Title: Use of stable isotope techniques to evaluate Vitamin A intervention programmes in children in Tanzania

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ACRONYMS

AGP	α1-acid glycoprotein
BCC	Behaviour Change Communication
CRP	C – Reactive Protein
D_2O	Deuterium oxide
ELISA	Enzyme Immuno-sorbert Assey
FAO	Food and Agricultural Organization of the United Nations
FFM	Fat free mass
FFQ	Food Frequency Questionnaire
FM	Fat mass
FQ	Food Frequency Questionnaire
FTIR	Fourier transform infrared spectroscopy
HAZ	Height-for-age
IAEA	International Atomic Energy Agency
IU	International Units
MOHSW	Ministry of Health and Social Welfare
MUAC	Mid-upper arm circumference
RBP	Retinol binding protein
SMEs	Small and Medium Enterprises
TBW	Total body water
TDHS	Tanzania Demographic Health Survey
TFNC	Tanzania Food and Nutrition Centre
UNICEF	United Nations Children's Fund
VAD	Vitamin A deficiency
WAZ	Weight-for-age
WHO	World Health Organizations
WHZ	Weight-for-height

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Executive Summary

Vitamin A deficiency (VAD) is a common form of micronutrient malnutrition affecting mainly children and women in developing countries. According to estimates by the World Health Organization (WHO), about 190 million children < 5 years and 19.1 million pregnant women are vitamin A deficient. In Tanzania, VAD is a chronic and widespread public health problem affecting mainly children, adolescent girls and women of reproductive age. According to the TDHS (NBS, 2010) data, the prevalence of VAD (as defined by serum retinol (SR) < 0.7 μmol/L) among children in Tanzania aged 6-59 months is 33%.

In order to prevent and control VAD and reduce childhood mortality, the government has focused on bi-annual vitamin A (VA) supplementation for children 6 – 59 months. Additionally, breastfeeding promotion, production and consumption of micronutrient rich foods, biofortification, use of micronutrient powder, nutrition education and more recently a VA oil fortification program, are available as complementary strategies. The goals of these intervention programmes is to raise the vitamin A status of deficient individuals yet at the same time does not lead to harmful or excessive intakes. However, monitoring and evaluating the impact of vitamin A interventions is challenging because liver VA concentrations which are considered the gold standard of VA status are difficult to assess. Serum retinol which is commonly used as a biochemical indicator is homeostatically regulated over a wide range of liver reserves and is only affected by status in overt deficiency, and thus is limited in responsiveness to supplementation especially when VAD is mild. Furthermore, in most developing countries infections are common, which may reduce SR, possibly independent of VA status. Thus, the assessment of VA status should be accompanied by measurements of inflammation markers such as C-reactive protein and plasma α1-acid glycoprotein.

Vitamin A supplementation and increased dietary intakes of vitamin A rich foods may result in excessive intake of vitamin A and larger liver reserves and lead to acute or chronic toxicity.

In view of existing multiple vitamin A intervention programmes in the country and the detrimental effects of vitamin A deficiency or toxicity levels on human health, accurate assessment of vitamin A status is necessary to make informed decisions regarding intervention programs. The aim of the proposed study is to assess the efficacy and effectiveness of the vitamin A intervention programme in children in Tanzania using ^{13}C-labeled vitamin A.

Research questions:

- Are children who have access to multiple vitamin A intervention programs at risk of excessive intake of vitamin A?
- Do infections affect vitamin A stores in children with access to vitamin A supplementation twice yearly?

Overall objective

To provide new knowledge and evidence on the application of the Retinol isotope dilution (RID) method to assess vitamin A status and the risk of excess vitamin A intake in children under the age of 3 - 5 years, where single and multiple interventions are in place to reduce vitamin A deficiency.

Specific Objectives:

- Determine vitamin A status in children aged 3 to 5 years using ^{13}C-retinol.
- Compare stable isotope techniques with conventional methods (serum retinol, RBP).
- Assess dietary vitamin A intake of children using 24 h recall methods.
- Assess inflammation indicators among children particularly CRP and α1-acid glycoprotein (AGP).

- Assess the effectiveness of vitamin A interventions in improving the status of children.

- Identify socio-economic and environmental factors affecting vitamin A status among children. Assess knowledge and practices of mothers on prevention and control of vitamin A deficiency.

Materials and Methods

Study area

The study will be carried out in Iringa and Bagamoyo districts. Iringa district is selected because of multiple vitamin A intervention programmes including supplementation twice a year and micronutrient powders and it is under UNICEF support. Bagamoyo district is selected because children are receiving only vitamin A supplementation. To evaluate the impact of vitamin A supplementation and other vitamin A interventions on vitamin A status of children under the age of 5, retinol isotope dilution (RID) method will be used. An oral dose of vitamin A labelled with the stable isotope of carbon (^{13}C) will be administered to subjects, a blood sample will be collected after the dose has mixed with endogenous vitamin A and the plasma isotopic ratio of labelled to unlabelled vitamin A will be measured, using gas-chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS). The total amount of vitamin A in the body will be estimated using a prediction equation.

This will be a longitudinal study that aims to recruit 110 children aged 3 – 5 years living in Iringa and Bagamoyo districts. The study will comprise two groups. Group one (GI) will be children exposed to high vitamin A intervention programmes while the second group (GII) will comprise children exposed to low vitamin A intervention programmes. All study participants will be screened for the presence of intestinal helminthes before administration of the stable isotope.Children with helminthiasis will be dewormed. On day 14, the the first blood samples will be obtained from each participant and thereafter they will receive an oral

dose of [$^{13}C_2$] retinyl acetate, which will be followed by a high-fat, low–vitamin A snack. Fourteen days later that is on day 28 the second blood sample will be drawn.

Inclusion criteria

Will be 3-5 y old children living in the study area who were considered relatively healthy (no clinical infection or fever, weight-for-age and weight-for-height Z-scores >-3, and hemoglobin >70 g/L at recruitment), who had received antihelminthic treatment the week prior to recruitment, and had not received a high-dose VA supplement in the past 6 months.

Data Collection and Research Instrument

A well-structured validated and pre-tested questionnaire will be administered to the child's parents/guardians to collect information on socioeconomic and demographic characteristics, living conditions of their child.

Blood collection, processing, and transport procedures

Trained laboratory technician will collect venous 5 – 7 ml blood samples from each child following standard procedures.

Assessment of inflammation markers (Acute phase proteins)

Serum C-reactive protein (CRP) and plasma α1-acid glycoprotein (AGP) will be assayed using enzyme immunoassay kits as per manufacturer's instructions.

Analysis of 13C-labeled vitamin A in blood samples

Analysis of blood samples for ^{13}C-retinol will be done by using a GC-C-IRMS method in collaboration with the University of Wisconsin-Madison.

Total body stores and liver concentrations of vitamin A

TBS and liver concentrations of VA will be determined using ^{13}C-RID and applying the mass balance equation.

Assessment of Inflammation and Biomarkers of Iron

CRP and α1-acid glycoprotein (AGP) will be determined by ELISA method. Hemoglobin will be measured by HemoCue System.

Anthropometry variable measurements

Anthropometric measurements will be taken following standard procedures. Measurements to be taken from study participants will include weight and height. Nutritional parameters of

height-for-age (HAZ), weight-for-height (WHZ) and weight-for-age (WAZ) Z-scores will be calculated.

Assessment of dietary intake

Dietary intake will be assessed using (1) FQ (Food Frequency Questionnaire) method and (2) a 24-h recall.

Helminthic infection assessment

Stool samples will be collected from each study participant and examined for helminthic infection. Stool samples will be microscopically examined for helminthic infection using the Kato-Katz technique – a cellophane faecal thick smear method.

1.0 Introduction

Vitamin A deficiency (VAD) is a major public health problem in developing countries. It is estimated that about 190 million preschool-age children, mostly from Africa and South-East Asia are vitamin A deficient (WHO, 2009). VAD alone is responsible for almost 6% of child deaths under the age of 5 years in Africa and 8% in South-East Asia (WHO, 2009). VAD can result in anemia, reduced resistance to infection, impaired cellular differentiation, xerophthalmia, and ultimately blindness and death. Major causes of VAD include inadequate intake of vitamin A to satisfy physiological needs and high frequency of infections (MOHSW, 2010). Other causes are those related to poverty, for example social underdevelopment (particularly affecting women); inadequate environmental sanitation; and insufficient water supply for growing food, drinking and maintaining adequate personal hygiene and malnutrition.

In Tanzania, VAD is a chronic and widespread public health problem affecting mainly children, adolescent girls and women of reproductive age. According to the TDHS (NBS, 2010) data, the prevalence of VAD (as defined by serum retinol < 0.7 μmol/L) among children in Tanzania aged 6-59 months is 33%. The prevalence does not vary significantly with the child's age; however, more boys are affected (35.3%) than girls (31%). Prevalence of VAD is only slightly higher among children residing in the rural areas (33.3%) than those in the urban setting (31.9%). VAD prevalence is highest in North Pemba (51%) followed by Kagera (46.7%). Furthermore, the data revealed that VAD prevalence is 37% in adolescent girls aged 15-19 years.

1.1 Control of vitamin A deficiency

In recent years, many countries have executed intervention programs to control and prevent VAD. Major interventions include periodic, high dose vitamin A supplementation, food fortification and food-based approaches that encourage diet diversification and promote vitamin A rich foods including bio-fortified foods (staple crops bred to make them richer in micronutrients). Other complementary interventions are promotion of exclusive breastfeeding, home fortification with multi-micronutrient powders or lipid-based nutrient supplements, and dietary diversification with vitamin A-rich foods.

In Tanzania, vitamin A supplements are delivered twice a year to children aged 6 to 59 months. Distributing vitamin A capsules twice a year is a cost-effective strategy to immediately improve the vitamin A status in children. Provision of high doses of vitamin A every 6 months until the age of 5 years was based on the principle that a single, large dose of vitamin A is well-absorbed and stored in the liver, and then mobilized, as needed, over an extended period of time (West and Sommer, 1987). A dose of 100 000 International Units (IU) in infants 6–11 months of age and 200 000 IU in children 12–59 months of age is considered to provide adequate protection for 4–6 months, with the exact interval depending on the vitamin A content of the diet and the rate of utilization by the body (WHO/UNICEF/IVACG, 1997; Swaminathan et al. 1970).

Food fortification, which adds essential vitamins and minerals to foods, is an effective strategy to increase the dietary intake of vitamin A and prevent micronutrient deficiencies in developing countries. Fortification of wheat and maize flour with iron, zinc and folic acid and vegetable oil with vitamin A has been ongoing in Tanzania since early 2013. It is expected that this approach will gain momentum because efforts are being made to involve as many Small and Medium Enterprises (SMEs) to embark on implementing food fortification.

Dietary diversification enhances the overall nutritional status of the population and is more sustainable in the long-term. This approach requires nutrition education to change dietary habits, as well as providing better access to vitamin A or provitamin A-rich foods, such as mangoes, papaya, or dark green leafy vegetables. Encouraging home gardening to grow such foods is part of extension services among communities in Tanzania. Within this approach for example, orange-fleshed sweet potatoes provide a locally-available, vitamin-A rich food which can contribute in combating VAD amongst children and women in Tanzania.

1.2 Factors that may affect plasma isotopic ratios of labelled to non-labeled retinol

Factors that may potentially affect the plasma isotopic ratio of labeled to non-labeled vitamin A, as well as estimates of vitamin A pool size, are dietary vitamin A intake during the mixing period, intestinal parasites, infection, inflammation, iron and zinc status, and intestinal malabsorption. Studies have shown that intestinal parasites, particularly roundworms, impair the absorption of many nutrients, including vitamin A and, thus, may render improvements in dietary intake of vitamin A ineffective (Ahmed, 1999; Mahalanabis, 1976). Serum vitamin A and carotenoids were significantly lower in Ascaris infected children in India, Nepal, and Panama (Taren et al., 1987; Curtale et al. 1994; Friis et al. 1997; Khandait et al. 2000). In India, school children infected with A. lumbricoides had significantly impaired absorption of retinol compared to normal controls (Sivakumar and Reddy 1975). In addition, the presence of fever (Mitra et al, 1998) and diarrhea (Akinyinka et al, 2000), with a consequent elevation of acute phase proteins, cause a reduction in serum retinol concentrations (Thurnham et al, 2002) that can mislead the interpretation of the results (Stephensen, 2000).

1.3 Assessing vitamin A status and deficiency

According to Handbook on Vitamin A Tracer Dilution Methods to assess status and evaluate intervention programmes (Haskell et al. 2005), vitamin A is stored primarily in the liver, and thus liver vitamin A concentration is considered the best indicator of vitamin A status. However, because obtaining liver specimens is difficult and usually not justified, indirect assessment techniques such as serum retinol concentration and the relative dose response

tests are commonly used to assess vitamin A status. Serum retinol concentrations have been used widely to identify populations at risk of vitamin A deficiency (Tanumihardjo, 1994). However, there are limitations associated with this indicator. For example serum retinol concentrations are homeostatically controlled and do not begin to decline until liver reserves of vitamin A are dangerously low. Furthermore, retinol-binding protein (RBP) is a negative acute phase protein; therefore, serum retinol and RBP concentrations will fall during times of infection. 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 (Tanumihardjo,1996).The status of other nutrients, particularly iron deficiency, may also negatively affect serum retinol concentrations (Rosales et al., 1999). Iron deficiency also may decrease the mobilization of vitamin A from liver storage (Jang et al., 2000).

Another indirect assessment method is the stable isotope dilution technique, which provides a quantitative estimate of the size of the exchangeable body pool of vitamin A. The stable isotope dilution technique has the advantage that it is the only indirect assessment method that provides a quantitative estimate of vitamin A status across the continuum of status, from deficient to excessive vitamin A pool sizes. Thus, the technique can also be used for assessing vitamin A pool size in populations at risk of excessive status because of exposure to too much vitamin A either through the use of dietary vitamin A supplements and/or excessive consumption of vitamin A-rich or fortified foods. The analogs of vitamin A that are used for assessing vitamin A pool size are labelled with stable isotopes (deuterium or ^{13}C) and are not radioactive. There is no known health risk associated with ingestion of stable isotope labelled vitamin A. The stable isotope dilution technique has been used successfully to assess total body vitamin A pool size in children and/or adults in the United States,

Vitamin A supplementation and increased dietary intakes of vitamin A rich foods may result in excessive intake of vitamin A and larger liver reserves and lead to acute or chronic toxicity. In view of existing multiple vitamin A intervention programmes in the country and the

detrimental effects of vitamin A deficiency or toxicity levels on human health, accurate assessment of vitamin A status is necessary to make informed decisions regarding intervention programs. Of all the methods that have been used to evaluate vitamin A status, only isotope dilution using vitamin A labelled with a stable isotope (^{2}H or ^{13}C) can quantitatively estimate total body and liver stores of vitamin A.

Therefore the proposed study aims at assessing the efficacy and effectiveness of the vitamin A intervention programme in children in Tanzania using ^{13}C-labeled vitamin A. Stable isotope techniques are the only methods that can quantitatively estimate total body stores of vitamin A. The generated data and information will be evidence for strong advocacy to policy makers and stakeholders, which will subsequently inform their decisions on, appropriate nutrition intervention programmes.

1.4 Research questions:

- Are children who have access to multiple vitamin A intervention programs at risk of excessive intake of vitamin A?

- Do infections affect vitamin A stores in children with access to vitamin A supplementation twice yearly?

1.5 Overall objective

To provide new knowledge and evidence on the application of the Retinol isotope dilution (RID) method to assess vitamin A status and the risk of excess vitamin A intake in children under the age of 3 - 5 years, where Low and high interventions are in place to reduce vitamin A deficiency.

1.6 Specific Objectives:

- Determine vitamin A status in children aged 3 to 5 years using ^{13}C-retinol.
- Compare stable isotope techniques with conventional methods (serum retinol, RBP).
- Assess dietary vitamin A intake of children using 24 h recall method.
- Assess inflammation indicators among children particularly CRP and α1-acid glycoprotein (AGP).
- Assess the effectiveness of vitamin A interventions in improving the status of children.
- Identify socio-economic and environmental factors affecting vitamin A status among children.
- Assess knowledge and practices of mothers on prevention and control of vitamin A deficiency.

2.0 Materials and Methods

2.1 Study area

The study will be carried out in Iringa and Bagamoyo districts. Iringa district is selected because of multiple vitamin A intervention programmes including supplementation twice a year and micronutrient powders and it is under UNICEF support. Bagamoyo district is selected because children are receiving only vitamin A supplementation.

2.2 Sample size

Sample size estimation will be according to Hedeker et al. (1999) as shown below:

$$N = \frac{2(2_{\alpha} + Z_{ß})^2 (1 + (n - 1)\rho)}{n [(\mu_1 - \mu_2)/\sigma]^2}$$

Where:-

σ^2 = is the assumed common variance in the two groups

$\mu_1 - \mu_2$ = is the difference in means of the two groups

n = is the number of the time points

ρ = is the assumed correlation of the repeated measures

$Z\alpha$ = 1.96 2 tailed 0.05 hypothesis test

$Z_{ß}$ = 0.842 power 0.8

Effect size $\mu_1 - \mu_2/\rho = 0.5$

n = 2 time points

ρ = 0.6 correlation of repeated measures

$$N = \frac{2(1.96 + 0.842)^2 \ (1 + (2 - 1) \times 0.6)}{2 \times (0.5)^2} = \frac{(15.7)\ (1.6)}{(2)\ (0.25)} = 50.3$$

Plus 10% attrition and the total sample size become 55 participants per group. In total about 110 participants will be required for this study.

2.3 Study design

This will be a longitudinal study involving children aged 3 – 5 years. The study will comprise two groups. Group one (GI) will be children with access to high vitamin A intervention programmes while the second group (GII) will comprise children who are exposed to low vitamin interventions. One month before supplementation the procedure for assessing impact of vitamin A programmes using stable isotope will begin. Recruitment will begin on day one and it will take three days. From day four up to day 9, participants will be screened for the presence of intenstinal helminthes. All participants with helminthes will be dewormed before administration of the stable isotope. On study day 14, blood samples will be obtained from each participant and thereafter they will receive an oral dose of [$^{13}C_2$] retinyl acetate, which will be followed by a high-fat, low–vitamin A snack. Fourteen days later that is on day 28 the second blood sample will be drawn (Fig. 1).

From the two districts, a simple random sampling method will be used to select villages and households that will be included in the study. Sample collections, and anthropometric measurements will be carried out at the nearby healthy facility. Prior to the commencement of the study, a pilot study will be conducted in Ilala district, Dar-es-Salaam to pre-test the validity of the questionnaire. Results obtained in this pre-test will be used for improving the questionnaire questions.

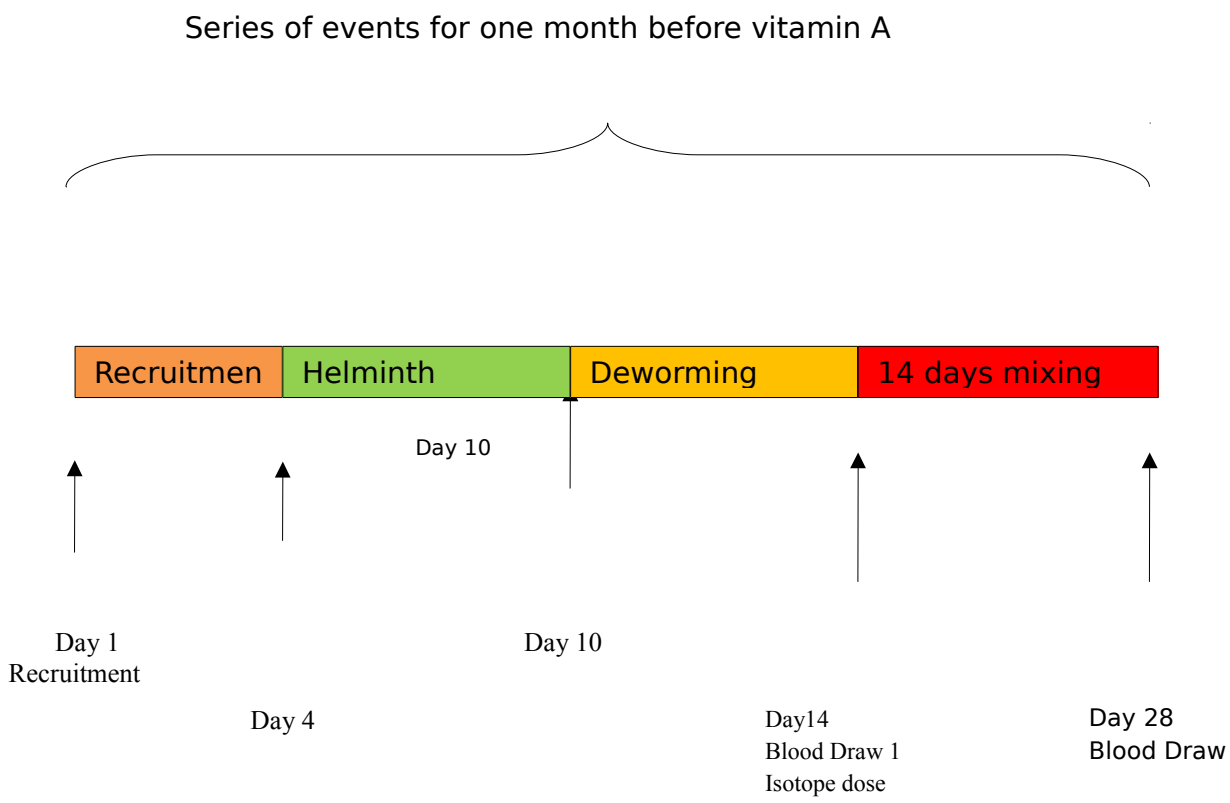

Figure 1: Study time line

2.4 Participant inclusion and exclusion criteria

Included in the study will be children aged 3 to 5 years. Severely sick children those with fever, chronic disease, diarrhea within the past week, malaria within the past 2 weeks, severe anemia, or signs or symptoms of xerophthalmia and those children who are not resident in the study area will be excluded from the study.

2.5 Recruitment

One month before vitamin A supplementation, children aged 3 -5 years will be recruited in the respective district at selected households following inclusion and exclusion criteria.

2.6 Data Collection and Research Instrument

The study will be carried out at health facilities in the selected villages in the study area. A well-structured validated and pre-tested questionnaire will be administered to the child's parents/guardians to collect information on socioeconomic and demographic characteristics, living conditions of their child. The questions will be in the local language (Kiswahili) to facilitate communication with parents/caregiver and children. To ensure that good quality data are collected, questionnaires will be cross-checked continuously. A team comprising investigators and medical personnel from the health facility will administer the questionnaire and conduct all the measurements. Data collection will be supervised by the research coordinator who will be in the field to ensure that all questionnaires are filled in correctly.

The children's dietary vitamin A intake will be assessed with the help of a semi quantitative food frequency questionnaire. A measuring cup and teaspoon will be used to estimate the usual portion sizes of locally available food items containing vitamin A. The mothers or caregivers will be asked if the children had consumed each food item during a 24 hour dietary

recall and the past seven days. The usual portion size and the frequency of consumption will be recorded. For each serving of the food items, the vitamin A content in retinol equivalents (RE) will be calculated with the help of food composition tables.

2.7 Administration of labelled vitamin A doses

After collection of the first blood sample, each child will be given an oral dose of ^{13}C-labeled vitamin A [1 mmol ($^{13}C_2$) retinyl acetate] dissolved in corn oil will be administered directly into the mouth using a positive displacement pipette with a disposable tip. A low vitamin A-high-fat snack will be provided immediately following administration of the labelled vitamin A to enhance absorption.

2.8 Blood collection, processing, and transport procedures

Trained phlebotomists and a medical laboratory technician will collect venous 5 – 7 ml blood samples from each child following standard procedures. Blood samples will be collected into evacuated, foil-wrapped, blood collection tubes specifically designed for the collection of serum (no additive). Blood samples will be protected from light to minimize photo degradation (by using foil-wrapped tubes and working in dim light), to keep the samples cool, and to handle the samples gently during all procedures to prevent hemolysis.

Blood samples will be allowed to clot prior to centrifugation. The foil will be removed and the tubes will be centrifuged at 2800 x g for 15 minutes to separate serum. Equal portions of serum will be transferred to two screw-cap vials, and flushed with nitrogen. The vials will then be wrapped in foil to protect the samples from light and stored in opaque boxes at $\leq -20^{o}C$. Two aliquots of serum will be prepared so that there is a back-up sample in case one sample is damaged or lost. It is very important to clearly label vials and boxes with waterproof markers. The samples will be packed in dry ice and shipped to USA for analysis.

2.9 Analysis of ^{13}C-labeled vitamin A in blood samples

Analysis of blood samples for ^{13}C-retinol will be done by using a GC-C-IRMS method in collaboration with the University of Wisconsin-Madison.

2.10 Procedures for estimating total body vitamin A pool size

^{13}C-labeled vitamin A will be used to estimate total body vitamin A pool size using the stable isotope dilution technique. Vitamin A total body reserve (TBR) will be calculated by using mass-balance equation (Goodman and Brenna, 1992).

$$(F_a \times a) + (F_b \times b) = (F_c \times c)$$

Where "a" is the μmol absorbed from the dose (dose × absorption rate), "b" is TBR in μmol at baseline, and "c" is TBR in μmol after dosing (c = a + b). F a, F b, and F c are the isotope abundance [^{13} C / total C; at % / 100; R/(R + 1) and R is ^{13} C / ^{12} C] of the dose, baseline serum, and post-dose serum, respectively.

2.11 Assessment of Inflammation and Biomarkers of Iron

CRP and α1-acid glycoprotein (AGP) will be determined by ELISA method. Hemoglobin will be measured by HemoCue System.

2.12 Anthropometry variable measurements

Anthropometric measurements will be taken following standard procedures. Measurements to be taken from study participants will include weight and height. Nutritional parameters of

height-for-age (HAZ), weight-for-height (WHZ) and weight-for-age (WAZ) Z-scores will be calculated.

2.13 Assessment of dietary intake

Dietary intake will be assessed using 24-h recall method. For the 24-h recall mothers/caregivers will be asked to recall all foods and beverages consumed by their children during the preceding 24-hours. To assist mothers/caregivers to recall accurately, household utensils will be used. Portion-sizes of consumed foods will be converted to grams, using household measures. Each food and beverage will then be coded according to prescribed protocol and analyzed for contents of energy and other nutrients, using Tanzanian food composition table. Mothers will be asked to recall the number of times water, tea, juice, fruits, and solids had been given during the past 24 hours.

2.14 Helminthic infection assessment

Stool samples will be collected from each study participant and examined for helminthic infection. Stool samples will be microscopically examined for helminthic infection using the Kato-Katz technique – a cellophane faecal thick smear method.

2.15 Statistics

Two-tailed t tests will be applied to compare means of the two groups. Pearson correlation, simple linear regression, and multiple linear regressions will be used to evaluate associations between variables. Analyses will be performed with SPSS version 20 (IBM). $P < 0.05$ will be considered statistically significant.

3.0 Project Administration Plan

This is a collaborative research project that will involve four institutions namely the Tanzania Food and Nutrition Centre and Ministry of Health and Social Welfare. TFNC will be the lead institution. It will be responsible for administrative aspects covering management of grants and research coordination, laboratory capacity to analyze body composition and training nutrition education aspects. Our work will be carried out with support from the International Atomic Energy Agency (IAEA) Vienna, Austria and the Government of Tanzania.

Elisaphinate Urio is a Senior Research Officer with expertise in assessing body composition using nuclear techniques, analysing saliva samples using Fourier Transformed Infra Red (FTIR), using nuclear techniques in detecting Helicobacter pylori infection, and also expertise in food safety. Also, has experience in assessment of nutritional status using anthropometric techniques and microbiological analyses in foods, feeds and body specimens. He holds MSc in applied microbiology from the University of Botswana, Botswana; MSc in food chemistry from the University of Gothenburg, Sweden; Post graduate diploma in nutrition for developing countries from Uppsala University, Sweden; Certificate if food safety, Japan, Post Graduate Diploma in Project Management. In the proposed study he will be Principal Investigator. He will also participate in the scientific aspect of the study.

Elifatio Towo is a Principal Research Officer with expertise in infant feeding and food fortification and experience in assessment of nutritional status, interventions on prevention and control of micronutrients deficiencies, food processing, and food product development. He is currently the acting Director, Food Science and Nutrition Department. Holds a PhD in food biotechnology from the University of Chalmers, Sweden and MSc in food chemistry from the University of Gothenburg, Sweden. In the proposed study he will be Co- Principal Investigator and will be responsible for the study administration. He will also participate in the scientific aspect of the study.

3.1 Project team members and their roles

Francis Modaha is a Senior Research Officer and leader of vitamin A supplementation programme and Integration of Nutrition Services into Health Services Delivery System in Tanzania through Nutrition Assessment Counseling and Support approach. He holds MSc in Post Harvest and Food Preservation Engineering. In the proposed study he will He will participate in the scientific aspect of the study.

Vincent Assey is the Assistant Director and Head, Nutrition Section in the Ministry of Health and Social Welfare. He holds a PhD in biochemistry from Bergen University in Norway. In the proposed study he will advice biochemical analysis and participate in the scientific aspect of the study.

Ladislaus Kasankala is a senior Research officer in the department of Food Science and Nutrition, Tanzania Food and Nutrition Centre. He holds a PhD in Food Technology from China. In the proposed study he will participate in the scientific aspect of the study.

Helen Semu is the Assistant Director, Health Promotion Section, Ministry of Health and Social Welfare. She holds MPH degree from UK and in the proposed study she will participate in the scientific aspect of the study.

Michael Maganga is an experienced technician. He holds an Advanced Diploma in Medical Technology from Muhimbili University of Health and Allied Sciences. He has also attended short courses on the application of isotopic techniques in determining body composition and diagnosing *Helicobacter pylori.* He will be responsible in administering stable isotopes and

analysis of saliva samples. Also he will oversee Hb measurements and stool samples examination.

Adam Hancy is a statistician at Tanzania Food and Nutrition Centre. He holds MA in statistics from the University of Dar es Salaam. He is involved in various researches and survey planning, development of data collection tools, sampling and data analysis at the Centre. In this study he will be responsible in data processing and analysis.

4.0 Ethical considerations

The research questions raised can only be answered by conducting a study in young children, for whom the study outcome is of considerable importance. Techniques that are going to be used are minimally invasive, and have been used in this age group elsewhere in developing countries.

A set of ethical principles that have been laid out to guide the execution of scientific research, studies (CIOMS, 2002) will be adhered to. These include individual informed consent, confidentiality of the information collected, and justice, protection of vulnerable groups and equitable distribution of burden and benefits of the interventions.

4.1 Information to the subjects

Before conducting the study, investigators will meet with the village leaders to explain the purpose of the study, objectives, methods, benefits and risks, right to abstain from participation in the study and right to terminate at any time from the study. Based on this information, parents/guardians will be able to make decisions on whether to allow their children to participate in the study or not.

4.2 Enrollment

Enrollment in the study will be voluntary and each parent/guardian will be required to provide informed consent.

4.3 Confidentiality

Strict confidentiality will be maintained. Efforts will be made to ensure that participation in this study will only be known to the researchers who will be obliged to abide strictly with the study ethics. Participants will not be identified in any reports or publications of this study.

4.4 The use of stable isotopic techniques

Stable isotopes are safe to use and do not present radiation hazards. They are naturally

present in all biological materials, such as foodstuffs that we eat; for example maize, bread, tomato, rice and sugarcane. Also, the applications of stable isotopes do not require special environmental conditions for use or disposal.

4.5 Use of anthropometric methods for nutritional status assessment.

Anthropometric methods are relatively non-invasive methods that assess the size or body composition of an individual. The measurements do not subject the participant to any danger.

4.6 Measurement of haemoglobin

This will involve collecting a venous blood sample. Whilst this causes a brief period of discomfort, this is minimal and children recover very quickly. Detection of vitamin A deficiency or existence of high levels of vitamin A and anaemia will be of benefit to affected children. Children will be referred to the clinic staff for further investigation, treatment and advice if clinical signs are present.

4.7 Compensation

There will be no special incentives to the research participants, such as allowances or stipends for participating in the study.

4.8 Risks and discomfort

Participants will feel slight pain during blood drawing and a small bruise may develop. These will cause mild and tolerable discomfort. In the case of researchers, they will be required to wear protective gear and adhere to recommended procedures for handling potentially infectious materials.

4.9 Benefits

Participants in the study will benefit directly by knowing their health and nutritional status, especially vitamin A status and anaemia. Also participants will benefit from medical and nutritional advice that will be provided. In addition, the findings of the study will be of great input to the prevention and control of vitamin A deficiency and the national food fortification programmes. Children found with intestinal parasites or malaria will be treated.

4.10 Beneficiaries of the project

The beneficiaries of the project will include:

- Preschool children.
- Policy makers of public health programmes.
- Community at large.

- Research institutions and NGOs working on prevention and control of vitamin A deficiency, anaemia and fortification programmes.

- Public health stakeholders.

Summary Budget for Bagamoyo

Grand Total	**19,535,000**

Summary Budget for Iringa

Grand Total	**20,615,000**

6.0 Work Plan and Implementation

No	Activity	2015					2016							
		ug	ep	ct	ov	ec	an	eb	ar	ril	ay	un	ul	ug
1.	Submission of proposal to Research and Ethics Committee (REC – NIMR)													
2.	Bagamoyo: Training of Research Assistants and Recruitment of participants													
3.	Baseline Data collection and Follow-up blood collection after 14 days													
	Baseline Data Analysis													
4.	Iringa: Training of Research Assistants and Recruitment of the study participants													
5.	Baseline Data Collection and Follow-up blood collection after 14 days													
6.	Data Analysis and Report writing													
6.	Dissemination of Results													

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Appendix 1: Questionnaire in English

Tanzania Food and Nutrition Centre (TFNC)

TANZANIA FOOD AND NUTRITION CENTRE
P. O. Box 977
DAR ES SALAAM

RAF 6047: Questionnaire to assess vitamin A status and impact of vitamin A intervention programmes in children 3 to 5 years

Questionnaire No._____

A-General Information

Name of the District…….…...

Name of the Ward…….……..

Name of the Health Facility……. ……..

Name of the Interviewer……..

Date of interview……..….

B-Subject information

Name of the subject ……

1. Sex: Female = 1 Male = 2

3. Date of birth__dd_____mons_____year_____

4. Age

5. Name of the Parent/Guardian

……..

Gender: Female = 1 Male = 2

C-Demographic and Household Characteristics

6. What is the Household size (number of HH members)?

7. What was the highest level of school the father of the child attended?

Pre-primary	0
Primary	1
Post-primary training	2
Secondary	3
Post-secondary	4

University 5

Don’t know 6

8. What was the highest level of school the mother of the child attended?

Pre-primary 0

Primary 1

Post-primary training 2

Secondary 3

Post-secondary 4

University 5

Don’t know 6

9. What is the main source of drinking water for members of your household?

Piped water 1

Water from open well 2

Water from covered well or bore hole 3

Surface water(river) 4

Other……

10. Do you do anything to the water to make it safer to drink?

Yes	1
No	2
Don't know	8

11. If yes, what do you usually do to make the water to make it safer to drink?

Boil	1
Add bleach/chlorine	2
Strain through cloth	3
Use water filter (ceramic/sand/composite etc)	4
Solar disinfection	5
Let it stand and settle	6
Don't know	7

Other (specify)……

12. What kind of toilet facility do members of your household usually use?

Flush	1

Pit latrine 2 ☐

No facility/bush/field 3

Other (specify)…….

13. Does your household have any mosquito nets that can be used while sleeping?

☐

Yes 1

No 2

14. If yes, how many mosquito nets does your household have?

☐

Number of nets ……

15. When you got the net, was it already treated with an insecticide to kill or repel mosquitos?

☐

Yes 1

No 2

Don't know 3

16. Since you got the mosquito net, was it ever soaked or dipped in a liquid to repel mosquitos or bugs?

☐

Yes 1

No 2

Not sure 3

17. Did your child slept under this mosquito net last night?

Yes 1

No 2

D- Week Morbidity Recall

18. Has your child had diarrhoea in the last 2 weeks? [If no or don't know go to QNo.24]

Yes 1

No 2

Don't know 8

19. If yes, was there any blood in the stool?

Yes 1

No 2

Don't know 8

20. Did you seek advice or treatment for diarrhoea from any source?

Yes 1

No 2

21. Where did you seek advice or treatment?

Referral Hospital	1
Regional Hosp	2
District Hosp	3
Health Centre	4
Dispensary	5
Traditional healers	6

Others……

22. Was he/she given any of the following at any time since he/she started having the diarrhoea?

ORS pkt 1 ☐

Zinc 2

Home made fluids 3

23. Was anything else given to treat diarrhoea?

☐

Yes 1

No 2

24. Has your child been ill with a fever at any time in the last 2 weeks?

Yes 1

☐

No 2

Don't know 3

25. Has your child had an illness with cough at any time in the last 2 weeks?

Yes 1

☐

No 2

Don't know 8

26. At anytime during the illness, did your child have blood taken from his/her finger for testing?

Yes 1 ☐

No 2

Don't know 8

27. When your child had an illness with a cough, did he/she breathe faster than usual with short, rapid breaths or have difficulty breathing?

Yes 1 ☐

No 2

Don't know 8

28. Was the fast or difficult breathing due to problem in the chest or to a blocked or runny nose?

Chest only 1 ☐

Nose only 2

Both 3

Other (specify)……

E- Vitamin A and deworming

29. Did your child receive vitamin A capsule during the last 6 months?

Yes 1 ☐

No 2

Don’t know 3

30. Where did your child get the vitamin A capsule? During the campaign with other children or during a sick visit or during a routine/healthy visit? Please circle/tick the appropriate choice

Vaccination campaign 1 ☐

Sick visit 2

Healthy visit 3

31. Do you have a card where vaccinations are written down? If yes, check to confirm ☐

Yes 1

No 2

32. Has your child taken any pill for intestinal worms in the last six months?

Yes 1 ☐

No 2

Don't know 8

F- Food eaten in the Household

Now I would like to ask you about the food your household eats.

33. How many meals does your household usually have per day?

Meals……..

34. In the past week, on how many days did the household eat meat?

Days……

35. In the past week, on how many days did the household eat fish?

Days……..

36. In the past week, on how many days did the household eat green leafy vegetables?

Days……

37. In the past week, on how many days did the household eat vitamin A rich fruits (papaya, mango)? Days……..

38. Did your household use oil to cook with in the past 7 days?

Yes	1
No	2

39. What kind of oil was it?

Simsim/sesame	1
Ground nut	2
Sunflower	3
Coconut	4
Read palm	5

Cotton seed 6

Cowfat 7

Ghee 8

Other fat (specify)…….

G- Dietary intake 24 h recall

What type of food or drink has your child been eating or drinking since this time yesterday?

Time	Food and drink consumed	Amount/portion by using standardized utensils	Weight in grams
Morning (Breakfast)			
Noon-time(Lunch)			

Evening(Dinner)			

H- Anthropometric measurements

40. Weight in kg ……

41. Height in cm ……

42. MUAC in cm…….

43. Weight for age z-score (WAZ)…….

44. Weight for Height z-score (WHZ)……

45. Height for age z-score (HAZ)…….

46. BMI for age z-score (BAZ)……

47. Weight for age percentile…….

48. Weight for Height percentile…….

49. Height for age percentile……

50. BMI for age percentile…….

I- Laboratory investigations

51. Hb (g/dL)_____

52. Retinol binding protein (RBP)_____

53. C-reactive protein (CRP) _____

54. α-1-acid glycoprotein_____

55. Stool examination for ova _____

Appendix 2: Questionnaire in Kiswahili

Tanzania Food and Nutrition Centre (TFNC)

TANZANIA FOOD AND NUTRITION CENTRE
P. O. Box 977
DAR ES SALAAM

RAF 6047: Questionnaire to assess vitamin A status and impact of vitamin A intervention programmes in children 3 to 5 years

Dodoso No._____

A-Taaarifa za jumla

Wilaya……..…...

Kata……..……..

Kituo cha Huduma ya Afya……. ……..

Jina la msahili……..

Tarehe ya usahili……..….

B-Taarifa za mshiriki

Jina la mshiriki ……

1. Jinsi: Kike = 2 Kiume = 1

3. Tarehe ya kuzaliwa siku_____mwezi_____mwaka_____

4. Umri

5. Jina la Mzazi/Mlezi

……..

6. Jinsi: Kike = 2 Kiume = 1

C-Hali ya jamii na Kaya (Demographic and Household C eristics)

6. Kaya yako ina watu wangapi pamoja na wewe?

7. Kiwango cha elimu ya baba wa mtoto

Elimu isiyo rasmi = 1

Elimu ya msingi = 2

Elimu ya Sekondari = 3

Elimu ya juu = 4

Sijui = 5

8. Kiwango cha elimu ya mama wa mtoto

Elimu isiyo rasmi = 1

Elimu ya msingi = 2

Elimu ya Sekondari = 3

Elimu ya juu = 4

Sijui = 5

9. Je, kaya yako inapata maji ya kunywa kutoka wapi?

Maji ya bomba 1

Maji ya kisima kilicho wazi 2

Maji ya kisima kilichofunikwa 3

Maji ya kwenye mto 4

Chanzo kingine, taja……

10. Mnafanya chochote ili kuyafanya maji hayo yawe safi na salama?

Ndiyo 1

Hapana 2

Sijui 8

11. Mnafanya nini ilikuyafanya maji yawe safi na salama

Kuchemsha	1
Kuweka dawa	2
Kuchuja kwa kitambaa	3
Kuchuja kwa kutumia mchanga	4
Kutibu kwa kutumia mwanga wa jua	5
Kuacha yatuame	6
Sijui	7

Njia nyingine, taja……

12. Je, kaya yako mnatumia choo cha aina gani?

Choo cha maji ya kuvuta	1
Choo cha shimo	2
Hakuna choo/Vichakani/shambani	3

Aina nyingine, taja…….

13. Je, kuna chandalua cha kuzuia mbu kwenye kaya yako?

Ndiyo 1

Hapana 2

14. Kwenye kaya yako kuna vyandalua vya mbu vingapi?

Idadi ya vyandalua ……

15. Ulipopata chandalua cha kuzuia mbu,kilikuwa kinadawa tayari?

Ndiyo 1 ☐

Hapana 2

16. Tangu umepata chandalua cha kuzuia mbu, kimekwisha kukitumbukiza kwenye dawa?

Ndiyo 1 ☐

Hapana 2

Sina Hakika 3

17. Je, mtoto wako alilala kwenye chandalua cha kuzuia mbu jana?

Ndiyo 1 ☐

Hapana 2

D- Kumbukumbu za Taarifa za magonjwa katika wiki (Week Morbidity Recall)

18. Je, mtoto wako ameharisha katika wiki mbili zilizopita?

Ndiyo 1

☐

Hapana 2

Sijui 8

19. Je, kwenye choo kulikuwa na damu?

Ndiyo 1 ☐

Hapana 2

Sijui 8

20. Je, ulitafuta ushauri wa kutibu kuharisha popote?

☐

Ndiyo 1

Hapana 2

21. Je, ulipata wapi ushauri wa kitabibu?

Hosipitali ya Rufaa 1

Hosipitali ya Mkoa 2

☐

Hosipitali ya Wilaya 3

Kituo cha Afya 4

Zahanati 5

Mganga wa kienyeji 6

Pengine, taja……

22. Je, tangu mtoto aanze kuharisha alipewa vitu vifuatavyo?

ORS pkt	1
Zinc	2
Home made fluids	3

23. Je, mtoto alipewa kitu kingine chochote kutibu kuharisha?

Ndiyo 1 ☐

Hapana 2

24. Je, mtoto wako ameugua homa katika kipindi cha wiki mbili zilizopita?

Ndiyo 1

Hapana 2 ☐

Sijui 3

25. Je, katika kipindi cha wiki mbili zilizopita aliumwa na kukohoa?

Ndiyo 1

Hapana 2 ☐

Sijui(Don't know) 8

26. Wakati motto anaumwa, Je, alichukuliwa vipimo vyovyote kupima damu?

Ndiyo 1 ☐

Hapana 2

Sijui 8

27. Wakati mtoto wako akiwa anaumwa pamoja na kukohoa alikuwa anapumua kwa shida na kwa haraka haraka?

Ndiyo 1 ☐

Hapana 2

Sijui 8

28. Kutokupumua vizuri kulitokana na matatizo kwenye kifua au niktuokana na pua kuziba?

Kifua peke yake 1

Pupua peke yae 2 ☐

Vyote viwili 3

Vingine, taja [Other (specify)]……

E- Vitamin A na dawa ya minyoo

29. Je, mtoto wako alipata vidonge vya Vitamin A katika kipindi cha miezi sita iliyopita?

Ndiyo 1 ☐

Hapana 2

30. Ni wapi mtoto wako amepatia vidonge vya vitamin A? Ni wakati wa Kampeni pamoja na watoto wengine au ni wakati alipokuwa mgonjwa au ni wakati wa kuhudhuria Kliniki? Tafadhali weka alama ya tiki au zungushia mduara kwenye jibu sahihi

Kampeni za chanjo 1

Wakati akiwa mgonjwa 2 ☐

Wkati wa kuhudhuria Kliniki 3

31. Je, unayo kadi inayooonyesha kwamba mtoto wako amepata chanjo? Kama jibu ni ndiyo, onyesha kuthibitisha

☐

Ndiyo 1

Hapana 2

32. Je, katika kipindi cha miezi sita iliyopita motto wako alipewa dawa ya minyoo?

Ndiyo 1

☐

Hapana 2

Sijui 8

F- Chakula kilicholiwa katika Kaya (Food eaten in the Household)

Sasa ningependa kukuuliza kuhusu vyakula vinavyoliwa katika kaya yako

33. Katika kaya yako mnakula milo mingapi kwa siku? Idadi ya milo……..

34. Ni siku ngapi katika wiki iliyopita kaya yako ilikula nyama? Days……

35. Ni siku ngapi katika wiki iliyopita kaya yako ilikula samaki? Siku……..

36. Ni siku ngapi katika wiki iliyopita kaya yako ilikula mboga za majani za kijani? Siku……

37. Ni siku ngapi katika wiki iliyopita ilikula matunda yenye vitamin A kwa wingi kama vile maembe na mapapai? Siku……..

38. Je, katika kipindi cha siku saba zilizopita, kaya yako ilitumia mafuta yoyote kupikia chakula?

Ndiyo 1

Hapana 2

39. Ni aina gani ya mafuta?

Ufuta (Simsim/sesame) 1

Karanga (Ground nut) 2

Halizeti(Sunflower) 3

Nazi (Coconut) 4

Mchikichi (Red palm oil) 5

Mbegu za Pamba(Cotton seed) 6

Ng'ombe (Cowfat) 7

Samli(Ghee) 8

Mengine, taja [Other fat (specify)]…….

G- Kumbukumbu ya chakula alichokula mtoto katika kipindi cha masaa 24 yaliyopita (Dietary intake 24 h recall)

Je, ni chakula au kinywaji gani ambacho mtoto wako alikula au kunywa tangu muda na wakati kama huu jana?

Muda	Maji /Vinywaji aliyokunywa na vyakula alivyokula	Kiasi alichula kwa kutumia kipimo maalum cha chombo	Uzito kwa gramu
Kifungua kinywa			
Chakula cha mchana			

Chakula cha jioni			

H- Vipimo vya kwenye mwili (Anthropometric measurements)

40. Uzito (Weight in kg)……

41. Urefu (Height in cm) ……

42. Mzungu wa mkono (MUAC in cm)…….

43. Uwiano wa uzito na umri [Weight for age z-score (WAZ)]…….

44. Uwiano wa uzito na urefu[Weight for Height z-score (WHZ)]……

45. Uwiano wa Urefu na umri[Height for age z-score (HAZ)]…….

46. BMI for age z-score (BAZ)……

47. Weight for age percentile…….

48. Weight for Height percentile…….

49. Height for age percentile……

50. BMI for age percentile…….

I- Uchunguzi wa Kimaabara

51. Hb (g/dL)[Wingi wa damu)_____

52. Retinol binding protein (RBP)_____

53. C-reactive protein (CRP) _____

54. α-1-acid glycoprotein_____

55. Kuangalia minyo kwenye choo/kinyesi(Stool examination for ova)_____

Appendix 3: Consent Form in English

Tanzania Food and Nutrition Centre (TFNC)

TANZANIA FOOD AND NUTRITION CENTRE

P. O. Box 977

DAR ES SALAAM

Consent form

Title of Research Project: RAF6047: Using Stable Isotope Techniques to Monitor and Assess the Vitamin A Status of Children Susceptible to Infection (AFRA)

Subproject: Use of stable isotope techniques to assess status and evaluate vitamin A intervention programmes in children in Tanzania

Invitation

Dear Parent/guardian,

Your child has been invited to participate in the above-mentioned study. The leaflet you are reading/I am reading to you provide information about this proposed study that aims at assessing status and evaluate vitamin A intervention programmes in children in Tanzania. Together with this leaflet is the Informed Consent Form. Please read/listen carefully the information in the leaflet, and if you accept your child to participate in this study, please put your signature, across the designated area in the Informed consent form. In case you need additional information or clarification you are welcome to ask for more explanations.

Purpose and description of the study

There are currently ongoing multiple vitamin A intervention programmes in the country that may result in excessive intake of vitamin A and larger liver reserves and consequently lead to acute or chronic toxicity. Stable isotope technique is a method that can quantitatively estimate total body stores of vitamin A. It has the advantage that it is the only indirect assessment method that provides a quantitative estimate of vitamin A status across the continuum of status, from deficient to excessive vitamin A pool sizes. The purpose of this study is to assess the efficacy and effectiveness of the vitamin A intervention programme in children in Tanzania using ^{13}C-labeled vitamin A and to determine the effect of intestinal worms infections on the status of vitamin A in children.

Results obtained from this study will be used as evidence for strong advocacy to policy makers and stakeholders, which will subsequently inform their decisions on, appropriate nutrition intervention programmes. You will be informed of the results of this study at a dissemination meeting. The dates for this meeting will be communicated to you later. The study results will also be disseminated to various stakeholders including the Ministry of Health and Social Welfare as well as through publications.

Study procedure

If you agree your child to participate in this study, please respond to our questionnaire and provide the required information. We will ask your permission to take body measurements, collect blood and stool specimens for laboratory analysis from your child. We would also like to determine vitamin A status. For us to be able to do this, we will request your child to drink few drops of liquid containing stable isotope of vitamin A. This liquid is safe and is not associated with any health hazard. After 14 days we will take again from your child blood sample for laboratory analysis.

Voluntary participation

Please note that your child's participation in this study is voluntary and you have a right to refuse to consent. If you consent for your child to participate you have the right to withdraw your child from the study at any time if you wish to do so.

Benefits

Participants in the study will benefit directly by knowing their health and nutritional status, especially vitamin A status and anaemia. Also participants will benefit from medical and nutritional advice that will be provided. In addition, the findings of the study will be of great

input to the prevention and control of vitamin A deficiency and the national food fortification programmes. Children found with intestinal parasites or malaria will be treated.

Risks and discomfort

Participants will feel slight pain during blood drawing and a small bruise may develop. These will cause mild and tolerable discomfort. In the case of researchers, they will be required to wear protective gear and adhere to recommended procedures for handling potentially infectious materials.

Compensation for time

There will be no compensation for the time your child contributes to the study. However, all children who participate will be provided with a snack upon completion of data collection.

Confidentiality

Strict confidentiality will be maintained. Efforts will be made to ensure that participation in this study will only be known to the researchers who will be obliged to abide strictly with the study ethics. Participants will not be identified in any reports or publications of this study.

Results

The results of the study will be made available to you through a planned dissemination meeting that will be held at the school. Results of this study will also be compiled in a research paper for publication.

Contacts for further information

For any question you may have regarding this study, please contact the Principal Investigator in charge of this study.

Mr. E. M. Urio.
Tanzania Food and Nutrition Centre,
P. O. Box 977,
Dar es Salaam.

If for any reason you want to talk to anyone else about this study call the office of:

The Chairperson,

Research and Ethics committee, National Institute for Medical Research,

Ocean Road,

P. O. Box 9653,

Dar es Salaam.

CONSENT FORM

I, _____

the parent/guardian of _____

confirm that I have read carefully and I have understood the information provided in the leaflet and consent for my child to participate in the study. I am aware that I can freely withdraw my child from this study any time I wish to do so.

Signature: _____

Date: _____

Appendix 4: Consent Form in Kiswahili

Tanzania Food and Nutrition Centre (TFNC)

TANZANIA FOOD AND NUTRITION CENTRE

P. O. Box 977

DAR ES SALAAM

FOMU YA KUKUBALI KUSHIRIKI KWENYE UTAFITI

Jina la Mradi wa utafiti:

RAF6047: Kutumia Isotopu kufuatilia na kupima hali ya Vitamin A kwa watoto wenye kuweza kuathirika na maambukizi ya magojwa [*Using Stable Isotope Techniques to Monitor and Assess the Vitamin A Status of Children Susceptible to Infection (AFRA)]*

Mradi mdogo: Kutumia Isotopu kufuatilia na kupima hali ya Vitamin A na kutathmini afua za vitamin A kwa watoto Tanzania *(Use of stable isotope techniques to assess status and evaluate vitamin A intervention programmes in children in Tanzania)*

Mwaliko kwa Wazazi/Walezi

Mpendwa mzazi/mlezi,

Mtoto wako amealikwa kushiriki katika utafiti uliyotajwa hapo juu. Your Fomu hii unayoisoma /Ninayokusomea inakupa taarifa zote kuhusu utafiti uliyopendekezwa, wenye lengo la kupima hali ya Vitamini A na kutathmini afua za Vitamin A kwa watoto hapa nchini Tanzania. Fomu hii imeunganishwa pamoja na maelezo ya kukubali kushiriki katika utafiti huu. Tafadhali soma/Sikiliza kwa makini maelezo yaliyoko katika fomu hii na kama utakubali mtoto wako ashiriki katika utafiti huu weka sahihi yako kwenye fomu hii hapo chini. Tafadhali, kama unahitaji maelezo zaidi au ufafanuzi, unakaribishwa kuuliza.

Kusudi la Utafiti:

Utekelezaji wa Mradi huu wa Utafiti

Taasisi ya Chakula na Lishe Tanzania, ndiyo inayohusika na kufanya utafiti huu. Aidha Taasisi itashirikia na Wizara ya Afya na Ustawi wa Jamii, Ofisi ya Idara za Afya Wilayani Bagamoyo na Iringa mkoani Pwani na Iringa na Shirika la Nguvu za Atomiki la Umoja wa Mataifa (IAEA), Vienna Austria.

Kuna afua mbalimbali zinazotekelezwa hapa nchini zenye lengo la kuzuia na kudhibiti matatizo yanayotokana na upungufu wa vitamin A. Pamoja na faida za afua hizo zinazotekelezwa kwa pamoja kunauwezekano pia wa kupelekea watoto kuwa na viwango vingi zaidi vya vitamini A kwenye miili yao na kuwaletea athari za kiafya. Njia pekee yenye uwezo wa kupima viwango vya vitamin A mwilini katika mweendelezo wake kuanzia viwango vya chini sana, viwango vya juu hata kwenye akiba inayokaa kwenye ini ni ile ya kutumia Isotopu thabiti. Kusudi la utafiti huu ni kupima hali ya vitamin A na kutathmini uwezo wa afua mbalimbali za vitamini A kuleta matokeo yanayotarajiwa kwa kutumia " ^{13}C-labeled vitamin A" na kupima athari za minyoo kwenye hali ya vitamin A kwa watoto.

Matokeo ya utafiti huu yatumika kama ushahidi wa kisayansi wa kutetea na kuhamasisha watunga sera na wadau wengine kufanya maamuzi sahihi kuhusu afua za vitamin A. Utajulishwa matokeo ya utafiti huu katika mkutano wa washiiki wote wa utafiti huu utakaofanyika mara baada ya kupata taarifa zote za matokeo. Tarehe, muda na mahali patakapofanyika mkutano huo mtajulishwa baadaye. Matokeo ya utafiti pia yatawasilishwa kwa wadau mbalimbali ikiwa ni pamoja na Wizara ya Afya na Ustawi wa Jamii na kwenye makala mbalimbali za kisayansi.

Taratibu za kushiriki:

Kama umekubali mtoto wako ashiriki katika utafiti huu, tutaomba ujibu maswali ya dodoso letu. Pia tutaomba idhini yako ili tuweze kuchukua vipimo vya mwili wa mtoto wako, (uzito na urefu) kuchukua sampuli ya damu kupima wingi wa damu yake, hali ya vitamini A na choo kwa ajili ya uchunguzi wa maambukizi ya minyoo na magonjwa mengine. Pia ilituweze kujua hali ya vitamini A tutampa mtoto wako matone ya Isotopu ya vitamini A. Matone haya ni salama kabisa na hayana madhara yoyote. Baada ya siku 14 tutarudi tena kuchukua sampuli za damu kwa ajili ya kupima hali ya vitamini A.

Hiari ya kushiriki:

Kushiriki kwako na kwa mtoto wako katika utafiti huu ni hiari. Unaweza kuamua wewe na mtoto wako kuingia na kushiriki katika utafiti huu au baada ya kushiriki ukaamua kumuondoa mtoto wako kwa sababu unazozifahamu wewe mwenyewe. Kumuondoa mtoto wako hakutamuathiri yeye kama mshiriki.

Faida zinazohusiana na utafiti huu:

Zipo faida ambazo utazipata moja kwa moja kutokana na utafiti huu. Utaweza kujua hali ya afya na lishe ya mtoto wako na pia utapewa ushauri wa kilishe. Watoto watakaokutwa wana minyoo watatibiwa.

Hatari na usumbufu:

Hakuna hatari zozote zinazohusiana na utafiti huu. Unaweza kujisikia maumivu kidogo tu wakati wa kutoa damu kwenye mkono lakini hili ni jambo la kawaida na hutokea kwa muda mfupi na kumalizika mara moja.

Motisha kwa kushiriki:

Hakuna motisha/ruzuku yoyote utakayolipwa kutokana na mtoto wako kushiriki katika utafiti huu. Hata hivyo, kila mtoto atapewa kitafunwa mara baada ya kumaliza kuchukuliwa vipimo.

Utunzaji wa taarifa za utafiti:

Taarifa zote za utafiti zitakuwa ni siri na jina lako pamoja na la mtoto wako halitajitokeza popote kwenye ripoti au makala zozote zitakazo andikwa. Ushiriki wa mtoto wako katika utafiti huu utakuwa unafahamika kwa watafiti tu ambao wanatenda kazi zao kwa kuzingatia maadili ya utafiti kwa makini. Ikitokea kwamba maadili ya utafiti yamekiukwa kwa namna moja au nyingine, hatua za kinidhamu zitachukuliwa dhidi ya watafiti wanaohusika. Hata hivyo matokeo ya utafiti yatatolewa kwa watafiti na wataalamu wengine kwa ajili ya kuelimishana.

Kwa Mawasiliano na Maelezo zaidi

Kama una swali lolote, tafadhali wasiliana na Mr. E. M. Urio yeye ndiye Mchunguzi Mkuu katika utafiti huu.

Anuani yake ni:

Taasisi ya Chakula na Lishe Tanzania,

S. L. P. 977,
Dar es Salaam. Simu: 2118138; 0754 31 05 86

Waweza pia kuwasiliana na:
Mwenyekiti,
Kamati ya Maadili ya Utafiti,
Taasisi ya Utafiti wa Magonjwa ya Binadamu,
S. L. P. 9653,
Dar es Salaam.

Simu: 2121338; 2126531; 2121391

Appendix 5 – Verbal Assent for children in English

Tanzania Food and Nutrition Centre (TFNC)

Verbal Assent for Children

Hi. My name is *[researcher's name]*. I'm a researcher from Tanzania Food and Nutrition Centre Dar-es-Salaam. We love children and we have passion for fostering child growth and development. Right now, we are trying to learn about how vitamin A supplementation and deworming initiatives are helping you in improving your health and nutrition status. I would like to ask you to help me by being in a study, but before I do, I want to explain what will happen if you decide to help me.

The study will involve taking measurements of your weight and height. These are common measurements that are taken when you go to clinic. Also we will collect your faecal sample to check for intestinal worms. Intestinal worms are not good for your health they eat food that you eat and leave you with nothing, as a result you become weak and malnourished. This will be followed by giving you treatment for intestinal worms. In addition we will take some blood sample to measure vitamin A and malaria. This will cause minor discomfort but it is tolerable. It is just like when you go to clinic to check for malaria. If malaria parasites are found you will also be treated.

When you join this study, nobody will know. Also when we tell other people about this study we will not use your name. Your *[mom/dad/guardian]* says it's okay for you to be in my study.

You can ask me questions about the study. If you have a question later that you don't think of now, you can ask *[your parents/guardian]* to call me or send me a message.

Do you have any questions for me now?

Would you like to be in my study and *[talk to me/answer some questions/draw some pictures/play a game/begin whatever activity is planned]*?

Name of Child: _____Parental Permission on File: ☐ Yes ☐ No

(If "No," do not proceed with assent or research procedures.)

Child's Voluntary Response to Participation: ☐ Yes ☐ No

Signature of Researcher: _____ Date: - _____

(Optional) Signature of Child: _____

NOTES TO RESEARCHER: The child should answer "Yes" or "No." Only a definite "Yes" may be taken as assent to participate.

Contacts for further information

For any question you may have regarding this study, please contact:
Mr. E. M. Urio.
Tanzania Food and Nutrition Centre,
P. O. Box 977,
Dar es Salaam.
Mobile: 0752 67 65 48

If for any reason you want to talk to anyone else about this study call the office of:

The Chairperson,

Research and Ethics committee, National Institute for Medical Research,

Ocean Road,

P. O. Box 9653,

Dar es Salaam.

Appendix 6 – Verbal Assent for children in Kiswahili

Tanzania Food and Nutrition Centre (TFNC)

Mtoto Kukubali Ushiriki kwa Maneno

Habari. Mimi ninaitwa *[Jina La mtafiti]*. Mimi ni mtafiti kutoka Taasisi ya Chakula na Lishe Tanzania yenye makao yake makuu Dar-es-Salaam. Tunawapenda watoto na kiu yetu kubwa ni kuwasaidia watoto wakue na kuendelea vizuri kiafya na kilishe. Kwa sasa hivi tunataka kufahamu ni kwa jinsi gani matone ya vitamin A na dawa za minyoo zinavyowasaidia kiafya na kilishe kila mnapopewa baaada ya kila miezi sita. Ningependa nikuombe utusaidie kwa kuwa sehemu ya utafiti huu, lakini kabla ya hapo ningependa nikuelezee kwa kifupi ni nini hasa tunataka kufanya kama utakubali kuwa sehemu ya utafiti huu na kutusaidia.

Utafiti huu utahusisha kuchukua vipimo vya uzito na urefu. Hivi ni vipimo vya kawaida tu ambavyo huchukuliwa kila unapopelekwa kliniki kufuatilia maendeleo yako ya ukuaji na lishe. Pia tutachukua sampuli za haja kubwa kupima kama unayo minyoo. Minyoo husababisha afya mbaya na huchangia kuleta utapiamlo. Baada ya kupima haja kubwa, utapewa dawa za minyoo. Vilele tutaomba kuchukuwa sampuli ya damu kidogo kwa ajili ya kupima viwango vya vitamin A na kuchunguza kama una malaria. Utapata maumivu kidogo sana ambayo hayatasabisha usumbufu au madhara makubwa ni ya kawaida tuu kamavile unavyokwenda kliniki na kupimwa malaria. Ukikutwa una vijidudu vya malaria utapewa dawa.

Kushiriki kwako kwenye utafiti huu hakuna mtu atakayejua. Pia tutakapokuwa tunawaambia watu wengine juu ya utafiti huu hatutatumia jina lako. Wazazi wako/walezi wako wamekubali wewe kujiunga na utafiti huu.

Kama una swali lolote kuhusu utafiti huu unaweza kuniuliza. La sivyo, kama swali litajitokeza baadaye unaweza kuwaambia wazazi/walezi wako wanipigie simu au waniandikie ujumbe mfupi wa simu.

Je, kwa sasa una swali lolote?

Je, ungependa kutusaidia kwa kujiunga kwenye utafiti huu?

Jina la mtoto: _____Ridhaa ya mzazi/mlezi**:** ☐ Ndiyo ☐ Hapana

(Kama jibu ni "Hapana" usiendelee na taratibu za ushiriki au utafiti.)

Ridhaa ya mtoto kukubali ushiriki: ☐ Ndiyo ☐ Hapana

Sahihi ya Mtafiti:_____Tarehe**:** _____

(Hiari)Sahihi ya Mtoto: _____

Ifahamike kuwa: Mtoto lazima ajibu "Ndiyo" au "Hapana". Ni "Ndiyo" dhahiri tu ndiyo itachukuliwa kama kukubali kwa mtoto kushiriki.

Kwa Mawasiliano Zaidi:

Kama utakuwa na swali zaidi kuhusu utafiti huu, tafadhali wasiliana na:-
Mr. E. M. Urio.
Tanzania Food and Nutrition Centre,
P. O. Box 977,
Dar es Salaam.
Mobile: 0752 67 65 48

Waweza pia kuwasiliana na Mwenyekiti wa Kamati ya Maadili ya Taifa ya Utafiti kama ifuatavyo:-

The Chairperson,

Research and Ethics committee, National Institute for Medical Research,

Ocean Road,

P. O. Box 9653,

Dar es Salaam.