Original Research

Quantification of food and nutrient intakes in Zambian children with and without malaria under controlled feeding conditions

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Abstract

Vitamin A supplementation improves status, which may protect against malarial infection. Provitamin A carotenoid biofortified staple crops may provide a more sustainable approach to alleviate vitamin A deficiency than supplementation, but the impact of febrile illness on food intake must be considered in malaria endemic regions. Morbidity data and food logs from a three-month efficacy trial on provitamin A biofortified (orange) maize in preschool Zambian children ($n = 181$, age 3–5 years) were systematically analyzed over time to determine the impact of malaria on food intake. Nutrients examined included macronutrients, iron, zinc, and vitamin A. Comparisons based on individual intakes in healthy and malarial states over three-day intervals were made including children from both the orange and white maize groups ($n = 100$). Malaria prevalence did not differ overall or between treatment groups over time (all $P > 0.05$). Lower nutrient intakes were observed for all variables during malaria outbreaks (food 289 ± 412 g; energy 248 ± 346 kcal; carbohydrate 42 ± 62 g; protein 8 ± 12 g; fat 5 ± 7 g; iron 1 ± 2 mg; zinc 1 ± 1 mg; vitamin A 58 ± 100 retinol activity equivalents; all $P < 0.05$). No differences were observed between nutrient decreases in orange and white maize groups ($P > 0.05$). Considering the impact of malaria on food and nutrient intakes and increased vitamin A utilization and excretion due to the acute phase response, biofortification targets for provitamin A carotenoids may need to be elevated in malaria endemic regions.

Keywords: Biofortification, carotenoids, maize, malaria, vitamin A

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Introduction

Malaria and micronutrient deficiencies are widespread health issues in Africa and are of particular concern in children.[1,2] Malaria is the most common reason for hospitalization in African children under five years of age,[3] and increased malaria morbidity and mortality are associated with micronutrient deficiency in this population.[4] In fact, certain micronutrient deficiencies appear to exacerbate malarial illness[4,5] epitomizing the importance of nutrition interventions in initiatives aimed at malaria prevention and treatment.

Vitamin A is essential for healthy growth, immune function, vision, and reproduction. Vitamin A deficiency compromises the immune function in 40% of children under five years of age, resulting in 1 million deaths annually.[6] Stürchler *et al.*[7] found low serum retinol concentrations to be associated with increased risk of malarial illness, and an inverse association between serum retinol and malarial parasitemia has been documented in subsequent studies.[8–12] Vitamin A supplementation reduced the frequency of malarial episodes by 30% in young children in Papua New Guinea[13] and promoted ponderal growth and protection against malaria-related death in Tanzanian children.[14]

The efficiency of vitamin A supplement distribution has doubled since 2000 with at least 80% of preschool-aged children in least developed countries receiving coverage with two vitamin A doses in 2011.[15] However, the introduction of provitamin A biofortified crops provides a potentially effective method for alleviating vitamin A deficiency in the rural poor, who are disproportionally missed by supplementation interventions.[16] Additionally, provitamin A carotenoid biofortified staple crops may provide a more feasible and long-term approach to alleviate vitamin A deficiency without the risk of hypervitaminosis A attributed to fortification with preformed vitamin A.[17,18] Plant sources of provitamin A carotenoids maintained total body vitamin A stores in Chinese and Filipino children,[19,20] demonstrating that dietary approaches to ameliorate vitamin A deficiency

are efficacious. However, malaria suppresses appetite and growth,[21–23] and therefore it is important to consider its potential impact on the effectiveness of biofortified crops in malaria endemic regions.

A recent study assessing the adaptation of children to biofortified maize was conducted in the Eastern Province of Zambia,[24] a region where malaria is >75% endemic.[25,26] In order to determine the impact of malaria on food and micronutrient intake in Zambian children during a three-month intervention, we systematically analyzed food intake and morbidity data as a function of time and then compared food and micronutrient intakes between healthy and malarial states from children receiving either biofortified orange or white maize.

Materials and methods

Subjects and maize

The feeding protocol used in this trial was previously published by Nuss *et al*.[24] and is briefly summarized below. All procedures involving human subjects were approved by the Tropical Diseases Research Center (TDRC) Ethics Review Committee in Zambia and by the Health Sciences Human Subjects Institutional Review Board at the University of Wisconsin-Madison. Written informed consent was obtained from parents or guardians of all participating children. The feeding trial was conducted from March to June of 2010 in the Nyimba District of the Eastern Province of Zambia with children 3–5 years of age ($n = 189$ initial participatory enrollment, Table 1). The target for study size was set to conservatively accommodate a 10% dropout rate and provided enough feeding observations for greater than 90% power to detect a difference between groups in the adaptation to orange maize.[24] Six study sites were chosen based on interest expressed by local communities during sensitization programs related to orange maize conducted by the National Food and Nutrition Commission of Zambia (Lusaka, Zambia) in 2009. Enrollment at each site was approximately 30 participants, who were randomly assigned to either orange or white maize treatments. Participants were screened and excluded from the study for severe anemia (hemoglobin <7.0 g/dL) and malnutrition (<−3 SD weight-for-age or height-for-age).[27]

Participating children were fed breakfast, lunch, and snack six days per week for a total of 70 days of feeding, excluding Sundays. Orange maize used in the trial was developed from conventional breeding programs that selected for high provitamin A kernel content; typically consumed white maize was sourced locally.

Project menu, food intake measurement, and environment

The project menu was designed to contain common Zambian foods and cooking methods as outlined by a national food intake survey.[28] A six-day rotation of traditional menu items with standardized serving quantities supplied similar energy and nutrient amounts to the orange and white maize treatment groups, with the exception of vitamin A, which was of primary interest. Caloric content and nutrient quantities, including carbohydrate, protein, fat, vitamin A, iron, and zinc were determined using food labels of locally procured ingredients and Nutritionist Pro™ (version 4.6.0). Vitamin A content of maize was determined using published methods.[29,30] Food intake was determined by subtracting uneaten food at the end of the meal from the initial serving using digital kitchen scales (My-weigh KD7000, China). Mealtime attendance was recorded so that zero food waste was not mistaken as complete consumption of the meal.

Table 1 Baseline and final anthropometric and biochemical measurements by treatment group (mean ± SD)

Measurement	Orange maize ($n = 95$)	White maize ($n = 86$)	Total ($n = 181$)
Baseline age (months)	52.9 ± 10.2	55.1 ± 11.3	54.0 ± 10.7
Weight (kg)*			
Baseline	14.8 ± 1.9	14.7 ± 2.1	14.8 ± 2.0
Final	15.8 ± 1.9	15.6 ± 2.0	15.7 ± 2.0
Height (cm)*			
Baseline	98.3 ± 7.0	98.5 ± 7.2	98.4 ± 7.0
Final	100.5 ± 6.9	100.9 ± 6.5	100.7 ± 6.7
Weight-for-height z-score†			
Baseline	0.11 ± 0.84	−0.10 ± 0.88	0.008 ± 0.86
Final	0.11 ± 0.84	−0.10 ± 0.88	0.010 ± 0.87
Baseline malaria prevalence	17.6%	24.1%	20.8%
Parasite load >0	14.3%	20.7%	17.4%
Parasite load >1000	3.3%	3.4%	3.4%
Final malaria prevalence	8.8%	16.1%	12.4%
Parasite load >0	8.8%	13.8%	11.2%
Parasite load >1000	0.0%	2.3%	1.2%

*Weight and height increases were seen in orange and white maize treatment groups and total study population ($P < 0.05$).
†World Health Organization Child Growth Standards, ages 2–5 years.[35]

Feeding practices were standardized across all six feeding sites. The orange and white maize groups were fed in separate rooms to avoid between-group influences that may have impaired acceptance of the orange maize. Each participant's identification number was confirmed at every meal to ensure accurate data collection. Mealtimes were monitored to prevent food sharing and all subjects were equally encouraged to finish their meals. All ingredients for the meals were measured and prepared on-site by Zambian nutritionists and locally recruited women.

Weekly intake

For weekly food and nutrient intake, days of feeding were converted into weeks of feeding to account for the six-day rotating menu. A ''week'' in this analysis includes six days of project feeding, excluding Sunday. Food and nutrient intakes were calculated for both orange and white maize groups. Due to staggered initial and final feeding days, weeks 1 and 12 were not matched in terms of feed intake across all study sites and were excluded from all analyses.

Malaria diagnosis

All blood collection and analysis were conducted by TDRC in Zambia. Blood was collected for malaria parasite identification and quantification at baseline and final measurements. Thick blood smear slides were prepared on-site, air-dried, and transported in slide boxes for analysis. Blood slides for malaria parasites were prepared in accordance with standard protocols using a Giemsa stain and compared under oil immersion by microscope against 200 white blood cells (Olympus CX31 IRBSFA).[31,32] A slide was termed positive for malaria if parasites were observed and negative if no parasites were observed in 100 fields. Quality control procedures included the re-examination of approximately 10% of the slides by a second laboratory technologist and the use of a third technologist if disparities existed between the first and second reading.

Malaria was diagnosed throughout the study on a weekly basis by local clinicians who were trained by TDRC to diagnose malaria based on symptoms of fever and general malaise. A standardized morbidity questionnaire was used to diagnose malaria during the previous week by day and was completed by clinicians based on reports from the participants' parents or guardians. Malaria treatment was provided to participants according to WHO recommendations and guidelines from the Ministry of Health in Zambia.

Malaria prevalence and association with food intake

Baseline and final malaria prevalence and parasite load quantification were determined from biochemical analysis of blood smears. To determine weekly malaria prevalence, morbidity records were analyzed and participants diagnosed with malaria for at least one day of the week were included in calculations. Prevalence calculations for weeks 4 and 5 were adjusted to account for missing morbidity logs from one of the study sites.

A total of 100 participants, 50 each from the orange and white maize groups, were randomly selected in order to establish an association between malaria and food intake. In order to account for increasing food intake over time,[22] 10 participants with malaria from the orange and white treatments ($n = 5$/group) were selected from each week and each participant was selected only once during the intervention phase of the study. The duration of malaria symptoms during an attack in young children is approximately 3–4 days.[33] In order to elucidate the relationship between malaria and food intake, the same three-day time intervals were compared from a week when the participant was experiencing a malaria episode and the preceding week when the participant was in a healthy, non-febrile state. For week 2 or if a participant was absent during one of the healthy state comparison days, food and nutrient intake values from the week following the malaria episode were used for comparison. Dietary intakes were compared between a malarial state to a previous healthy state for the following reasons: (1) the incubation period for malaria, during which *Plasmodium* reproduces in the liver, can vary with the earliest onset of symptoms occurring between 5 and 12 days,[34] so it is unlikely that participants displayed symptoms the week before the outbreak was identified by clinicians; (2) malaria attacks reoccur every 2–3 days, when the parasites reproduce in blood cells,[34] so participants may have experienced symptoms the week following the initial outbreak; and (3) participants were provided a course of antimalarial medication once diagnosed with fever, which may produce side effects the following week.[34]

Statistical analysis

SAS statistical package (version 9.2) was used to analyze data. To investigate differences between treatments and malaria prevalence across weeks, repeated measures ANOVA followed by least square difference of variance testing was used to account for autocorrelated errors due to repeated measures on each participant with a first-order autoregressive structure. For malaria prevalence, an arcsine square root transformation was used to homogenize variance. Paired *t*-test was used for comparison of baseline and final anthropometric measurements and for in-group comparisons of food and nutrient intake between a malarial and healthy state. Independent *t*-test was implemented for between-group comparison of intake differences. All tests were two-sided, and $P \leq 0.05$ was considered significant. Values for food and nutrient intake are rounded to the nearest unit to reflect the precision of the field scales.

Results

Subject data

Baseline and final anthropometric data and malaria prevalence based on blood smear are given individually for orange and white maize groups and all study subjects in Table 1. Although no differences were observed in weight-for-height z-score ($P > 0.05$), increases were observed in weight and height for each treatment and overall ($P < 0.05$). No differences were observed in these

measurements between treatments ($P > 0.05$). Malaria prevalence in the white maize group was approximately 5% higher than the orange maize group at the beginning and end of the study; prevalence decreased in both treatments by approximately 9%. Eight children dropped out of the study (4%). Primary reasons for discontinuing study participation included non-compliance with study requirements and severe illness.

Comparison of weekly intakes

No differences were observed in food or energy intake reflecting equal acceptance of the project menu; participants consumed 90% of the food and the total kcal served each week. Of the total amount served each week, participants consumed 91% of carbohydrates, 89% of protein, 92% of fat, 92% of vitamin A, 90% of iron, and 75% of zinc. As expected, weekly vitamin A intake was higher in the orange maize group ($P < 0.05$). Intakes of food and all nutrients, except vitamin A, increased by week ($P < 0.05$), supporting previous findings by Nuss *et al.*,[24] which reported increasing intakes of menu items by week and similar intakes of all menu items after week 2 of the study.

Comparison of weekly malaria prevalence

During weeks 2 through 11 of the study, 94.5% of all study participants were diagnosed with at least one case of malaria. Malaria prevalence did not differ by maize treatment or week, and no interactions were observed between these variables (Figure 1, $P > 0.05$). The mean weekly malaria prevalence was 25.6%. Comparison of malaria prevalence at weeks 2–11 demonstrated a significant decrease from the beginning to the end of the examined study period ($P < 0.05$), which corresponded to the end of the rainy season.

Intake patterns associated with malarial status

Malarial status was associated with decreased intake of food, energy, and all nutrients ($P < 0.05$), which did not differ between treatments (Table 2). Food and kcal intake decreased during a malaria episode by 11%. Carbohydrate, protein, and fat intake decreased by 10%, 11%, and 10%, respectively, in children with malaria. During a three-day period with malaria, vitamin A intake decreased by 58 ± 100 retinol activity equivalents (9% decrease), iron intake decreased by 1 ± 2 mg (13% decrease), and zinc intake decreased by 1 ± 1 mg (14% decrease).

Discussion

Appetite suppression is a common symptom of malaria, which may impact the deliverance of micronutrients using biofortified staple crops to target populations where the infection is endemic. The findings presented in this study are important, as little work has been done to estimate the impact of malarial infection on dietary intake. In this study, the impact of malarial infection on food, energy, and nutrient intake was examined in rural Zambian children during a three-month feeding trial with provitamin A carotenoid biofortified maize. Intakes of food and all examined nutrients were suppressed during malarial infection in these children. The amount of decrease in food, energy, and nutrient intake was similar in the orange and white maize groups, which is expected because overall dietary intakes were similar and malaria prevalence did not differ between groups. Vitamin A intake was suppressed by 9.5% during the three-day malarial intervals assessed. This equates to a vitamin A loss of 19 ± 33 retinol activity equivalents/day or a 5–6% of the Recommended Dietary Allowance (RDA) in the study population due to malaria-related appetite suppression.

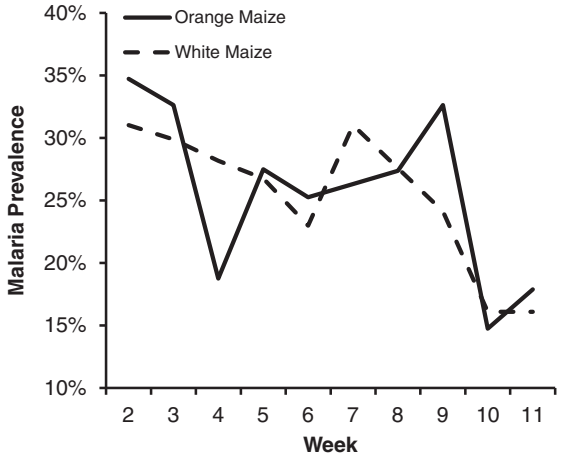

Figure 1 Weekly malaria prevalence by treatment group in rural Zambian children. No overall differences were observed in malaria prevalence by treatment group or week and no interactions were observed (*all* $P > 0.05$). A decrease in total malaria prevalence was found when comparing week 2 to week 11, reflecting seasonal effects ($P < 0.05$)

Ninety percent of the maize crop produced in Africa is white carotenoid-poor maize, which accounts for >70% of daily calories consumed in the Eastern Province of Zambia.[36–39] Poorly diversified diets can lead to subclinical micronutrient deficiencies, wasting, and stunting,[40,41] which can increase risk of infection and weaken immune response. Micronutrient deficiencies are associated with increased malaria morbidity and mortality in children.[4] Additionally, energy and protein intakes and weight gain are inversely proportional to febrile infections in Guatemalan and Bangladeshi children.[42,43] Stunting, a reflection of protein energy malnutrition, can be predicted by malaria in rural African settings and is associated with more frequent malarial infection and greater related morbidity.[43] Because of this, integration of nutrition and malaria initiatives may prove effective in malaria endemic regions. According to WHO criteria, the children in this study were in the healthy weight-for-height range and not malnourished (>−2 SD weight-for-height z-score)[27]; however, significant increases in weight and height during the three-month study period confirm that the nutritious study menu was beneficial to the children despite a weekly malaria prevalence ≥14%.

Malaria is endemic in the Eastern Province of Zambia with seasonal effects from December to April.[25,26,45–47] This study took place from March to June of 2010, during the transition out of ''malaria season.'' Although no overall

Table 2 Three-day comparison of nutrient intakes during a malaria episode and healthy state in rural Zambian children (mean ± SD)*

Total population studied	Food (g)	Energy (kcal)	Carbohydrate (g)	Protein (g)	Fat (g)	Vitamin A (RAE)	Iron (mg)	Zinc (mg)
Healthy state	2597 ± 321	2263 ± 274	395 ± 45	67 ± 12	48 ± 8	608 ± 84	9 ± 1	8 ± 1
Malarial state	2308 ± 467	2015 ± 380	352 ± 68	59 ± 15	43 ± 8	550 ± 113	8 ± 2	7 ± 1
Difference	289 ± 412	248 ± 346	42 ± 62	8 ± 12	5 ± 7	58 ± 100	1 ± 2	1 ± 1

*$n = 100$ comparisons between healthy and malarial states. Data for both treatments were pooled because no differences were observed between malaria-related decreases in food, energy, and nutrient intakes for orange and white groups ($P > 0.05$). Decreases were observed in all food, energy, and nutrient intakes during a malarial state when compared to a healthy state ($P < 0.05$).

differences were observed in weekly malaria prevalence throughout the study, a comparison of the extremes revealed a decrease in malaria prevalence from weeks 2 to 11 due to the seasonal effect. With the exception of baseline and final biochemical analysis to determine malaria parasitemia, clinicians diagnosed malaria symptomatically throughout the study. Without confirmation of parasitemia, it is possible that misdiagnosis of malaria occurred, inflating or underestimating weekly malaria prevalence. In fact, malaria is commonly misdiagnosed in resource-poor clinics for other febrile illnesses, such as pneumonia.[48,49] The implementation of malaria-specific rapid diagnostic tests can improve the proper diagnosis of malaria where microscopy is not available; however, these tests are costly and do not provide information on parasite density.[48,50] Nonetheless, the children were ill with fever, which does not negate our findings.

Malaria exacerbates vitamin A deficiency in at-risk populations.[51] The acute phase response to infections leads to a drop in serum retinol that is typically restored after the infection resolves.[52,53] This results in serum retinol measurements that do not accurately reflect vitamin A status during infection. For example, Thurnham and Singkamani[54] found Thai patients suffering from malarial infection to have serum retinol values indicating deficiency, although the patients had no clinical signs and a diet high in provitamin A carotenoids. It is paramount to measure and adjust for elevated positive acute phase proteins in the serum to avoid misclassification of vitamin A deficiency during infection when more reliable measurements, such as the modified relative dose response test or retinol isotope dilution, are not possible.[55,56]

The decrease in serum retinol during the acute phase response results from the suppression of retinol binding protein and transthyretin (prealbumin) in order to increase vitamin A availability to tissues.[9,57] Increased vitamin A utilization during malarial infection is also supported by reductions in serum provitamin A carotenoids.[54,58,59] Furthermore, significant urinary retinol losses occur during infection.[60,61] This increase in vitamin A utilization coupled with appetite suppression and urinary loss may result in increased vitamin A requirements in malaria endemic regions. Based on other febrile disease states and illnesses, we quantified the projected loses of vitamin A in the urine during a malaria episode. In children with infections that cause fever, up to 500 μg retinol can be lost per day,[60] for a total of 1.5 mg for a three-day malarial attack. Up to 1.7 mg has been estimated to be lost in children with a severe infection that lasts for 10 days.[61] Therefore, a single malarial episode could result in urinary losses that are greater than the weekly intake of vitamin A from foods as quantified in this controlled study. This implies that vitamin A requirements may be higher in areas with endemic febrile illness.

In conclusion, provitamin A biofortified staple crops can increase vitamin A intake in regions where deficiency is prevalent. However, as malarial infection negatively impacts vitamin A status through decreased food intake, urinary loss, and increased utilization, biofortification targets may need to be elevated to meet vitamin A requirements in endemic regions. The provitamin A biofortified maize target level is currently set at 15–17 μg β-carotene equivalents/g dry weight, which is projected to meet 50% of the estimated average requirement for vitamin A or 140 μg/day for a child.[62] Raising the target level by 3–5 μg/g in countries with endemic malaria and high maize intakes could act as a buffer to assist in maintaining adequate vitamin A stores during febrile episodes. This target level, which has already been surpassed in some experimental maize genotypes, is achievable with traditional breeding techniques (SAT personal observations). Considering that maize intakes of preschool children in Zambia are 150–200 g dry weight/day, a 3-μg/g increase in target level would provide approximately 100 extra retinol activity equivalents during a three-day malaria episode, which would cover the loss from appetite suppression found in this study. The introduction of provitamin A biofortified staple crops in coordination with supplementation programs may be particularly beneficial to children in malaria endemic regions.

Author contributions: KAB was responsible for training staff, facilitating field research, data entry and input into statistical analysis, and writing the paper; JC was responsible for coordinating blood sample collection and storage, analyzing blood slides for malaria parasitemia, and training local clinicians for collection of morbidity data; and SAT designed research, provided input into data analysis, and revised the paper. All authors read and approved the final manuscript.

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