoko					
Crab					
Mushroom					
Dark Green Leafy Vegetables					
Ayoyo					
Nkontomire					
Allefu					
Garden eggs					
Tomatoes					
Bean leaf					
Carrots					
Cabbage					
Okro					
Other vegetables					
Fruits					
Mango					
Pawpaw					
Orange					
Banana					
Guava					
Watermelon					
Cereals/Grains					
Maize					
Rice					
Millet					
Guinea corn					
Legumes					
Cowpea					
Groundnuts					
Soy beans					
Bambara groundnuts					
Other (specify)					
Roots and Tubers					

90

Sweet potato					
Cocoyam					
Yam					
Cassava					
Plantain					
Fats and Oils					
Palm oil					
Palm kernel oil					
Groundnut oil					
Shea butter					
Other (specify)					
Other foods					
Toffees					
Ice cream					
Biscuits					
Meat pie/doughnuts					
Koose/Agawu					
Roasted plantain					
Fried plantain					
Roasted cassava					
Vitamin, herbs and other supplements					
Drinks					
Asana					
Fruit Juice					
Coke, fanta etc Other (specify)					

F. 24 Hour Dietary Recall

Meal Type	Foods and Beverages consumed. Describe in detail.	Amount Eaten (g) or L	Serving Description(Cup, Teaspoon, Slice, Finger, Fist, Tablespoon)
Morning			
Mid-Morning			
Noon			
Afternoon			
Evening			
Late evening			

G. Anthropometry of Child

Characteristics	Measurement		
	1	2	3
Weight (kg)			
Height (cm)			

<center>**APPENDIX II: CONSENT FORM**</center>

<center>**CONSENT FORM**</center>

DIETARY INTERVENTION ON SERUM VITAMIN A AND NUTRITIONAL STATUS OF CHILDREN

Introduction

(This form provides adequate information about the above study to help you make an informed decision about your participation in the study. We therefore entreat you to read (or have read to you) the information contained in the form. After you have studied the information contained in the form, you will be required to sign/initial it (in the presence of a witness) if you wish to participate in our study. A copy of the signed form will be given to you for your reverence. You will also be asked to sign it (or make your mark in front of a witness). Please feel free to ask us to explain anything you may not understand in the form).

Purpose/General Information about Study

The main objective of this study is to determine the effect of cowpea and palm oil based diets on the serum vitamin A and nutritional status of school children. Hence, we will assess your food intake, weight, height and mid-upper arm circumference. We will also draw 5ml of blood from you to enable us determine the effect of the intervention on the serum vitamin A as well as the nutritional status of the school children. If you and your parent/guardian agree to participate in the study, we will only spend 45 minutes of your time at your convenience.

Possible Risks and Discomforts

This study poses little (in terms of blood collection) or no discomforts other than giving us thirty minutes of your time.

Possible Benefits

There may be no direct benefit to you for your participation in our study. However, the results of the study will benefit society.

Confidentiality

We assure you that any information you may provide will be protected and used solely for academic purposes and will be treated confidentially. Your name will not be in any of our reports. However, a member of the IRB may want to ask you questions concerning your participation in the study. Should this happen, you may choose not to respond to such questions. A competent court of law could order medical records shown to other people, but this is not likely to happen in this study.

Compensation

This is student research and as such, it is budget constrained. We are therefore unable to pay you any compensation for your participation in our study.

Leaving the Study
You are free to withdraw from our study at any time if you choose to participate in it.

If You Have a Problem or Have Other Questions/Concerns
Please call Dr Matilda Steiner-Asiedu on telephone number 0541260704/0266142105 if you have questions about the study.

Your rights as a participant
This study has been reviewed and approved by the IRB of the Noguchi Memorial Institute for Medical Research at the University of Ghana, Legon. An IRB is a committee that reviews studies in order to ensure the protection of participants. If you have any questions about your rights as a participant you may contact (Rev. Dr. Ayete-Nyampong, Chairperson, NMIMR-IRB, mobile 0208152360)

Participant Agreement
This form which describes the benefits, risks and procedures for the study **"Dietary Intervention on Serum Vitamin A and Nutritional Status of Children"** has been read and explained to me. All my questions/doubts about the study have been addressed to my satisfaction. I therefore agree to participate in the study.

_____   _____
Date   Signature or initials of participant

If volunteers cannot read the form by themselves, a witness must sign here:

I certify that the benefits, risks and procedures of the study were read and explained to the participant in my presence. All questions/doubts were addressed to the participant's satisfaction and the participant has agreed to take part in the study.

_____   _____
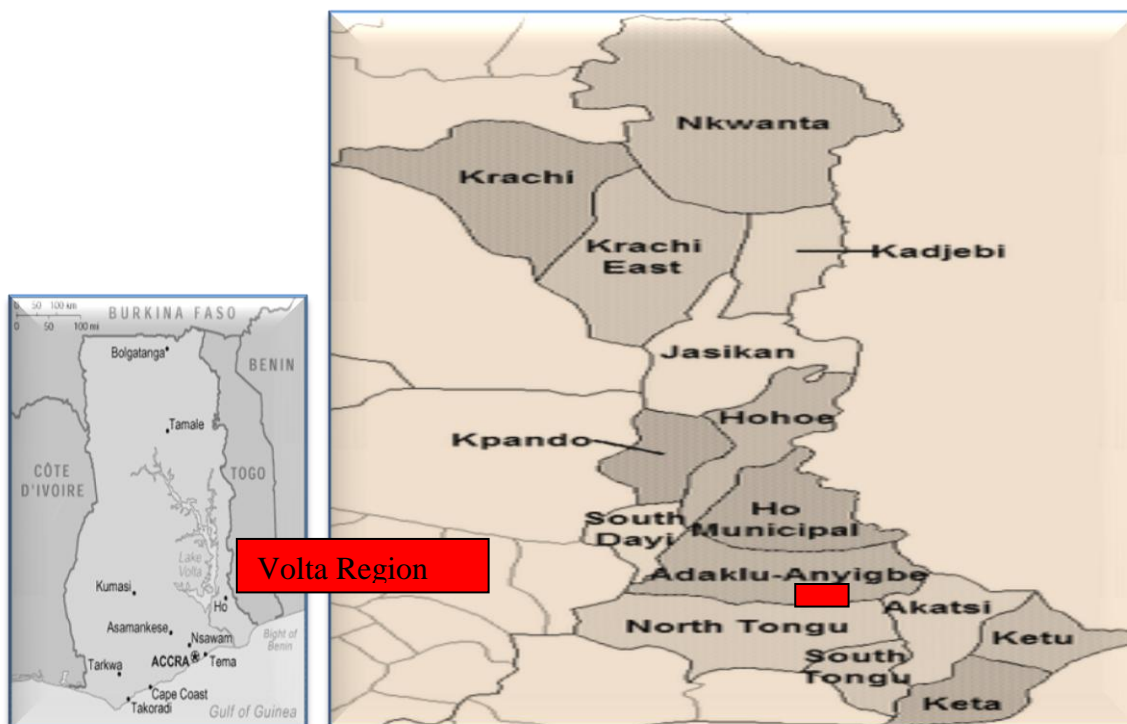Date   Signature of Witness

I certify that the nature and purpose, the potential benefits, and possible risks associated with participating in this study have been explained to the above individual.

_____   _____
Date   Signature of Person Who Obtained Consent

APPENDIX III: SURVEY CHECKLIST

SURVEY CHECKLIST FOR AVAILABILITY AND ACCESSIBILITY OF VITAMIN A RICH FOODS IN KODZOBI COMMUNITY

Food	Availability Yes, no, seasonal or all year round	Price
Animal and Animal Products		
Fish		
Milk		
Eggs		
Poultry		
Meat		
Vegetables		
Wole/ayikoko		
Mushroom		
Ayoyo		
Nkontomire		
Allefu		
Tomatoes		
Bean leaf		
Carrots		
Other vegetables		
Fruits		
Mango		
Pawpaw		
Orange		
Banana		
Guava		
Watermelon		
Fats and Oils		
Palm oil		
Other (specify)		

APPENDIX IV: MAP OF STUDY AREA

Map of Ghana   **Map of study setting**