EFFECT OF NUTRITION INFORMATION ON FEEDING FIRST FOOD ENRICHED WITH ORANGE FLESHED SWEET POTATOES ON VITAMIN A STATUS IN 6-12 MONTHS CHILDREN IN MOROGORO REGION, TANZANIA.

By

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ABSTRACT

EFFECT OF NUTRITION INFORMATION ON FEEDING FIRST FOOD ENRICHED WITH ORANGE FLESHED SWEET POTATOES ON VITAMIN A STATUS IN 6-12 MONTHS CHILDREN IN MOROGORO REGION, TANZANIA.

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Vitamin A deficiency is widespread and has severe consequences for young children in the developing world. Food-based approaches may be an appropriate and sustainable complement to supplementation programs. We assessed the effect of nutrition information on feeding first food enriched with orange fleshed sweet potatoes on vitamin A status of 6-12 months children in Morogoro region of Tanzania. This one month intervention did not improve vitamin A status of the subjects as measured by Retinol Binding Protein: Transthyretin ratio. All subjects were vitamin A deficient at baseline where two subjects had severe vitamin A deficiency. The subjects remained vitamin A deficient post intervention with more subjects having severe VAD than those at baseline (cut off value $\leq$0.7uM).We observed a significant reduction of RBP:TTR ratio concentration during the intervention (p-value; 0.001). The subjects gained an average weight of 0.82 $\pm$ 0.14kg and average growth of 2.07 $\pm$ 0.27cm.There was no significant difference in total protein and C-reactive protein levels (p-value;0.4,0.37 respectively) between baseline and post intervention periods. However, we did observe a significant reduction in TTR levels (p-value; 0.02) at baseline and post intervention. Although this intervention study did not have a positive effect on vitamin A status of the children; it has shed some light on the fact that it is possible to enrich maize porridge which is the main first food for 6-12mo children in this country with orange fleshed sweet potatoes as other possible food based approach in combating VAD.

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KEY TO ABBREVIATIONS

CIC	Conjuctival Impression Cytology
CRBP	Cellular Retinol Binding Protein
CRP	C - Reactive Protein
DBS	Dried Blood Spot
EBF	Exclusive Breast Feeding
EDP	Essential Drug Program
HIV	Human Immunodeficiency Virus
HPLC	High Pressure Liquid Chromatography
LRAT	Lecithin Retinol Acyltransferase
MRDR	Modified Relative Dose Response
NIH	National Institute of Health
OFSP	Orange Fleshed Sweet Potatoes
PEM	Protein Energy Malnutrition
RAE	Retinol Activity Equivalent
RBP	Retinol Binding Protein
RDR	Relative Dose Response
RDA	Recommended Dietary Allowances

TTR	Transthyretin
UNICEF	United Nations Children's Fund
VAD	Vitamin A Deficiency
VADD	Vitamin A Deficiency Disorder
VAS	Vitamin A sufficiency
WHO	World Health Organization

1. INTRODUCTION

1.1. Vitamin A

Vitamin A, or retinoid, is a generic term that refers to fat-soluble compounds that are similar in structure and exhibit biologic activity of retinol. The term refers to any compound structurally similar to retinal (aldehyde), retinol (alcohol), and retinoic acid or any other substance that exhibits vitamin A activity. The term also refers to precursors of vitamin A including the carotenoids, mainly β-carotene. Two main dietary sources of vitamin A exist: pre-formed vitamin A, commonly found in foods of animal origin, and provitamin A carotenoids, found in yellow and orange fleshed fruit and vegetables as well as dark-green leafy vegetables [1]. Of the ≈600 carotenoids found in nature, only 3 are important precursors of vitamin A in humans, namely, β-carotene, α-carotene, and β-cryptoxanthin. β-carotene is the major provitamin A component of most carotenoid-containing foods [2].

Several proteins participate in the digestion and absorption of dietary vitamin A. Dietary retinyl esters are hydrolyzed in the intestine by the pancreatic enzyme, pancreatic triglyceride lipase, and intestinal brush border enzyme, phospholipase B. Unesterified retinol taken up by the enterocyte is complexed with cellular retinol-binding protein (CRBP) type 2 and the complex serves as a substrate for re-esterification of the retinol by the enzyme lecithin:retinol acyltransferase (LRAT) [3]. Carotenoids and other fat soluble micronutrients are hypothesized to follow the fate of lipids in the upper gastrointestinal tract and their absorption presumably occurs in the upper half of the small intestine [4]. The first phase of the processes of digestion and absorption is the dissolution of carotenoids and vitamin A in the fat phase of the meal. This phase is emulsified into lipid droplets in the stomach and duodenum [5]. The size of the droplets has apparently no effect on the efficiency of the absorption of vitamin A

in healthy humans, and no degradation or absorption of vitamin A has been detected at the stomach level [6]. The retinyl esters are then incorporated into chylomicrons, intestinal lipoproteins containing other dietary lipids, such as triglycerides, phospholipids, free and esterified cholesterol, and apolipoprotein B. Chylomicrons containing newly absorbed retinyl esters are then secreted into the lymph. Although under normal dietary conditions much of the dietary vitamin A is absorbed via the chylomicron/lymphatic route, it is also clear that under some circumstances there is substantial absorption of unesterified retinol via the portal route [3]. Retinol bound to a cellular RBP (CRBP or CRBP-II) can be esterified by the enzyme lecithin:retinol acyltransferase (LRAT), the resulting retinyl ester being stored primarily in liver stellate cells. LRAT provides a readily retrievable storage form of vitamin A, as well as regulating its availability for other pathways [7]. Approximately 90% of the vitamin A in the body is stored in the liver as retinyl-esters (retinaldehyde esterified with fatty acids), and an adult liver can contain enough vitamin A to last more than 1 year [7].

Circulating retinol is primarily bound to retinol-binding protein (RBP), and can enter and leave the liver several times per day in a process known as retinol recycling, which acts to regulate the amount of retinol in circulation and protects cells from the damaging effects of free retinol or retinoic acid. This also facilitates cellular uptake of retinol via Stra6 proteins. This 74 kDa multi-transmembrane transporter has been identified as a specific receptor for RBP. Among other tissues, this protein is found in the intestine during development, although it is not clear whether it persists in adults [8].

Several factors affects vitamin A metabolism. Example zinc status influences several aspects of vitamin A metabolism, including its absorption, transport, and utilization. Two common mechanism that can explain this are the regulatory role of zinc in vitamin A transport

mediated through protein synthesis, and oxidative conversion of retinol to retinal that requires the action of a zinc-dependent retinol dehydrogenase enzyme [9].

Zinc deficiency is commonly associated with low plasma concentrations of vitamin A, even when hepatic vitamin A stores are normal, suggesting that there is a defect in mobilization of vitamin A rather than in its absorption or transport to the liver. With zinc deficiency there is impaired synthesis of proteins that turnover rapidly, such as RBP. This impairment affects retinol transport from the liver to the circulation and other tissues because retinol is transported as a retinol-RBP complex in association with transthyretin [10].

Decreased serum vitamin A concentrations has been described in children with protein energy malnutrition (PEM) .When such children were treated with high protein diets these patients demonstrated highly significant increases in serum vitamin A concentrations [11]. These findings suggest that the low serum vitamin A levels in kwashiorkor largely reflect a functional impairment in the hepatic release of vitamin A rather than vitamin A deficiency per se. Hepatic release of vitamin A is apparently impaired because of defective hepatic production of plasma proteins, including the plasma transport proteins for retinol because of a limiting supply of substrate for protein synthesis. When substrate is provided by dietary calories and protein, the hepatic production of plasma proteins increases, plasma RBP and concentrations rise, and hence, plasma vitamin A concentration increases [11].

Iron-deficiency anemia and VAD share several features. VAD is a potential cause of iron deficiency [12]. It has been hypothesized that VAD promotes the sequestration of iron in tissues, making it unavailable for erythropoiesis, resulting eventually in anemia and abnormal iron indices[13]. Iron deficiency inhibits mobilization of vitamin A stores and may decrease the absorption and irreversible utilization of vitamin A [14]. In an experimental study of children with anemia, it was observed that iron supplementation alone increased serum

retinol compared to placebo-treated children. Although these differences were not significant, it is important to consider that children receiving elemental iron, alone or in combination with VA supplements, had higher serum retinol concentrations than their respective controls [15].

Vitamin A has three active forms (retinal, retinol and retinoic acid) and a storage form (retinyl ester) [7]. Vitamin A is essentially required in the body to maintain visual system, sustain normal cellular differentiation, develop resistance against infections, and uphold epithelial integrity, red blood cell production, and reproduction [16].

The most accurate measure of an individual's vitamin A status is the hepatic vitamin A content. To assess this requires an invasive technique that is impractical for population studies. Alternative methods for estimating vitamin A deficiency (VAD) at population level include measuring breast milk vitamin A, dark adaptometry, pupillary responses, conjunctival impression cytology and serum or plasma retinol concentration [17]. More over xerophthalmia classification was traditionally used to identify populations with vitamin A deficiency [18].

Conjunctival impression cytology (CIC) has been used in field surveys. The equipment necessary for conducting CIC is not expensive. However the method seem to be negatively impacted in drier African countries [19]

Biochemical methods that are currently available for assessment of vitamin A include serum retinol and breast milk retinol concentrations, relative dose response and modified relative dose response tests and the deuterated retinol isotope dilution test [18].

Serum retinol concentrations have been used extensively to identify populations at risk of vitamin A deficiency. The major drawback of serum retinol is that blood samples are required. Moreover, in healthy individuals, serum retinol concentrations are homeostatically

controlled and do not begin to decline until liver reserves of vitamin A are dangerously low. Furthermore, RBP is a negative acute phase protein; therefore, serum retinol and RBP concentrations will fall during times of infection [18].

Because of the high degree of infection in children at risk of vitamin A deficiency and the homeostatic mechanism, serum retinol does not always respond to vitamin A intervention strategies [19]. The status of other nutrients, particularly iron deficiency, may also negatively affect serum retinol concentrations [20]. Iron deficiency also may decrease the mobilization of vitamin A from liver storage [14].

The ratio of plasma Retinol Binding Protein:Transthyretin (RBP:TTR) has been proposed as a means to improve the assessment of vitamin A status of individuals with concurrent infection or inflammation [21].

Several biologic properties of the RBP: TTR ratios support its use as an indicator of VA status. The formation and secretion of holo-RBP into plasma requires adequate hepatic VA stores; otherwise, RBP is poorly secreted and accumulates in the liver. Transthyretin is important in maintaining circulatory levels of holo-RBP. It forms a large transport complex, thus reducing the glomerular filtration of the smaller-sized holo-RBP [22].

Studies of the binding and dissociation capacities of these proteins showed that retinol's selectivity for RBP might originate from its interaction with TTR, which enhances retinol's uptake by peripheral tissues [22]. Using reverse-phase HPLC and molecular exclusion techniques, Burri et al, was able to demonstrate that the retinol-RBP-TTR concentration was a better linear predictor of hepatic VA stores than unbound retinol, or retinol bound only to RBP [23].

1.2. Vitamin A deficiency

Health consequences that are a result of vitamin A deficiency are collectively known as Vitamin A deficiency disorders (VADD). These conditions range from ocular manifestations of xerophthalmia, to less specific disorders such as impaired mechanisms of host resistance, severe infectious illnesses, poor growth in children and mortality attributable to vitamin A deficiency in a population [24]. Vitamin A deficiency remains a leading public health problem in the developing world, with its health consequences most apparent and severe among infants, young children, and women of reproductive age [25].

Estimates of extent and severity of vitamin A deficiency are imperfect, as they depend on the frequency of use of valid indicators of vitamin A status across populations, and draw on diverse sources of data of varying representativeness. In chronically deficient regions, night blindness in women and xerophlmia in children can be used as indicators to provide data on the extent of moderate to severe vitamin A deficiency [17]. It is estimated that Vitamin A deficiency globally affects 140 million children aged <5 y, of whom nearly 100 million live in South Asia or sub-Saharan Africa. Countries of eastern and southern Africa have the highest prevalence (37%) of preschool children with low serum retinol concentrations, followed by South Asia (35%) and Western and Central Africa (33%) [26]. In Tanzania it is estimated that VAD and xerophthalmia occur in about 6% of the Tanzanian population, 98% of those affected being children under 6 years of age. This deficiency is a problem of public health significance mainly in the dry regions of central Tanzania [27].

Vitamin A deficiency is the leading cause of severe preventable visual impairment and blindness among children in developing countries. It is also a great contributor to severe infection and death particularly from measles and diarrhea. VAD also increases vulnerability

to other infections and might be an important contributing factor to growth deficits in children [28].

Childhood VAD is firstly caused by maternal vitamin A deficiency which is caused by mothers consuming diets low in vitamin A and they experience high fertility with prolonged breast-feeding. The magnitude and implications of VAD among reproductive aged women was not fully appreciated as compared to the problem among children because the clinical signs of xerophlamia are very rare in women. Data from the past decade however reported that xerophlamia as an indicator of VAD may be very common among women than in children [25]. This causes substantial maternal and infant mortality and maternal morbidity[29]. Furthermore, deficient mothers produce breast milk with very low concentrations of vitamin A which puts the next generation at risk [30].

Exclusive breastfeeding (EBF) provides infants nourishment at a lower risk of contamination than does the mixing and storage of formula with water, especially in poor households [31],however maternal vitamin A deficiency contributes to mother-to-child transmission of HIV [32] and thus posing a greater risk for micronutrients deficiency including VAD to these children. WHO adopted new guidelines in 2010 with respect to mother-to-child transmission of HIV through breastfeeding. The guidelines provided 3 choices to HIV infected mother to either; EBF for the first 6 mo of life, introduction of appropriate complementary foods thereafter, and the continuation of breastfeeding for the first 12 mo of life; formula feeding, if acceptable or the use of expressed heat-treated breast milk in specific circumstances [33].

Children also become vitamin A deficient because they consume diets; during the weaning and after weaning periods that contain insufficient vitamin A. In many developing countries the intake of Vitamin A containing foods is limited. A large number of populations consume

diets that lack in animal products. This is almost always the case because a young child cannot possibly consume sufficient dietary sources of β-carotene to satisfy their vitamin A needs from vegetables and grains alone [34].

Another important contributing factor to vitamin A deficiency is children in developing countries often get sick. The prevalence of diarrhea among young children in developing countries ranges from 10 - 19%. In this case a child from developing country spends an average of 36-70 days each year with diarrhea [35]. The prevalence of acute respiratory illness among young children in developing countries is even higher, 25 - 60% [35]. In a longitudinal study done in Bangladesh to study the interactions between infectious diseases and the nutritional status of children showed that illnesses of the upper respiratory tract, such as purulent rhinitis and pharyngitis, had the highest prevalence in children. Diarrheas were the second most common illnesses, with a peak prevalence rate in children 6–11 months of age. Diarrhea was also the most frequent reason for hospitalization of study children [36].

Vitamin A deficiency can be considered a nutritionally acquired immunodeficiency disease characterized by pathological changes such as those of the mucosal epithelia of the respiratory, gastrointestinal, and genito-urinary tracts; impaired antibody responses to protein antigens, altered cell-mediated immunity, and alterations in T-cell subsets [37]. Even mild deficiency in vitamin A increases the incidence of respiratory disease and diarrhea as well as a higher rate of mortality from infectious disease [38].

The onset of infection reduces blood retinol levels very rapidly. This phenomenon is generally believed to be related to decreased synthesis of RBP by the liver. In this manner, infection stimulates a vicious cycle, because inadequate vitamin A nutritional status is related to increased severity and likelihood of death from infectious disease [37].

Vitamin A deficiency can be controlled as a public health problem by maintaining adequate intakes of the nutrient in high-risk groups through direct supplementation, fortification, dietary diversification and educational efforts to the community on how to improve diets [26].

Foods of animal origin are the best source of vitamin A, however in developing countries a large number of the population cannot afford them [39]. Thus local production of fruits and vegetables may be a potential source of foods rich in provitamin A. For example, in Bangladesh, locally produced fruit and vegetables that are rich in provitamin A provide a valuable contribution to vitamin A intake in communities where alternative dietary sources of vitamin A are scarce [40].

Strategies focusing on food diversification aim to increase the production and availability of, access to and subsequent consumption of foods that are rich in vitamin A and provitamin A carotenoids. Home-garden interventions are most effective when combined with promotional and educational interventions [41]. In a review that assessed the potential for food based strategies to reduce vitamin A and Iron deficiencies Marie *et al,* summarized a number of studies that showed significant increase on vitamin A as a result in home garden interventions which had strong education and behavior change component[42].

Vitamin A supplementation has also been found to decrease both the severity and incidence of deaths related to diarrhea and measles in developing countries, where vitamin A deficiency is common [43].

1.3. Orange fleshed sweet potatoes

Food-based approaches have been reviewed and judged to have a promising role in integrated strategies to reduce the burden of vitamin A deficiency. Orange flesh sweet potatoes (OFSP) are a particularly promising food, because levels of β-carotene are extremely high in many varieties [100–1600 μg retinol activity equivalent (RAE)/100 g for varieties in Africa] and it is generally well accepted by young children [44].

OFSP is also a good source of energy (293 to 460 kJ/100 g), easy to cultivate, vegetatively propagated, and fairly drought resistant once established; these characteristics make OFSP an excellent food security crop. Sweet potato is less labor intensive than most other staple crops and can be planted over a broad range of time without considerable yield loss [45].

The consumption of OFSP increased the vitamin A intake of Kenyan women and children [45]. A recent assessment indicated that OFSP could make a major contribution to controlling vitamin A malnutrition in sub Saharan Africa [46]. Replacing white-fleshed varieties with high-carotene varieties that meet local preferences could benefit an estimated 50 million children aged 6y who are currently at risk of diseases associated with vitamin A deficiency. The consumption of diets containing mostly plant sources of β-carotene, the primary source being red sweet potato, increased serum retinol concentrations in Indonesian children marginally deficient in vitamin A [47].

The most common sweet potato cultivars in Eastern and Southern Africa are white-fleshed varieties that contain negligible amounts of β-carotene [48]. Studies have confirmed that African mothers can readily accept orange-fleshed varieties, thus dispelling the notion that African tastes preclude the use of all but white-fleshed cultivars. Recent estimates done in six East and Central African countries showed the magnitude of the potential impact of replacing white-fleshed varieties with orange-fleshed cultivars. Overall, over 50 million children under the age of 6 stand to benefit from this effort [49].

1.4. Feeding practices of children under 5 years of age

The WHO recommends that women breastfeed their infants exclusively in the first 6 months of life to two years to maximize the benefits of breastfeeding [50]. However many infants in sub-Saharan Africa begin to receive cereal-based supplemental feeds well before the age (6 months) recommended for the introduction of 'safe and nutritionally adequate' complementary foods, or in rare instances, do not receive these until the second year. Only 30% of children, 6 months of age are exclusively breastfed [39].

Complementary foods given to the children even before reaching 6 months are mainly monotonous and bulky watery cereal or maize porridges of low energy and nutrient densities that rarely cover the shortfall left by breast milk in providing the energy and nutrients required to support rapid growth, build nutrient stores and assure resistance to infection. The foods are often prepared, served and stored under conditions that expose the child to frequent infection [39].

Inappropriate complementary feeding practices are said to be major causes of under nutrition in children under 5 years in Tanzania. Infants in Tanzania are particularly vulnerable to under-nutrition during transition from breast milk (as the only source of nourishment) to solid foods [51].

Data from Tanzania Demographic health survey (TDHS 2010) identified risk factors that are associated with inappropriate feeding practices in Tanzania as young child's age (6–11 months), lower level of paternal/maternal education, limited access to mass media, lack of post-natal check-ups and poor economic status of the parents [52].

A cross-sectional study done in Kilosa district in Tanzania to study the feeding practices and the extent of wasting, stunting, and iron-deficiency anemia in 3-23 months children showed

that children consumed mainly a thin porridge prepared from maize flour as complementary food. Carbohydrates contributed most energy (on average 69%), followed by fats (18.6%) and protein (on average 12.1%). The complementary food covered only 15%, 20%, and 27% of the recommended iron intake for children aged 6-8, 9-11 and 12-23 months respectively [53].

Complementary feeding practices in Tanzania, as measured by dietary diversity, meal frequency and acceptable diet, are not adequately met, and there is a need for interventions to improve the nutritional status of young children in Tanzania [51].

2. MATERIALS AND METHODS

2.1. Subjects

A convenience sample of 25 children aged 6-12 months were randomly recruited for the study. We used the data provided by Rosales and Ross, Low *et al*, and Van Jaarsveld *et al*, to calculate the number of subjects required for our study. In the study by Rosales and Ross[21], VAD was characterized by a plasma retinol concentration <0.35uM, marginal VAD as plasma retinol of 0.35-0.7uM, and vitamin A sufficiency (VAS) as plasma retinol >0.7uM. The mean RBP: TTR ratio of VAD individuals, plasma retinol <0.35, was 0.3 with a standard deviation of 0.12, therefore we used this value as our indication of VAD in our population. Even though there is no data on the increase in RBP: TTR ratio upon consumption of orange fleshed sweet potatoes, there is data on the change in serum retinol concentrations. Two independent groups show an increase in serum retinol of about 0.10-0.12uM upon consumption of orange fleshed sweet potatoes which elevates the classification of the population from marginal vitamin A deficiency to vitamin A sufficiency[26, 44]. Hence in this study an effect size of 0.08 – 0.12 was used to increase the children from being classified as VAD to VAS with a standard deviation of 0.14. We used the online calculator developed Russel Lenth on July 25, 2013 found at statpages.org/#Power.

Subject demographic data is shown in Table 1 and weight for age, length for age, and weight for length z-scores and percentiles were calculated using WHO Anthro for personal computers (version 3.2.2, 2011, Software for assessing growth and development of the world's children. Geneva, WHO 2010). Subjects were obtained from maternal and child health clinic at Mbuyuni village in Kilosa district in Morogoro region, Tanzania. The study was approved by the Michigan State University IRB and parental consent was obtained.

2.2. Nutrition Information

25 minutes nutrition education was provided to the subject's mothers who assembled at Mbuyuni primary school twice per week. The education session covered general nutrition information on feeding practices for children under five years as well as information on nutritional benefits of sweet potatoes as a source of vitamin A. The subject's mothers were also taught how to process orange fleshed sweet potatoes into flour so as to make the composite orange fleshed sweet potato and maize flour. The education session also involved a demonstration on how to prepare enriched porridge form the composite flour of maize and orange fleshed sweet potatoes.

2.3. Feeding Sessions

Since orange fleshed sweet potatoes are not commonly consumed in this community, the subjects assembled at Mbuyuni primary school twice per week from 8.00-11.00 am to receive portions of enriched porridge. Subject's mothers participated in the preparation of enriched porridge. This gave them the chance to learn how to prepare the enriched porridge and it ensured that the subjects were fed the porridge prepared in a standard method and a known quantity. Orange fleshed sweet potato flour which was used in the preparation of enriched porridge was obtained from Sokoine University of Agriculture, orange fleshed sweet potato processing plant.

2.4. 24 hours dietary recall, weight and height

24 hour dietary recall was used to assess what the subjects consumed prior to blood draw. The subject's mothers were asked to remember and report all the foods and beverages

consumed in the preceding 24 hours. The subject's mothers were asked probing questions to help them provide correct dietary information [54].

Baseline and final weight and height were measured at the beginning and end of the intervention for all 23 subjects participating in the intervention. Weight was measured with subjects wearing light clothing to the nearest 0.1kg. Length (for children under 2 yrs) was measured to the nearest 0.1 cm [55].

2.5. Dried blood spots samples

Due to lack of advanced technology for storage of blood samples in most parts of Tanzania, dried blood spot (DBS) samples were used to obtain blood for assessment of the ratio of RBP to TTR. Whatman 903 Protein Saver Cards (GE Healthcare Bio-Sciences Corp., Westborough, MA, USA) were used.

Samples were collected from free-flowing capillary blood obtained by finger or heel stick with sterile lancets at baseline and at the end of the intervention [56]. DBS samples were then stored under cool temperature in total darkness before shipped to U.S. to avoid oxidation of analytes by light. Duplicate blood spots were obtained from each subject, and the best quality samples were used in analyses. Quality was visually assessed by analyzing each spot for smearing or streaking, overlapping blood drops, and size of blood drops. Hole punches of mm, approximately 15uL, were taken and proteins were extracted by overnight incubation in 500uL 0.05% Tween 20 (Sigma-Aldrich, St. Louis, MO, USA) in Dulbecco's Modified Phosphate Buffered Saline (Sigma-Aldrich) at 4C for a dilution of approximately 1 to 33.

2.6. DBS Analyte Analysis

Post-extraction all samples were stored at -20C until analysis. Samples were diluted appropriately to be within the range of each assay. Total protein was assessed using a commercial BCA assay per manufacturer instructions at a total sample dilution of 1 to 133 (Thermo Scientific, Pittsburgh, PA, USA). Transthyretin (sample dilution of 1 to 33,000), RBP4 (sample dilution of 1 to 990), and CRP (sample dilution of 1 to 33,000) were assessed by ELISA according to manufacturer instructions (Abcam, Cambridge, MA, USA).

2.7. β- Carotene Analysis

Samples were analyzed in triplicate on three different days following a modified protocol [57]. Briefly, aliquots of each sample were weighed and extracted in 50:25:25 hexane (Sigma)/acetone (Sigma)/ethanol (Sigma, St. Louis, MO, USA). Samples were extracted by periodic vortexing for 30 minutes in the dark. One fourth of the extract was transferred to amber HPLC vials, dried under nitrogen, and reconstituted in an equal volume of 15:30:55 tetrahyrdofuran (Sigma)/acetonitrile (Sigma)/methanol (Sigma). One tenth of the prepared sample was injected and analyzed by HPLC. Conditions of HPLC analysis include an isocratic mobile phase of 90:10 methanol/acetonitrile, 0.9 mL/min flow rate, over a Prism reverse phase column (150mm x 3mm, 3 micron particle size, Thermo Electron Corp).In addition, a standard solution of β-carotene dissolved in hexane was prepared. Concentration of β-carotene standard was determined using 448nm spectrophotometry and Beer's law [58]. The ratio of β-carotene standard area under the curve to unknown area under the curve was used to calculate the concentration of unknowns. β-carotene concentration of standard and sample was used to calculate the micromoles per sample and micrograms β-carotene per 100 g sample.

2.8. Statistical Analysis

All data is presented in scatter plots and mean $\pm$ standard error of the mean reported in text. Microsoft Excel was used to calculate paired two-tailed t-tests to compare baseline data to post-intervention data with statistical significance set at $p \leq 0.05$.

3. RESULTS

3.1. Demographics

Our study population was convenience sample of 19 healthy children under 12 months of age (**Table 1**). We had 55% male and 45% female participants with an average age of 8 ± 0.49 months at the start of a one month intervention resulting in a post-intervention age of 9 ± 0.49 months. The children were generally healthy at baseline based on analysis of z-scores for w/l (z-score; 0.85 ± 0.23, %tile; 71.72 ± 5.80), w/a (z-score; -0.54 ± 0.22, %tile; 36.21 ± 5.74), and l/a (z-score; -1.98 ± 0.21, %tile; 6.80 ± 2.09) and post intervention w/l (z-score; 1.24 ± 0.19, %tile; 83.01 ± 3.64), w/a (z-score; -0.01 ± 0.19, %tile; 50.40 ± 6.14), and l/a (z-score; -1.72 ± 0.20, %tile; 9.34 ± 2.74). A significant change was observed in w/a percentile (p-value; 0.006) and z-score (p-value; 0.002), w/l percentile (p-value; 0.028) and z-score (p-value; 0.048), and l/a z-score (p-value; 0.034), but change in l/a percentile (p-value; 0.076) was not significantly different between baseline and post-intervention. As expected due to rapid growth of this age group, there was a significant difference in baseline and post-intervention length (baseline; 65.6 ± 0.8, post-intervention; 67.7 ± 0.7, p-value; <0.0001) and weight (baseline; 7.9 ± 0.2, post-intervention; 8.7 ± 0.2, p-value; <0.0001). In the one month time frame of the intervention children grew 2.07 ± 0.26cm and gained 0.82 ± 0.14Kg. Three children either gain no weight (n=2) or lost weight (n=1) and one child had no change in length (**Fig 1**).

Table 1: Demographic Characteristics[1,2]

	Baseline	Post-intervention	p-value[3]
Age (mo)	8.0 ± 0.49	9.0 ± 0.49	N/A
Sex (#)	11M/9F	11M/9F	N/A
Length (cm)	65.6 ± 0.8	67.7 ± 0.7	<0.0001
Weight (Kg)	7.9 ± 0.2	8.7 ± 0.2	<0.0001
L for A (z score)[4]	-1.98 ± 0.21	-1.72 ± 0.20	0.034
Percentile[4]	6.80 ± 2.09	9.34 ± 2.74	0.076
W for A (z score)	-0.54 ± 0.22	-0.01 ± 0.19	0.002
Percentile	36.21 ± 5.74	50.40 ± 6.14	0.006
W for L (z score)	0.85 ± 0.23	1.24 ± 0.19	0.048
Percentile	71.72 ± 5.80	83.01 ± 3.64	0.028

[1] Mean ± SEM
[2] All subjects received vitamin A supplementation before the intervention.100, 000IU (30µg RE) vitamin A for subjects 6-11months and 200,000IU (60µg RE) vitamin A for subjects 12 months.
[3] paired t-test to compare change in demographics during the study
[4] Calculated using WHO anthro software

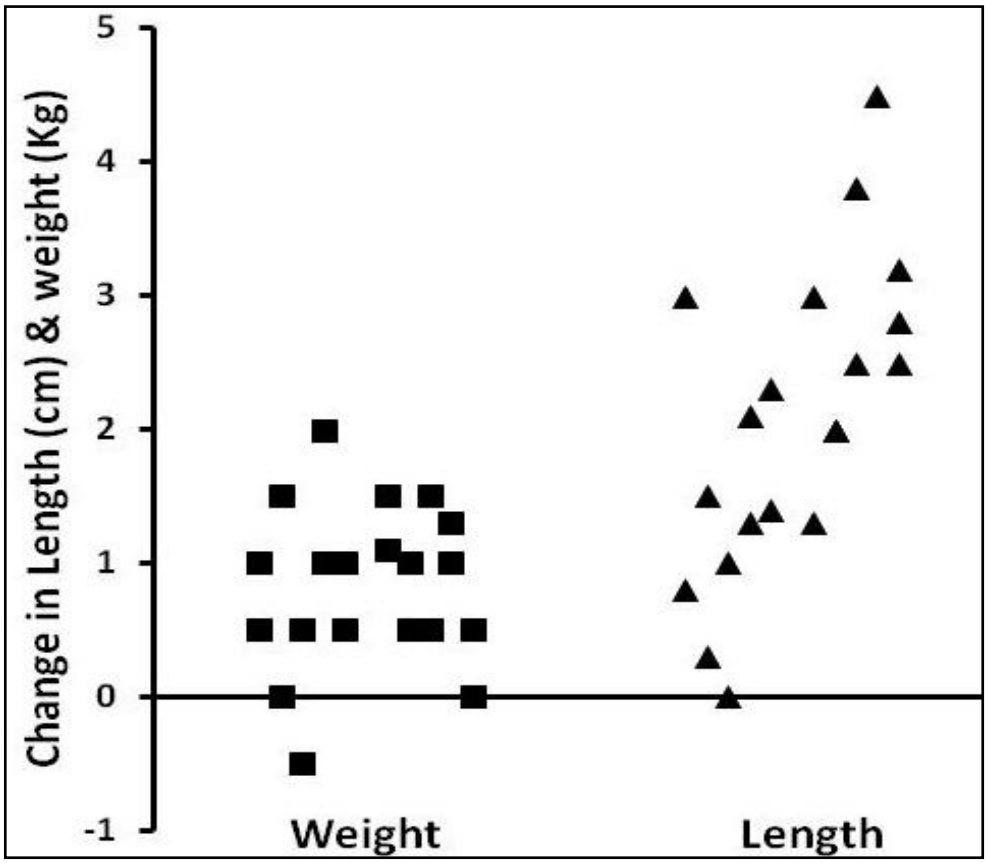

Figure 1: Change in subject weight and length during the intervention.

All but 3 subjects gained weight during the study, 2 subjects had no change in weight and 1 subject lost weight. The average weight gained was 0.82 $\pm$ 0.14Kg. All but one subject grew. The average growth was 2.07 $\pm$ 0.27cm.

3.2. Biochemical indicators of subject health status

To determine the effects of consuming OFSP-fortified porridge on vitamin A status, we relied on measuring serum analytes including total protein, TTR, RBP, and CRP, we could determine if any children were malnourished at baseline or post-intervention (**Fig 2**).

The subjects had total protein that ranged from 63.63mg/ml to 83.91mg/ml with the mean value of 67.51 mg/ml $\pm$ 2.41 at baseline. Six of the subjects (n=6) had high protein values at baseline based on the normal protein ranges (43-69mg/ml) where as only one subject had low protein value (42.31mg/ml) before the intervention. All but three subjects had normal total protein values post intervention with the mean value of 63.44 $\pm$ 2.87mg/ml. Three subjects had high protein ranges with one of the subjects having a very high protein value of 102.20mg/ml post intervention. A negative phase reactant and indication of malnutrition [59] TTR analysis indicates all children were healthy at baseline and post intervention based on the cut off value that ranges from 0.002-0.005uM [60]. The mean TTR at baseline was 0.54 $\pm$ 0.03uM and 0.41 $\pm$ 0.03uM post intervention, respectively.

Analysis of CRP indicated that no subject had infection/inflammation at the beginning or at the end of intervention based on the 0.25uM CRP cut off value[61]. The mean CRP at baseline was 0.03 $\pm$ 0.01uM and 0.04 $\pm$ 0.01uM post intervention.

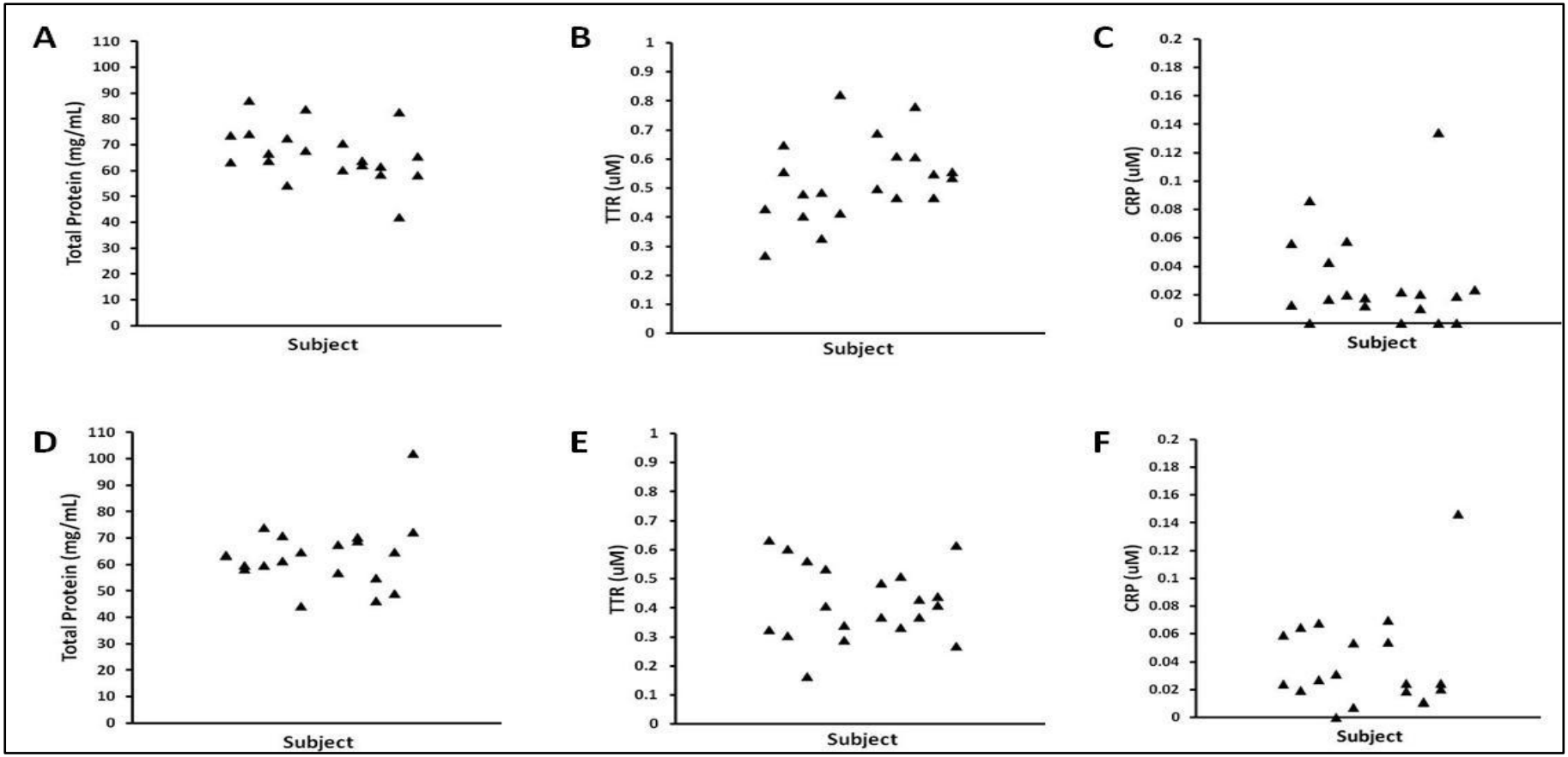

Figure 2: Biochemical indicators of subject health.

A-C: Baseline total protein (A), TTR (B) and CRP (C). D-F: Post intervention total protein (D), TTR (E), and CRP (F).Six of the subjects had high protein values that were not within the normal range (43-69mg/ml) at baseline and one subject had low total protein at baseline. The mean protein at baseline was 67.51 mg/ml $\pm$2.41 .One of the subjects had a very high protein value (102.20mg/ml) post intervention. The average post intervention total protein was 63.44$\pm$2.87mg/ml. All subjects were healthy based on TTR values at baseline and post intervention with the mean TTR of 0.54 $\pm$ 0.03uM and 0.41 $\pm$ 0.03uM at baseline and post intervention, respectively. All subjects were healthy based on CRP cut off 0.25uM at baseline and after intervention with the mean CRP value of 0.03 $\pm$ 0.01uM at baseline and 0.04 $\pm$ 0.01uM post intervention

3.3. Change in biochemical parameters during the intervention

Within the one month intervention period, we did not observe any significant change in total protein between the post and the pre intervention periods (p-value 0.4). There was no significance difference between baseline and post intervention CRP (p-value; 0.37). There was a significant reduction in TTR levels (p-value; 0.02) during the one month intervention period **(Fig 3)**

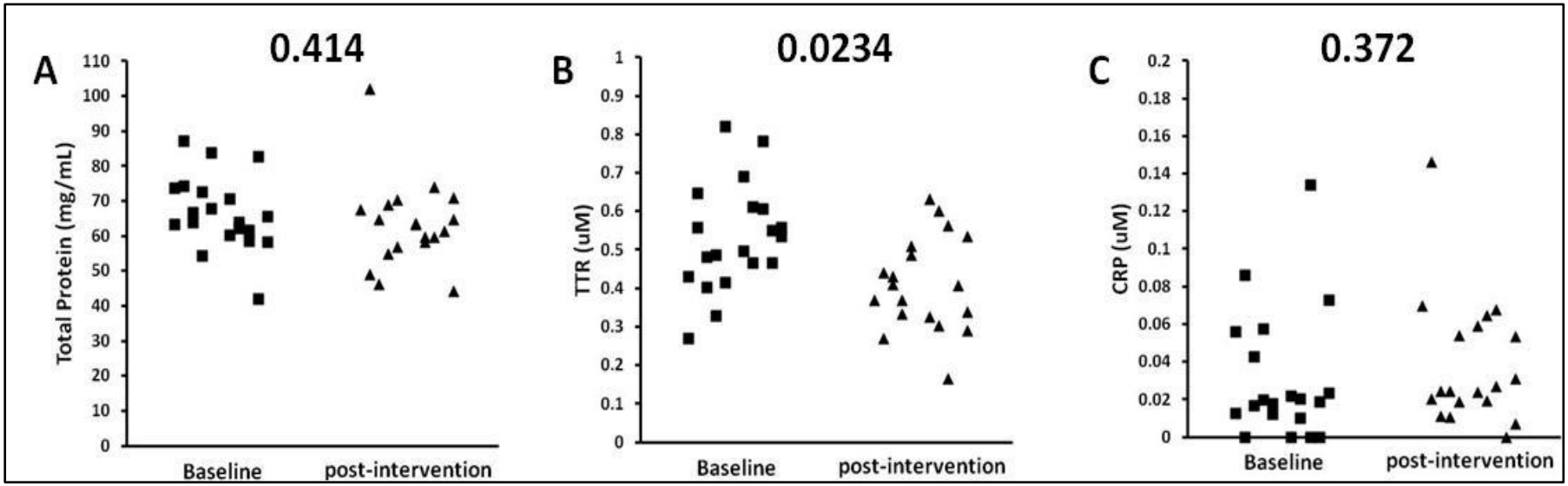

Figure 3: Change in biochemical parameters during the intervention.

A: total protein, B: TTR and C: CRP.Within the one month intervention period, we did not observe any significant change in total protein between the post and the pre intervention periods (p-value 0.4). There was no significance difference between baseline and post intervention CRP (p-value; 0.37). There was a significant reduction in TTR levels (p-value; 0.02) during the one month intervention period.

3.4. Intervention effects on vitamin A

We observed that all children were vitamin A deficient at baseline based on RBP concentration alone; cut off value of ≤0.7 μM. Two of the subjects had severe VAD at baseline. All subjects remained vitamin A deficient post-intervention with more subjects having severe VAD in comparison to those at baseline. A significant reduction in RBP concentration during the intervention was observed (p-value; 0.001) **(Fig 4A)**

At baseline all children except one were vitamin A-sufficient based on the RBP: TTR ratio (cut off value≤ 0.36μM).The mean RBP: TTR ratio at baseline was 0.91±0.06.and 0.71±0.05 post intervention. A different child from that of the baseline (n=1) was observed to be vitamin A deficient post-intervention. A significant reduction in the RBP TTR ratio was detected between baseline and post intervention samples (p-value; 0.04). Based on this finding and this ratio being a good vitamin A indicator when infection is taken into account, we can say that there was no significant intervention effect of fortified porridge on vitamin A status on the children (**Fig 4B**).

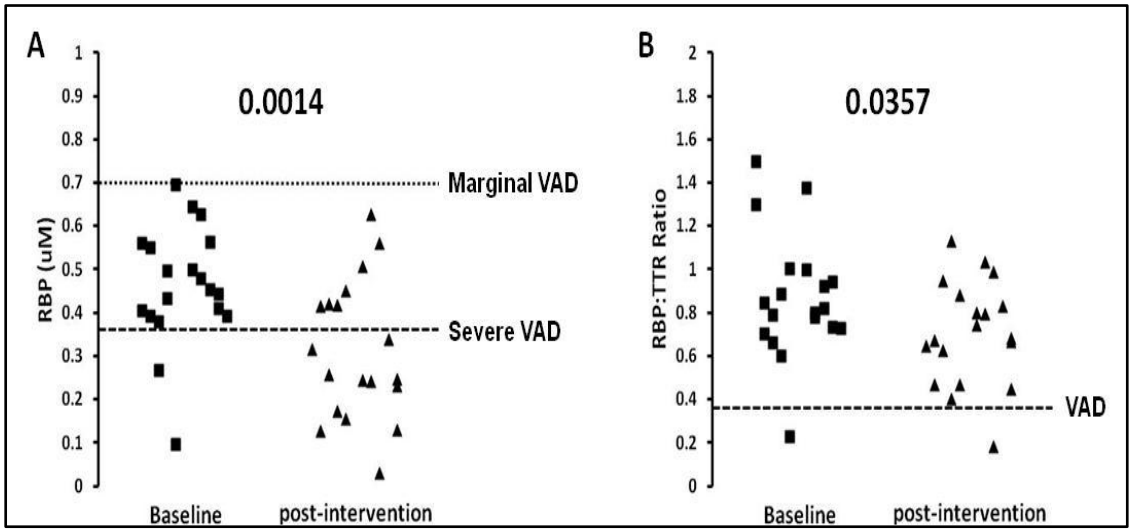

Figure 4, A and B:-Intervention effect of OFSP on vitamin A

Intervention did not improve vitamin A status of subjects. A: RBP (uM). All subjects were vitamin A deficient at baseline where two subjects had severe VAD based on blood RBP concentration. The subjects remained vitamin A deficient post intervention with more subjects having severe VAD than those at baseline (cut off value ≤0.7uM). A significant reduction of RBP concentration was observed during the intervention (p-value; 0.001). B: RBP: TTR ratio. All subjects except one were vitamin A sufficient at baseline based on the RBP: TTR ratio with a cut off value of ≤0.36 ratio. The mean RBP: TTR ratio at baseline was 0.91±0.06. A different subject was vitamin A deficient post intervention; the mean RBP: TTR ratio was 0.7±0.05 post intervention. A significant intervention effect was observed in the one month intervention period (p-value; 0.04).

3.5. OFSP-fortified porridge

The various samples of orange flesh sweet potatoes were processed in Tanzania and sent to Michigan State University for analysis. These samples consisted of two different whole orange flesh sweet potatoes, sundried sweet potatoes, sweet potato flour, and two different sweet potato-fortified porridges. The sweet potato fortified porridges contained either 5 tablespoons of sweet potato to 10 tablespoons of maize (OFSP-Porridge 1:2) or 10 tablespoons sweet potato to 5 tablespoons of maize (OFSP-Porridge 2:1). Using HPLC we assessed the β-carotene content in triplicate on three different days, relevant comparisons were analyzed by t-test (**Table 2**). There was no significant difference (p-value; 0.057) in β-carotene content of Whole A (8929 $\pm$ 549.0 µg/100g) versus Whole B (7455 $\pm$ 467.2 µg/100g). Therefore, we combined the data for these two samples (8149 $\pm$ 392.0 µg/100g) and obtained a new RAE value (679.1 $\pm$ 32.7 µg/100g) for comparison to remaining samples. Compared to published USDA analysis of OFSP (raw, unprepared, #11507) β-carotene (8509 $\pm$ 369.5 µg/100g) we did not observe significantly different β-carotene content in whole OFSP (p-value; 0.409) from Tanzania. Sundried sweet potatoes (4000 $\pm$ 207.9 µg/100g) were compared to the combined Whole A and Whole B data, which was significantly reduced by about one half ($p<0.0001$). Sweet potato flour (659.1 $\pm$ 25.19 µg/100g) was significantly reduced by roughly 80% compared to sundried ($p<0.0001$). Additionally, OFSP-Porridge 2:1 (76.59 $\pm$ 4.965 µg/100g) and OFSP-Porridge 1:2 (50.76 $\pm$ 5.482 µg/100g) were found to be significantly different from each other (p-value; 0.003). Therefore, there are significant losses in preparing and processing of OFSP for porridge fortification.

Table 2: β-carotene and RAE content of OFSP samples from Tanzania[5]

	(µg/100g)[6]	RAE/100g[7]
Whole OFSP sample A	8929 ± 549.0	744.1 ± 45.8
Whole OFSP sample B	7455 ± 467.2	621.3 ± 38.9
Combined Whole OFSP	8149 ± 392.0	679.1 ± 32.7
US OFSP[8]	8509 ± 224.1	709.1 ± 18.7
Sundried OFSP	4000 ± 207.9	333.3 ± 17.3
OFSP Flour	659.1 ± 25.19	54.9 ± 2.1
OFSP-Porridge 2:1[9]	76.59 ± 4.965	6.4 ± 0.4
OFSP-Porridge 1:2[10]	50.76 ± 5.482	4.2 ± 0.5

[5] Values are mean ± SEM, n=8-9 (triplicate samples on three different days).
[6] HPLC analysis of β-carotene from samples.
[7] Calculated RAE by dividing by 12[62]
[8] Reported β-carotene content of US OFSP (USDA nutrient database; sweet potato, raw, unprepared, #115)
[9] 10 Table spoons OFSP flour: 5 Table spoons Maize flour
[10] 5 Table spoons OFSP flour: 10 Table spoons Maize flour

4. DISCUSSION

Vitamin A supplementation is one of the best proven, safest and most cost-effective interventions in public health. In populations where vitamin A deficiency is of public health importance , vitamin A supplements are recommended as prophylaxis and as treatment for at-risk groups and sick individuals respectively[29]. A meta-analysis of several large vitamin A trials has shown that improving vitamin A status reduces mortality rates in subjects between 23–34% among children six months to five years of age if vitamin A supplementation is given at least twice per year at coverage rates of at least eighty percent[63].In Tanzania, efforts to combat VAD started through a disease-targeted approach in 1987.Primary health care facilities were the centers providing supplementation which targeted children between 6–59 months suffering from xerophthalmia or diseases precipitating VAD. This strategy caused many young children at risk of VAD in Tanzanian communities not to be treated with vitamin A[64].

Ten years later, Vitamin A supplementation was introduced through the routine Essential Drugs Programme (EDP) for post partum mothers and children at 6–59 months together with measles vaccination. The supplementation coverage levels were estimated at 94% and 99% in 1999 and 2000 respectively[64]. These findings and similar lessons from the Philippines[65], led UNICEF to initiate and support the national bi-annual implementation of vitamin A supplementation in children 6 months to 5 years in Tanzania starting in 2001[27]. In the present study all subjects received vitamin A supplementation before the intervention.100, 000IU (30µg RAE) vitamin A for subjects 6-11months and 200,000IU (60µg RAE) vitamin A for subjects 12 months. The reason RBP: TTR ratio decreased even after supplementation could be likely due to the subjects having other nutrient deficiencies such as zinc and Iron.

The existence of concomitant nutrient deficiencies may impair the bioavailability of the supplements, since some of these nutrients (including fat, protein, and zinc) could be limiting factors for the absorption and utilization of the lipid-soluble vitamin[3]. The effect also varies with the regimen characteristics, such as the dosage and interval of administration: Smaller and more frequent doses of vitamin A seem to be more protective than large periodic doses[66].

The goal of this study was to determine whether enriching maize porridge with orange fleshed sweet potatoes could improve vitamin A status of 6-12 months children. We observed a significant intervention effect on vitamin A status of the subjects within the one month period as measured by RBP: TTR ratio (p-value 0.04). Unexpectedly, the intervention reduced TTR, RBP and the RBP: TTR ratio which resulted in reduced vitamin A status. However, the β-carotene content of the OFSP and porridge samples was significantly reduced compared to raw OFSP samples. Therefore, the high losses in β-carotene in OFSP fortified porridge did not provide vitamin A to the subjects. During the intervention period, we did not observe any significant changes between baseline and post intervention biochemical indicators of the subjects except that TTR levels were reduced post intervention (p-value;0.02).There was no significant change in total protein (p-value;0.4) and CRP (p-value;0.37) at baseline and after the intervention. The subjects gained the average weight of 0.82 $\pm$ 0.14Kg and had the average growth of 2.07 $\pm$ 0.27cm during the intervention period.

Since children in developing countries are high risk for infectious disease [35], RBP:TTR ratio was a good method for vitamin A assessment in this population. The extent to which indicators of micronutrient status are affected by the acute phase response depends not only on the specific indicator used, but also on the severity of infection, the time phase of the acute phase response and the nutritional status of the individual [67]. It was demonstrated in rats

that the molar ratio of plasma RBP:TTR selectively diagnosed VA deficiency during inflammation [68]. Several studies have examined vitamin A status in both clinically ill subjects and in apparently healthy subjects in relation to the acute phase response. Retinol concentrations decreased during illness and increased again during recovery. In healthy children, plasma retinol was related to the concentrations of acute phase proteins [69, 70]. The subjects in our study were apparently healthy with total protein, CRP, and TTR within the reported normal range for this age group. Therefore, the RBP: TTR ratio should provide an accurate indication of vitamin A status.

Our analysis of RBP however indicated that the children remained vitamin A deficient post-intervention with more subjects having severe VAD in comparison to those at baseline. We observed a significant reduction in RBP concentration during the intervention (p-value; 0.001). Although serum levels of RBP have been suggested as a surrogate for serum retinol, the validity of their use to evaluate relative vitamin A status needs careful consideration. Several other factors, such as energy balance, protein nutriture affect RBP levels and could confound their interpretation[71]. RBP is also affected negatively by the presence of infection or inflammation[72]. Different methods have been proposed to assess vitamin A status of the populations with VAD problem. Conventionally, clinical signs and symptoms of xerophthalmia were used to identify populations with VAD. While these tests are still used in areas where vitamin A depletion is severe, a subclinical vitamin A deficiency is more prevalent in these areas.

Rapid dark adaptation method is considered as a good marker of early physiological impairment in VAD [73]. This method has the advantage of being noninvasive, portable, quick and does not require handling of biologic specimens also correlates well with other indicators of VAD such as serum retinol and night blindness; The biggest drawback of this

method however, is that it may not be suitable for young children (<2 y) and that more is still needed to validate the proposed cut-off values (>−1.11 log cd/m^2) for purposes of screening and evaluation [74].

As an indicator of VAD , serum retinol levels of <0.7 μmol/L in children under five years can reflect vitamin A status of the individual especially when stores are limited [11]. The major drawback of serum retinol is that blood samples are required and healthy individuals, serum retinol concentrations are homeostatically controlled and do not begin to decline until liver reserves of vitamin A are dangerously low [47]. Moreover, serum retinol is affected by infection and protein energy malnutrition. [70]. High pressure liquid chromatography (HPLC) was recognized as the method of choice for serum retinol estimation, but the problem is the need for quality and external controls for laboratories especially in developing countries [75]. RBP plays a central role in the uptake and transport of retinol. RBP has been used as a surrogate marker for serum retinol. Correlations coefficients (r^2) between serum RBP and serum retinol range from 0.4 to 0.8 [76]. It is easier to measure since it requires less blood and has lower costs, however it is also affected by infection and protein energy malnutrition [72].

The relative dose response (RDR) test is a gold standard for assessment of vitamin A status. It involves giving a small dose of retinyl ester and taking a blood sample at time 0 and 5 h after the dose and calculating a percent increase [77].The RDR test operates on the principle that during vitamin A depletion *apo*-RBP accumulates in the liver. By providing a challenge dose of retinyl ester, the retinol will bind to the excess RBP and be transported out into the serum as the *holo*-RBP-retinol complex [18]. A modification of this method was made by using 3,4-didehydroretinyl acetate as the challenge dose and subsequently termed the modified relative dose response (MRDR) test because circulating concentrations of 3,4-

didehydoretinol are very low in human plasma, a single blood sample is all that is required 4 to 6 h after dosing and a ratio of 3,4-didehydoretinol to retinol is calculated [77].

Due to lack of advanced technology for storage of blood samples in most parts of Tanzania, DBS samples were used these being the most feasible method for blood collection given the conditions of the study area. DBS offers a number of advantages over conventional blood collection. As a less invasive sampling method, DBS offers simpler sample collection and storage and easier transfer, with reduced infection risk of various pathogens, and requires a smaller blood volume. The use of DBS samples has eliminated the need for a cold chain and refrigeration of specimens in the field, reducing considerably the complexity of storage in remote areas [41]. Until recently, it was considered not possible to measure VA in DBS due to its instability. However, this assumption was dismissed when Shi *et al* demonstrated that holo-RBP could be measured in DBS using capillary electrophoresis with laser-excited fluorescence detection and that it correlated with serum retinol [78]. This demonstration was an important achievement since the holo-RBP complex was thought to be unstable when exposed to air and iron from the red blood cells. Hence vitamin A, like many other biochemical markers, could now be measured in blood samples collected from a finger- or heel-prick directly onto collection cards[78]. It has also been demonstrated that DBS have the advantage of preserving some unstable analytes from degradation or delaying the degradation process[79]. Moreover proteins may remain immunologically active in DBS for long periods and thus dismissing the question of stability of our different proteins(CRP,TTR and total protein) that were analyzed in this study [76].

Orange fleshed sweet potatoes have been shown to improve vitamin A status of 13mo children in extremely poor resource area in rural Mozambique. After controlling for infection/inflammation and other confounders, mean serum retinol of the children increased

by 0.100 μmol/L (SEM 0.024; $P < 0.001$) [44]. β-carotene rich orange fleshed sweet potatoes also improved vitamin A status of primary school children assessed with relative modified dose response (RMDR). In this study the estimated intervention effect for the ratio of 3,4-didehydroretinol to retinol (DR:R) was −0.008 (95% CI: −0.015, −0.001; $P = 0.0203$), which indicated a greater improvement in vitamin A liver stores in the treatment group than in the control group [26]. However it is unknown whether this improvement in vitamin A status would also be true for children under one year of age studied in our analyses.

Human TTR is a protein with a dual transporting function. It is a carrier protein for thyroxine and an indirect vehicle for vitamin A by the binding of retinol-binding protein. In malnutrition, plasma TTR is markedly reduce and returns toward normal on repletion more rapidly than other visceral proteins [80]. In this study, our TTR analysis indicated that all subjects were health at baseline and post intervention with a significant increase within the one month intervention period (p-value; 0.0234). The increase in TTR and reduction in RBP results in a reduced RBP: TTR ratio and hence an indication of reduced vitamin A status.

Different processing methods cause losses of β-carotene in OFSP. In our study there were substantial losses of β-carotene in the OFSP samples from Tanzania. β-carotene was significantly reduced in sundried chips of OFSP by about one half ($p<0.0001$) as compared to whole OFSP. β-carotene was significantly reduced from 744.1 $\pm$ 45.8 RAE/100g in whole OFSP to 4.2 $\pm$ 0.5 RAE/100g in enriched porridge mixed in 1:2 ratio (OFSP: maize flour). In a study done to assess the effects of various traditional processing methods on β-carotene content of OFSP; the retention of all-*trans*-*β*-carotene was 78% when OFSP were boiled in water for 20 min. When OFSP were steamed for 30 min the retention was 77%, whereas deep-frying OFSP roots for 10 min resulted in retention levels of 78%. Drying slices of OFSP roots at 57°C in a forced-air oven for 10 h reduced the all-*trans*-*β*-carotene content by 12%.

Solar drying and open-air sun drying OFSP slices to moisture content of ⩽10% resulted in all-trans-β-carotene losses of 9% and 16%, respectively. These losses of β-carotene during processing might account for small decreases in vitamin A status of the subject within the one month intervention period.

According to the U.S National Institute of Health (NIH), the Recommended Dietary Allowance (RDA) for vitamin A for children 0-6 and7-12 months is 400 and 500µg RAE respectively for both male and female. The amount of β-carotene in a jar of 2.5oz Gerber first food, vegetables and sweet potatoes is 2742.73µg which is equivalent to 228.56µgRAE. In this light, 0-6 and 7-12 months children need to consume 4.4oz and 5.5oz respectively of Gerber first food to reach the RDA .In this study, the analysis of β-carotene indicated that for children to reach the RDA for vitamin A, they need to consume at least 10kg of 1:2 OFSP porridge to meet the RDA

OFSPs, which are naturally rich in β-carotene, are an excellent food source of provitamin A[45].Coupled with nutrition education OFSP varieties can make a significant contribution to a viable long-term effective and sustainable food-based approach to prevent vitamin A deficiency in developing countries not only in young children but also in other populations .

5. FUTURE DIRECTIONS

Food diversification through the production of yellow-orange β-carotene–rich vegetables is seen as a viable long-term strategy to complement supplementation and fortification programs in combating vitamin A problem in Sub Sahara Africa. Acceptability and ease of introduction of OFSP should be explored in areas where there is the potential to produce OFSP but where there has been no previous cultivation of any type of sweet potato [46]. Not only will OFSP aid in amelioration of vitamin A deficiency, but OFSP contains several other nutrients that will supplement diets. OFSP is considered as an excellent source of natural health-promoting compounds, such as anthocyanins. The protein content of sweet potato leaves and roots range from 4.0% to 27.0% and 1.0% to 9.0%, respectively[81].

OFSP is a promising and healthier alternative to synthetic coloring agents in food systems given its high concentration of anthocyanin and β-carotene, combined with the high stability of the color extract it make.

Starch and flour processing from sweet potato can create new economic and employment activities for farmers especially women and rural households, and can add nutritional value to food systems. Repositioning sweet potato production and its potential for value-added products will contribute substantially to utilizing its benefits and many uses in human food systems.

Despite the American Association of Pediatrics recommendation to breastfeed for at least one year, this recommendation is not currently feasible in Tanzania. It is unlikely that this will change in the near future. Therefore, finding a sustainable and adequate nutrient dense porridge formulation is of utmost importance. Adequate nutrition in these early

developmental has implications for health later in life. This particular group is important to study because children in Tanzania are particularly vulnerable to under-nutrition during transition from breast milk (as the only source of nourishment) to solid foods. It is hence of significance importance to find ways to ameliorate this problem.

Research is also needed to find ways to minimize β-carotene losses during processing of OFSP. Education should be provided to the community especially women on how to process OFSP in order to retain as much β-carotene as possible. Addition of antioxidants during processing and minimizing exposure to heat and light will reduce degradation of β-carotene. However access to these antioxidants and technologies remains limited, so other complementary foods should be incorporated to reduce the loss of β-carotene, but also provide additional nutrients that are lacking from the diet.

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