Justus Liebig University Giessen

Institute of Nutritional Sciences

Professorship of Nutrition in Prevention and Therapy

Impact of baobab (*Adansonia digitata L*) fruit pulp consumption on hemoglobin and iron status in Kenyan schoolchildren: A randomized, controlled trial

DISSERTATION

for the degree of Dr. oec. troph.

in the Faculty of Agriculture, Nutritional Sciences, and Environmental Management

Justus Liebig University Giessen

Submitted by

Esther C. Evang, M.Sc., M.Sc.

Cologne, 2021

First supervisor: Prof. Dr. Gunter P. Eckert

Second supervisor: Prof. Dr. Bernd Honermeier

DOI: http://dx.doi.org/10.22029/jlupub-423

Contents

List of tables

List of figures

Abbreviations and acronyms

AGP	Acidic glycoprotein
BFP	Baobab fruit pulp
CRP	C-reactive protein
FER	Ferritin
FtFF	Food-to-food fortification
Hb	Hemoglobin
KNBS	Kenya National Bureau of Statistics
MUAC	Mid-upper arm circumference
NACOSTI	National Commission for Science, Technology, and Innovation research permit
sTfR	Soluble transferrin receptor

Acknowledgements

First of all, I would like to express my sincere thanks to my advisor and the principal investigator of the *Baobab Nutrition Intervention Study*, Prof. Michael Krawinkel, for his professional guidance and widespread backing. Our discussions helped me shape and refine ideas and I highly appreciate the constant and always very timely support. I would like to express my gratitude for encouraging my research and for allowing me to grow as a researcher.

I highly appreciate the input from my former colleagues from the International Nutrition Working Group at the Justus Liebig University Giessen who gave feedback. I am grateful for the support and exchange with Dr. Tsige-Yohannes Habte, Dr. Irmgard Jordan, Dr. Christine Ludwig, Dr. Sandra Habicht, Dr. Jan Makurat and Maria Gracia Glas who guided me along way. I also want to thank Daniela Rühl for the great administrative support and Dr. Johannes Herrmann for the statistical support. In particular, I would like to thank Maike Schnermann who spent months with me in the field in Kitui. I would like to express my appreciation for her excellent support during the implementation of the study, data collection and data analyses.

Thanks to the BAOFOOD team, particularly to Kathrin Meinhold, for their friendliness and support. My special thanks goes to Dr. Willis Omondi Owino for his facilitation and guidance in the preparatory and implementing phase of the field work. The kind assistance of George Kinyua Kaimba, Joyce Chepngeno, Dorah Momanyi, and Victoria Nyutu during my time in Kenya is highly appreciated.

I have received tremendous support from the field assistance. *Asante sana* and *nimuvea muno* to Eline Ressa, Rousemarie Awour, Grace Nzulu, Peter Kyalo, and Phidelis Mwanza. I would like to express my gratitude to the head and deputy head teachers of Heidemarie Primary School and Kakumuti Primary School. Their creativity, flexibility, and cultural understanding were crucial to the success of my field work.

I would like to thank the team of the Baraka Health Centre and Kauwi Sub-District Hospital for their assistance during data collections. In addition to the hard work of the enumerators, nurses, and drivers.

A heartfelt thank you to the school children and their caregivers who participated in the study. This study would not have been possible, without their patience and kindness.

The abundant emotional support, motivation and inspiration I have received from my friends and family have helped me throughout my PhD. I would like to thank everyone who has shared this exciting and demanding journey with me over the past five years.

1. Introduction

The baobab tree (*Adansonia digitata L.)* is native to many regions in Sub-Saharan Africa [1, 2]. The fruits, leaves, and seeds of the indigenous tree are rich in vitamins, essential amino acids, minerals, and bioactive components [3]. Baobab products are consumed by some communities in Sub-Saharan Africa as part of their diet, drinks, and medicine [4]. The baobab fruit pulp (BFP) (see Figure 1) is one of the most important parts of the tree. It is eaten raw, dissolved in water, or added to porridge [5].

© Evang

Figure 1: Picture of a baobab tree (left) and a cracked baobab fruit (right)

In Kenya, which is located in East Africa, baobab trees grow in dry areas along two belts, one in the inland from the Tanzanian border towards the northeast and a second one along the whole coastal region [4]. These marginalized areas are prone to droughts and high levels of food and nutrition insecurity, particularly among children and women [6]. In addition to environmental challenges, a predominantly cereal based Kenyan diet predisposes the population to micronutrient deficiencies due to inadequate dietary intake, low dietary diversity, and poor quality food [7, 8]. Iron deficiency is one of the major dietary deficiencies in Kenya [8], and it is associated with slow cognitive and physical developments in children, low immunity, and high mortality [9–13].

Several studies reported an elevated prevalence of iron deficiency related to inadequate dietary intake in Kenyan children [14–16]. A subnational analysis among schoolchildren in Kenya confirmed that inadequate food intake is an important driver of anemia in schoolchildren [17], especially in combination with a high intake of anti-nutrients [18] and a low intake of heme iron [18, 19]. Therefore, dietary means to improve iron uptake itself or absorption, e.g. via vitamin C, are warranted.

BFP is a good source of minerals, trace elements, and particularly vitamin C [3, 20, 21] and has a relatively high nutrient content compared to legumes, vegetables, and other fruits [22]. The

high vitamin C content is of particular interest as it is known to be the most notable and widely distributed plant-based promotor of iron absorption [23]. Furthermore, vitamin C prevents the dose-dependent effects of inhibitory compounds of iron absorption [24, 25].

Therefore, it is assumed that BFP consumption has the potential to improve the bioavailability of iron from plant based foods [26, 27], thereby addressing anemia and iron deficiency. This assumption fits into the concept of food-to-food fortification (FtFF), an emerging food-based strategy with the potential to address micronutrient deficiencies at once, with little dietary change required by consumers. FtFF encourages the utilization of local resources and creates market opportunities for locally produced foods [26].

Within the framework of FtFF, BFP could have a high potential for enhancing food security by providing nutrients to the diet and income generation to rural communities in Kenya [4]. Lately, the role of baobab in contributing to food security has focused on availability, access and/or stability [28–30], but little is known about the utilization of BFP in the human body. Therefore, the current thesis determines the impact of BFP consumption on the nutritional status of Kenyan schoolchildren, using the example of hemoglobin (Hb) and iron status.

1.1 Scope of the doctoral research

Research conducted for this dissertation was embedded in the BAOFOOD research project, which was funded by the *German Federal Ministry of Food and Agriculture* from 2016-2019. BAOFOOD aimed to promote the domestication, market development, processing, and consumption of baobab *(Adansonia digitata L.)* in order to improve food and nutrition security and rural livelihoods in Kenya. Rhine-Waal University of Applied Sciences (Germany), Justus Liebig University of Giessen (Germany), Jomo Kenyatta University of Agriculture and Technology (Kenya), the University of Kordofan (Sudan), and the University of Khartoum (Sudan) jointly implemented this interdisciplinary research project. This dissertation focuses on the utilization of BFP and its role in nutrient provision in Kenya. Specifically, it aimed to determine the impact of BFP in alleviating iron deficiency through the *Baobab Nutrition Intervention Study.*

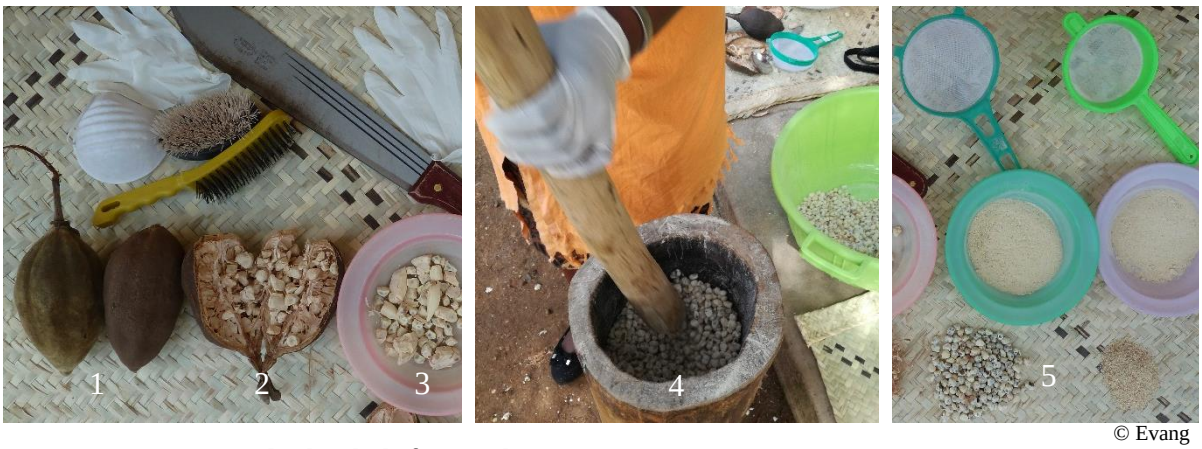
The *Baobab Nutrition Intervention Study*

The single-blind placebo-controlled, parallel group study was conducted among schoolchildren aged 6-12 years over 12 weeks. The first round was carried out in 2017 in Mathare, Nairobi County, an urban resource-poor setting. One year later, the study was repeated in Kakumuti, a rural area in Kitui County (see Figure 2). Both purposely selected schools had school meal

programs in place, which offered a local dish, *githeri*, a mix of maize and beans. *Githeri* is part of the Kenyan National School Meals and Nutrition Strategy [31] and contains a significant amount of plant-based iron.

Figure 2: Map of Kenya with Nairobi County (left) and Kitui County (right) *highlighted in green* (created with https://mapchart.net/africa-detailed.html)

Prior to the intervention, school children were screened for lowest Hb levels. In addition to the non-heme iron-rich school meal, children received either a drink with BFP (intervention group) or an isoenergy drink without BFP (control group). Due to the high vitamin C intake, the intervention group was expected to utilize more non-heme iron from the school meal than the control group. Processing the baobab fruit to powder is shown in Figure 3.



© Evang

Figure 3: Processing the baobab fruit pulp.
1. Cleaning baobab fruits with a wire brush first and with a soft brush afterwards to remove the hair from the outer shell. 2. Cracking the fruits with a machete. 3. Removing the pulp–seed mix from the shell. 4. Grounding the pulp–seed mix in a mortar to separate the pulp (which is in the form of a powder) from the seeds. 5. Sieving the powder twice in succession.

A detailed description of the inclusion and exclusion criteria are presented in Figure 4, page 7. Children who met the inclusion criteria were randomly allocated into intervention and control groups. At baseline and endline, anthropometric measurements and blood samples were taken.

Table 1 presents an overview of the main blood parameters. In the first (t1), fifth (t2), and eleventh (t3) week, 24h-recalls with primary caregivers were conducted to monitor the dietary intake outside the controlled school setting. An assessment of the nutritional intake is described elsewhere [32].

Table 1: Overview of the input, expected outcomes, and control parameters of the *Baobab Nutrition Intervention Study*

	Intervention group	**Control group**
Input	BFP drink	Isoenergy drink
Expected outcomes		
Main blood parameter	**Mean tissue iron stores** (sTfR)	
	↓ (improve)	→ (no change)
Other blood parameters	**Mean FER and Hb**	
	↑ (improve)	→ (no change)
Control parameters	Sickle cell and α-thalassemia traits AGP and CRP	

BFP: baobab fruit pulp; HFIES: household food insecurity experience scale; SES: socioeconomic status; WASH: water, sanitation, and hygiene; AGP: acidic glycoprotein; CRP: C-reactive protein; sTfR: soluble transferrin receptor; FER: ferritin; Hb: hemoglobin

The vitamin C content of the BFP was determined via HPLC using the method of Vikram, Ramesh, and Prapulla [33] with slight modifications as described by Evang et al. [32]. Iron, zinc, calcium, and magnesium were analyzed with an inductive coupled plasma-optic emission spectrometer [34].

The expected outcome was an improvement in the tissue iron stores in the intervention group determined by a decrease of the inverse indicator soluble transferrin receptor (sTfR). Hemoglobinopathies are associated with higher sTfR levels [35, 36]. Therefore, the study was controlled for the common Hb disorders in Kenya: sickle cell and α-thalassemia traits. Homozygous carriers were excluded, as well as combined cases of sickle cell and α-thalassemia traits.

An inflammation infection is known to elevate ferritin (FER) levels [37, 38]. Therefore, the inflammation markers acidic glycoprotein (AGP) and c-reactive protein (CRP) were accessed and used for adjusting FER concentrations and removing the effects of subclinical inflammation [38]. Hb was adjusted for altitude [37].

Blood analysis of sTfR, FER, AGP, and CRP were analyzed at the VitMin Lab in Germany, Hb disorders at the KEMRI-Wellcome Trust research laboratories in Kilifi, Kenya and Hb was directly assessed in the field with HemoCue [32].

The institutional review board of the Faculty of Medicine at Justus Liebig University Giessen, Germany (197/16) and the AMREF Ethics and Scientific Review Committee (AMREF—ESRC

P313/2017) Kenya, approved the *Baobab Nutrition Intervention Study* under the Kenyan National Commission for Science, Technology and Innovation research permit (NACOSTI/P/17/60305/15018 and NACOSTI/P/18/60305/20841). The study was registered with the German Clinical Trials Registry (DRKS00011935). Furthermore, municipal and governmental authorities in Kenya granted official permission for the implementation of the study.

1.2 Objective of the doctoral research

This dissertation had two major aims. The first was to identify the population that benefits most from a nutrition intervention with BFP by assessing and comparing malnutrition and micronutrient deficiencies linked to dietary intake among urban and rural Kenyan schoolchildren from food-insecure settings (Chapter 2). The second aim was to study the impact of BFP consumption on Hb level and iron statuses in Kenyan schoolchildren (Chapter 3). Additional data not presented in Chapters 2 or 3 is used in the discussion to support the extensive interpretation of results and explain the limitations of impact. Finally, the research results are discussed within the broader context of food security (Chapter 4).

1.3 The first round of the Baobab Nutrition Intervention Study

In 2017, the first round was conducted in Mathare, a resource-poor setting in Nairobi. Due to several challenges, a second round was implemented in 2018 in a rural area with an adjusted study design. The first and second rounds differed in the study design, therefore it was impossible to merge the data in a meaningful way. The results of the adjusted study design serve as the basis of this thesis. The following paragraphs describe the first round, its results, and derived adjustments for the second round.

No changes have been made to the sample size calculation, blood sample collection and analysis, anthropometric measurements, or to the vitamin C, iron, zinc, magnesium, and calcium analysis of the BFP. They are described in Chapter 3. Study sites and ethical approval are presented in Chapter 2.

Recruitment of participants and randomization

Schoolchildren in classes 2 and 3 from the Primary Heidemarie School were screened for signs of malnutrition, undernutrition, Hb level and sickle cell or α-thalassemia traits. Details of the inclusion and exclusion criteria are shown in Figure 4, page 7. In order to allocate participating children into either the intervention or control group, the Hb levels at screening were sorted in

ascending order. Starting with the lowest Hb, children were assigned to either the intervention or control groups in alternating order.

Intervention

The first round of the randomized controlled trial lasted 11 weeks (57 regular school days) from May to July 2017. In addition to a standardized school meal, study children received either one cup of a drink with BFP (n = 28) or one cup of a drink without BFP (n = 29). The standardized portion of the regular school meal had an estimated iron content of 3.3 mg per portion, mainly from beans and maize (NutriSurvey2007) [19] and consisted of kidney beans, maize, cabbage, carrots, iodized salt, and vegetable oil. The company *Wild Living Resources* in Kilifi processed the BFP every two weeks, packed it into plastic bags opaque to light and air, and sent it to the study side.

A weighted cup of intervention drink contained 30 g BFP, 10 g honey, 110 ml of bottled water and 70 ml of mango juice. The control drink consisted of 10 g honey, 110 ml water and 70 ml mango juice. About 20-30 minutes prior to distributing the drinks to the children, all ingredients for the intervention and control drink were blended. As mango juice itself contains vitamin C, the juice was boiled for 20 minutes the day before consumption to remove it.

The cups were coded with different colors to differentiate between intervention and control drinks. Two trained field assistants observed the children during the consumption to avoid any exchange of food and drinks. They recorded the amount of food and drink consumed by each child. Even though the study team only provided the regular school meal to the children, some children brought their own home-cooked meal. The field assistants recorded the food and estimated the portion sizes with the photo book, as described in Evang et al. [19]. The acceptance of the school meal decreased towards the end of the intervention; some children only took the drink without additional food.

Statistical analyses

Data management and statistical analysis were performed using SPSS software. The mean intake of energy and nutrients, determined through NutriSurvey, at time points t1, t2, and t3 was calculated for each child. Normality of distributions was evaluated using the Shapiro–Wilk test. As most continuous variables (micronutrient status and energy and nutrient intake) had heavily skewed distributions, descriptive statistics for continual variables are presented in the median and interquartile range (IQR). Outliers in development (baseline to endline) of Hb, FER, and sTfR were identified as described by Tukey [39] and excluded from the analysis (outliers

control group: n(Hb) = 1). The baseline and endline data on FER and sTfR were log transformed and used to calculate the development between baseline and endline to apply the independent samples' t test for differences between groups and the paired *t test* for development within the group.

Results

Of the 209 eligible schoolchildren in classes 2 and 3, a total of 118 were screened. The exclusion criteria are presented in Figure 4; a total of 50 children were available for the analyses.

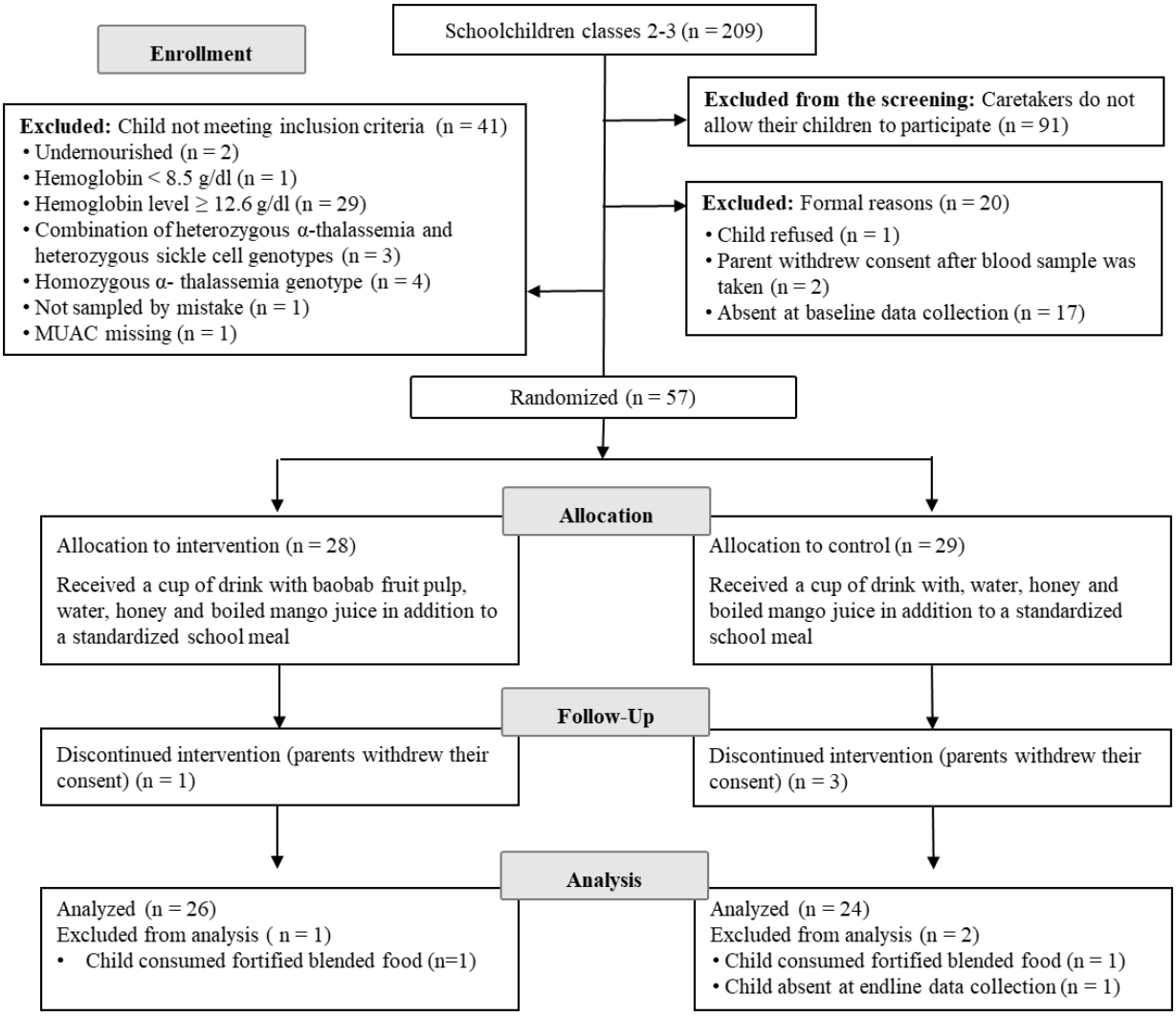


Figure 4: Consort flow diagram of the first round of the *Baobab Nutrition Intervention Study*

MUAC: mid-upper arm circumference

The Hb levels were much higher than expected. In order to include the required number of children, a cut-off for low Hb levels was set at 12.6 g/dl, i.e. 9% above the normal cut-off at 11.5 g/dl used in Kenyan schoolchildren otherwise. The majority of apparently healthy

participants were girls with 73.1% in the intervention and 70.8% in the control group. Table 2 shows the baseline characteristics of participants.

Table 2: Median (IQR) of anthropometric characteristics for intervention and control group at baseline

		Intervention		**Control**	*Median Test*
	n	Median (IQR)	n	Median (IQR)	
Age					
Age in years	26	8 (8-9)	24	8 (8-9)	*0.802*
Nutritional status					
Weight (kg)		23.8 (22.8-26.5)		24.9 (23.2-27.9)	*0.571*
Height (cm)		128.6 (120.7-133.1)		129.4 (124.6-131.3)	*0.571*

At baseline, the median test did not show any significant differences between the intervention and control groups in terms of median Hb (non-adjusted and adjusted), FER (non-adjusted and adjusted), sTfR, CRP, and AGP (data not presented). Elevated inflammation markers were detected as follows: (a) one child with incubation in the intervention group, (b) two children with early convalescence, and one child with late convalescence in the control group.

In the intervention group, 30.8% were carriers of heterozygous α-thalassemia and 7.7% of heterozygous sickle cell traits. In the control group, 25.0% were carriers of heterozygous α-thalassemia and 8.3% of heterozygous sickle cell traits.

Impact of baobab intake on hemoglobin and iron status

For the blood analysis of FER and sTfR, two participants in the intervention and three in the control group were excluded due to false tube labeling during data collection.

Table 3 shows the baseline and endline values for Hb, FER, and sTfR.

Between baseline and endline, changes in blood parameters showed a better tendency of Hb (mean), FER (geometric mean), and sTfR (geometric mean) in the intervention than in the control group. In the intervention group, the geometric mean of FER values improved (6.9%), while mean Hb (0.4%) and the geometric mean of sTfR (1.1%) remained unchanged. None of the changes were at a significant scale. In the control group, mean Hb levels significantly worsened by 3.2% ($t(22) = 2.218$, $p = 0.037$). The geometric mean of FER worsened by 7.3%, while sTfR remained unchanged (0.6%). As presented in Figure 5 (page 11), the independent *t test* did not show significant differences in changes of blood parameters between the intervention and control groups for Hb, FER, and sTfR, respectively.

Table 3: Hemoglobin level and iron status at baseline and endline

	Intervention		Control	
	Baseline	Endline	Baseline	Endline
Hb (g/dL)	n = 26		n = 23	
Hb, mean ± SD	12.9 ± 0.76	12.9 ± 0.87	13.1 ± 0.78	12.7 ± 0.77
Hb, adj [a] mean ± SD	12.4 ± 0.76	12.4 ± 0.87	12.6 ± 0.78	12.2 ± 0.77
Hb, adj [a] < 11.5, n (%)	3 (11.5)	5 (19.2)	1 (4.2)	6 (25)
FER (ug/L)	n = 24		n = 21	
FER, geometric mean ± SD	50 ± 1.89	53.1 ± 1.62	66.1 ± 1.76	60.0 ± 1.74
FER adj [b], geometric mean ± SD	49.6 ± 1.90	50.2 ± 1.58	60.6 ± 1.60	56.1 ± 1.84
FER adj [b], < 15 µg/L, n (%)	1 (3.8)	1 (3.8)	0	1 (4.8)
sTfR (mg/L)	n = 24		n = 21	
sTfR geometric mean ± SD	6.1 ± 1.29	6.1 ± 1.26	5.8 ± 1.18	5.8 ± 1.20
sTfR > 8.3, n (%)	4 (16.0)	4 (16.0)	1 (4.8)	0

[a] Hemoglobin adjusted for altitude, [b] ferritin adjusted for inflammation stage, Hb: hemoglobin; FER: Ferritin, sTfR: soluble transferrin receptor

Notably, the statistically significant decrease in Hb levels of the control group does not automatically translate into a biologically meaningful significance. The mean difference in Hb measurements with HemoCue of successive blood drops from the same donors was reported higher (0.5 g/dl) [40, 41] than the actual change in Hb from baseline to endline (0.4 g/dl) observed in the control group.

Lessons learned

During the first intervention study in 2017, several challenges occurred that were addressed in an adjusted study design in 2018 (Table 4, page 12).

Setting-related challenges

Hb levels at screening were much higher than expected (expected anemia prevalence 25% among children aged 6 months to 12 years) [42]. Even though children with the lowest Hb were selected, the prevalence of anemia at baseline was only 8.2%. Intervention effects on improved Hb levels were expected to be higher in a population with a lower Hb levels [43, 44].

The intervention effect was further reduced as it was impossible to conduct *Baobab Nutrition Intervention* on a daily basis. Participants in this urban setting were unable to attend on weekends or public holidays. Also, the low compliance of caretakers to attended interviews during the course of the intervention made it difficult to monitor the nutrient intake of participants outside of the school. The low compliance was mainly due to busy working

schedules of caretakers. Caretakers were very busy generating their income and were unable to spare time for the interviews.

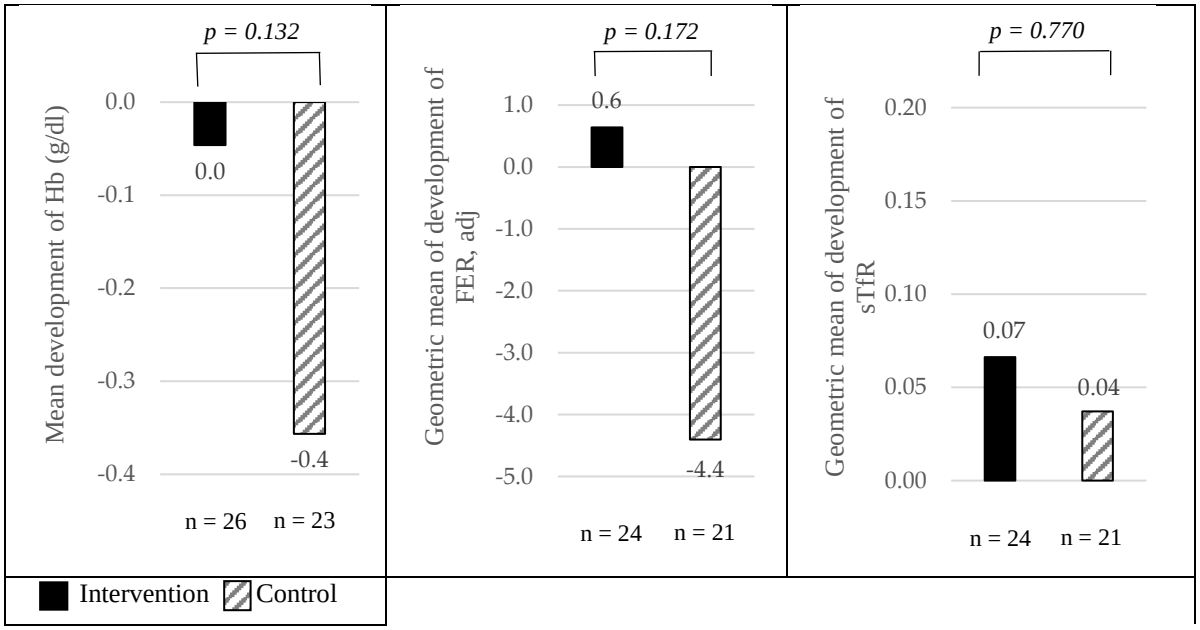


Figure 5: Changes in hemoglobin and iron status from baseline to endline

An independent t-test was applied for the difference in development between the intervention and control groups. (a) Mean development of Hb with non-significant differences between the intervention and control groups. (b) Development of the geometric mean of FER and with non-significant difference between the development of LN(FER) in the intervention and control groups. (c) Development of the geometric mean of sTfR with a non-significant difference between the development of LN(sTfR) in the intervention and control groups. Hb: hemoglobin, FER: ferritin, sTfR: soluble transferrin receptor.

Standardized intervention

A standardized iron intake together with intervention or control drinks were only partly achieved, as children occasionally brought their own home-cooked meals, particularly towards the end of the trimester. Their motivation to eat the regular school meal decreased over the course of their studies and some children even took the drink without any school meal. Hence, the compatibility of the participants in terms of iron intake, in addition to the intake of intervention and control drinks, was limited.

Low vitamin C content of BFP

Moreover, the vitamin C content of the BFP from Kilifi was lower (mean 65 mg / 100 g, N = 12, sd = 29, min = 24, max = 126) than expected from the literature (> 200 mg / 100 g) of vitamin C [3, 20].

1.4 Adaptation of the study design and second round of the *Baobab Nutrition Intervention Study*

Based on the lessons learned, it was decided to conduct the *Baobab Nutrition Intervention Study* in a rural area, particularly in Kitui County. In Kitui the BAOFOOD project was well known, and collaborations with ministries already existed because of several research activities in 2017.

Moreover, the anemia prevalence of schoolchildren in rural areas is higher than in urban areas [45]. Relatively high levels of food insecurity in Kitui are likely to contribute to a lower nutritional and health status. For example, the stunting rate among children under five is among the highest in the country [6]. There was no national data available on the prevalence of hemoglobinopathies, but due to low malaria prevalence in Kitui, hemoglobinopathies were expected to be low. Furthermore, the Kenyan Demographic and Health Survey states that the willingness to participate in interviews is generally higher in rural than in urban settings.

Table 4: Adaptation of the study design from 2017 to 2018

	2017	**2018**
Increase number of children enrolled at screening		
Enrollment procedure	**Classes 2 and 3**	**All children aged 6-12**
Improved randomization approach		
Allocation into intervention and control groups	With ascending Hb levels, alternately allocated into intervention and control groups	Stratified random sampling according to sex, Hb level, and age
Adaptation to improve vitamin C content of the BFP		
Location of harvest *(inland has higher vitamin C content*	Kilifi (coast)	Kitui (inland)
Duration between harvest and consumption in months (min-max)	9-12	1-3
Processing of baobab pulp	Every two weeks	Daily, 2-3 hours prior to consumption
Adaptation of the recipe to improve acceptance of the intervention and control drink		
Amount of BFP in the drinks	30 g	20 g
Recipe of intervention drink	30 g BFP, boiled mango juice, water	20g BFP, mango flavor, honey, water
Recipe of control drink	Boiled mango juice, honey, water	Starchy corn flour, mango flavor, honey, water
Modification of the school meal to increase iron content and acceptance		
Modification of the school meal (iron content per portion)	No modification of the regular school meal (3.3 mg)	Modification of the school meal by increasing amounts of beans and portion sizes (7.6 mg)

BFP: baobab fruit pulp

Furthermore, preliminary results of BAOFOOD partners showed higher vitamin C content of baobab pulps from Kitui than from Kilifi, which was later confirmed [21]. Baobab pulp for the first *Baobab Nutrition Intervention Study* came from Kilifi. Baobab suppliers from Kibwezi/Kitui were identified for providing baobab pulp samples for the second *Baobab Nutrition Intervention Study*.

Unfortunately, the official permission to ship blood samples to Germany for analyses of FER, sTfR, CRP, and AGP took more than a year, even though authorities such as NACOSTI and AMREF previously approved the study design, which clearly stated this procedure. The complete evaluation of the study in 2017 was conducted after the intervention 2018 had already been completed. Only the impact of Hb levels and anthropometric measurements was evaluated prior to the intervention in 2018.

References

1. Jama BA, Mohamed AM, Mulatya J et al. (2008) Comparing the “Big Five”: A framework for the sustainable management of indigenous fruit trees in the drylands of East and Central Africa. Ecological Indicators 8:170–179. https://doi.org/10.1016/j.ecolind.2006.11.009
2. Venter SM, Witkowski ETF (2013) Fruits of our labour: Contribution of commercial baobab (Adansonia digitata L.) fruit harvesting to the livelihoods of marginalized people in northern Venda, South Africa. Agroforest Syst 87:159–172. https://doi.org/10.1007/s10457-012-9532-6
3. Chadare FJ, Linnemann AR, Hounhouigan JD et al. (2009) Baobab food products: a review on their composition and nutritional value. Crit Rev Food Sci Nutr 49:254–274. https://doi.org/10.1080/10408390701856330
4. Gebauer J, Adam YO, Sanchez AC et al. (2016) Africa’s wooden elephant: The baobab tree (Adansonia digitata L.) in Sudan and Kenya: a review. Genet Resour Crop Evol 63:377–399. https://doi.org/10.1007/s10722-015-0360-1
5. Zahrau B, Mohammed AS, Ghazali HM et al. (2014) Baobab Tree (Adansonia digitata L) Parts: Nutrition, Applications in Food and Uses in Ethno-medicine – A Review. Ann Nutr Disord & Ther 1:1011
6. Kenya National Bureau of Statistics (KNBS) and ICF Macro (2015) Kenya Demographic and Health Survey 2014. https://dhsprogram.com/pubs/pdf/fr308/fr308.pdf. Accessed 18 Oct 2019
7. Kenyan Ministry of Public Health and Sanitation (2012) National Nutrition Action Plan 2012-2017. https://extranet.who.int/nutrition/gina/sites/default/files/KEN%202012%20National%20Nutrition%20Action%20Plan%202012%20-%202017.pdf. Accessed 16 Apr 2020
8. Food and Agriculture Organization (2004) Nutrition Country Profile: Republic of Kenya. Accessed 08 Sep 2016
9. Kumar V, Choudhry VP (2010) Iron deficiency and infection. The Indian Journal of Pediatrics 77:789–793. https://doi.org/10.1007/s12098-010-0120-3
10. Grantham-Mc Gregor S, Ani C (2001) A Review of Studies on the Effect of Iron Deficiency on Cognitive Development in Children. J Nutr 131:649S-668S
11. WHO (2001) Iron Deficiency Anaemia: Assessment, Prevention and Control. Version current 2001
12. Scott SP, Chen-Edinboro LP, Caulfield LE et al. (2014) The impact of anemia on child mortality: an updated review. Nutrients 6:5915–5932. https://doi.org/10.3390/nu6125915
13. Soliman AT, Al Dabbagh MM, Habboub AH et al. (2009) Linear growth in children with iron deficiency anemia before and after treatment. J Trop Pediatr 55:324–327. https://doi.org/10.1093/tropej/fmp011
14. Grant FKE, Martorell R, Flores-Ayala R et al. (2012) Comparison of indicators of iron deficiency in Kenyan children. Am J Clin Nutr 95:1231–1237. https://doi.org/10.3945/ajcn.111.029900
15. Suchdev PS, Ruth LJ, Woodruff BA et al. (2012) Selling Sprinkles micronutrient powder reduces anemia, iron deficiency, and vitamin A deficiency in young children in Western Kenya: a cluster-randomized controlled trial. Am J Clin Nutr 95:1223–1230. https://doi.org/10.3945/ajcn.111.030072
16. Siekmann JH, Allen LH, Bwibo NO et al. (2003) Kenyan school children have multiple micronutrient deficiencies, but increased plasma vitamin B-12 is the only detectable micronutrient response to meat or milk supplementation. J Nutr 133:3972S-3980S
17. Pullan RL, Gitonga C, Mwandawiro C et al. (2013) Estimating the relative contribution of parasitic infections and nutrition for anaemia among school-aged children in Kenya: A subnational geostatistical analysis. BMJ Open 3. https://doi.org/10.1136/bmjopen-2012-001936

18. Neumann CG, Bwibo NO, Murphy SP et al. (2003) Animal Source Foods Improve Dietary Quality, Micronutrient Status, Growth and Cognitive Function in Kenyan School Children: Background, Study Design and Baseline Findings. J Nutr 133:3941S-3949S
19. Evang EC, Habte T-Y, Owino WO et al. (2020) The Nutritional and Micronutrient Status of Urban Schoolchildren with Moderate Anemia is Better than in a Rural Area in Kenya. Nutrients 12. https://doi.org/10.3390/nu12010207
20. Stadlmayr B, Charrondiere UR, Eisenwagen S et al. (2013) Nutrient composition of selected indigenous fruits from sub-Saharan Africa. J Sci Food Agric 93:2627–2636. https://doi.org/10.1002/jsfa.6196
21. Stadlmayr B, Wanangwe J, Waruhiu CG et al. (2020) Nutritional composition of baobab (Adansonia digitata L.) fruit pulp sampled at different geographical locations in Kenya. Journal of Food Composition and Analysis 94:103617. https://doi.org/10.1016/j.jfca.2020.103617
22. Habte T-Y, Krawinkel MB (2017) Metaphysical Analysis of the Nutritional and Therapeutic Value of Baobab (Adansonia Digitata L.). J Nutr Health Sci 5:101. https://doi.org/10.15744/2393-9060.5.101
23. Gibson RS, Perlas L, Hotz C (2006) Improving the bioavailability of nutrients in plant foods at the household level. Proc Nutr Soc 65:160–168. https://doi.org/10.1079/PNS2006489
24. Siegenberg D, Baynes RD, Bothwell TH et al. (1991) Ascorbic acid prevents the dose-dependent inhibitory effects of polyphenols and phytates on nonheme-iron absorption. Am J Clin Nutr 53:537–541
25. Lynch SR, Cook JD (1980) Interaction of Vitamin C and Iron. Ann NY Acad Sci 355:32–44. https://doi.org/10.1111/j.1749-6632.1980.tb21325.x
26. Kruger J, Taylor JRN, Ferruzzi MG et al. (2020) What is food-to-food fortification? A working definition and framework for evaluation of efficiency and implementation of best practices. Comprehensive Reviews in Food Science and Food Safety 19:3618–3658. https://doi.org/10.1111/1541-4337.12624
27. van der Merwe R, Kruger J, Ferruzzi MG et al. (2019) Improving iron and zinc bioaccessibility through food-to-food fortification of pearl millet with tropical plant foodstuffs (moringa leaf powder, roselle calyces and baobab fruit pulp). J Food Sci Technol 56:2244–2256. https://doi.org/10.1007/s13197-019-03711-y
28. Jäckering L, Fischer S, Kehlenbeck K (2019) A value chain analysis of baobab (Adansonia digitataL.) products inEastern and Coastal Kenya. Journal of Agriculture and Rural Development in the Tropics and Subtropics 120:91–104
29. Momanyi DK, Owino WO, Makokha A et al. (2019) Gaps in food security, food consumption and malnutrition in households residing along the baobab belt in Kenya. Nutrition & Food Science 138:47. https://doi.org/10.1108/NFS-11-2018-0304
30. Kaimba GK, Mithöfer D, Muendo KM (2021) Commercialization of underutilized fruits: Baobab pulp supply response to price and non-price incentives in Kenya. Food Policy 99:101980. https://doi.org/10.1016/j.foodpol.2020.101980
31. Ministry of Education, Science and Technology, Ministry of Health, and Ministry of Agriculture, Livestock and Fisheries (2016) School Nutrition and Meals Strategy for Kenya. https://extranet.who.int/nutrition/gina/sites/default/files/KEN_school%20nutrition%20and%20meals%20strategy%20for%20Kenya.pdf. Accessed 25 Nov 2018
32. Evang EC, Habte T-Y, Owino WO et al. (2020) Can the supplementary consumption of baobab (Adansonia digitata L.) fruit pulp improve the hemoglobin levels and iron status of schoolchildren in Kenya? Findings of a randomized controlled intervention trial. Eur J Nutr. https://doi.org/10.1007/s00394-020-02447-2

33. Vikram VB, Ramesh MN, Prapulla SG (2005) Thermal degradation kinetics of nutrients in orange juice heated by electromagnetic and conventional methods. Journal of Food Engineering 69:31–40. https://doi.org/10.1016/j.jfoodeng.2004.07.013
34. Habte TY, Suleiman I, Adam HE et al. (2019) The potential of Baobab (Adansonia Digitata L) leaves from North- and West Kordofan in Sudan as mineral complement of common diets. J Nutrition and Food Processing 2. https://doi.org/10.31579/2637-8914/015
35. George J, Yiannakis M, Main B et al. (2012) Genetic hemoglobin disorders, infection, and deficiencies of iron and vitamin A determine anemia in young Cambodian children. J Nutr 142:781–787. https://doi.org/10.3945/jn.111.148189
36. Gebremedhin S (2020) Soluble transferrin receptor level, inflammation markers, malaria, alpha-thalassemia and selenium status are the major predictors of hemoglobin in children 6-23 months in Malawi. Food Sci Nutr 8:4601–4610. https://doi.org/10.1002/fsn3.1780
37. WHO (2017) Nutritional anaemias: tools for effective prevention and control. https://www.who.int/nutrition/publications/micronutrients/anaemias-tools-prevention-control/en/. Accessed 16 Apr 2020
38. Thurnham DI, McCabe LD, Haldar S et al. (2010) Adjusting plasma ferritin concentrations to remove the effects of subclinical inflammation in the assessment of iron deficiency: a meta-analysis. Am J Clin Nutr 92:546–555. https://doi.org/10.3945/ajcn.2010.29284
39. Tukey JW (1977) Exploratory Data Analysis. Addison-Wesley, Reeading, MA
40. Bond MM, Richards-Kortum RR (2015) Drop-to-drop variation in the cellular components of fingerprick blood: implications for point-of-care diagnostic development. Am J Clin Pathol 144:885–894. https://doi.org/10.1309/AJCP1L7DKMPCHPEH
41. Conway AM, Hinchliffe RF, Earland J et al. (1998) Measurement of haemoglobin using single drops of skin puncture blood: is precision acceptable? J Clin Pathol 51:248–250
42. Kenya Ministry of Health (2016) Kenya Malaria Indicator Survey 2015. https://dhsprogram.com/pubs/pdf/MIS22/MIS22.pdf. Accessed 16 Apr 2020
43. Hunt JR (2005) Dietary and physiological factors that affect the absorption and bioavailability of iron. Int J Vitam Nutr Res 75:375–384. https://doi.org/10.1024/0300-9831.75.6.375
44. Andang'o PEA, Osendarp SJM, Ayah R et al. (2007) Efficacy of iron-fortified whole maize flour on iron status of schoolchildren in Kenya: A randomised controlled trial. The Lancet 369:1799–1806. https://doi.org/10.1016/S0140-6736(07)60817-4
45. Kenya Ministry of Health (2015) The Kenya National Micronutrient Survey 2011. http://www.nutritionhealth.or.ke/wp-content/uploads/Downloads/The%20Kenya%20National%20Micronutrient%20Survey%202011.pdf. Accessed 16 Apr 2020

2. The nutritional and micronutrient status of urban schoolchildren with moderate anemia is better than in a rural area in Kenya

nutrients

MDPI

Article

The Nutritional and Micronutrient Status of Urban Schoolchildren with Moderate Anemia is Better than in a Rural Area in Kenya

Esther Charlotte Evang [1], Tsige-Yohannes Habte [1], Willis Omondi Owino [2] and Michael Bernhardt Krawinkel [1,*]

[1] Institute of Nutritional Sciences, Justus-Liebig-University Giessen, Wilhelmstr. 20, D-35392 Giessen, Germany; esther.c.evang@ernaehrung.uni-giessen.de (E.C.E.); tsige-yohannes.habte@ernaehrung.uni-giessen.de (T.-Y.H.)

[2] School of Food and Nutritional Sciences, Jomo Kenyatta University of Agriculture and Technology, Juja, Nairobi 62000-00200, Kenya; willis@agr.jkuat.ac.ke

* Correspondence: michael.krawinkel@uni-giessen.de; Tel.: +49-641-99-39034

Received: 19 November 2019; Accepted: 8 January 2020; Published: 13 January 2020

check for updates

Abstract: Low diet quality is a driver of general and micronutrient malnutrition in urban and rural areas. The objective was to compare malnutrition and micronutrient deficiencies linked to dietary intake among urban and rural schoolchildren from food insecure settings in Kenya. The cross-sectional study was conducted among urban and rural schoolchildren aged 7–9 years. Height and weight were measured, venous blood samples were assessed and data on dietary intake was collected. After screening out children with hemoglobin >12.2 g/dL and moderate or severe undernutrition,a total of 36 urban and 35 rural children participated. The prevalence of moderate underweight, wasting, and stunting were lower in urban than in rural children, with significant differences in median *z*-scores for underweight ($p < 0.001$) and wasting ($p < 0.001$). Significantly higher values forserum ferritin ($p = 0.012$) and zinc ($p < 0.001$) were found in urban children. Yet, the median adequacy ratios were higher for vitamin C ($p = 0.045$), iron ($p = 0.003$), and zinc ($p = 0.003$) in rural than in urban children. General nutritional, iron, and zinc status were significantly better in slightly anemic urban children than in rural ones. Improving the nutrition of schoolchildren in urban and rural settings requires different dietary approaches.

Keywords: nutritional status; micronutrients; dietary intake; urban; rural; schoolchildren

1. Introduction

Adequate nutrient and energy intake through diverse diets are important drivers for healthy child development. In sub-Saharan Africa, diverse diets contribute to overall nutritional adequacy [1,2] and are associated with a good nutritional status [3]. Monotonous, cereal-based diets with low nutrient density are often linked to a lack of access to vegetables, fruits, and animal-sourced food (ASF), causing deficiencies in essential micronutrients [4]. Often, these deficiencies are not apparent, hence they are also called hidden hunger [5].

Living environment, access to clean water, sanitary facilities, health facilities, and food differs between urban and rural settings in Kenya. Food consumers in urban resource poor setting are primarily depended on the market supply, while people in rural settings mainly depend on self-produced agricultural commodities. In urban resource poor settings, the most available and affordable diets are often unhealthy [6]. Likewise, typical street foods in resource poor settings in Nairobi tend to be high in calories and low micronutrient content [7]. In rural Kenyan settings, access to these street foods is limited. The supply of vegetables (kales, tomatoes, onions) in Nairobi is fairly constant across the

year [8]. Nevertheless, the poorest people in urban areas tend to be the lowest consumers of fruit and vegetables, which already accounts for 17.1% for vegetables and 6.5% for fruits on their share of food expenditure [9]. In rural settings, the consumption of vegetables varies by season [10].

Children from households affected by food insecurity are particularly vulnerable to micronutrient deficiencies. These do not allow them to reach their full physical and cognitive potential, and their educational and professional achievements in later life are impaired [11]. These children are often underweight and stunted, with higher prevalence in rural than urban children [12]. This also applies for Kenya, where stunting and underweight affect 20% and 7% of urban, and 30% and 13% or rural children, respectively [13]. Urban/rural differences in micronutrient status in Kenyan children are less distinct, nevertheless prevalence or micronutrient deficiency tend to be higher in rural settings [14].

Even though urban/rural differences are investigated, disparities within urban or rural groups are often neglected. Disparities within groups are higher in urban than in rural groups [15], which is challenging for the interpretation of mean values that are usually reported. When comparing prevalence of malnutrition of lowest socioeconomic group for urban and rural settings, prevalence of stunting is still lower in urban than rural children, yet the difference is extremely small [16]. Accordingly, when adjusting for socioeconomic status, urban/rural differences in child malnutrition reported in Demographic and Health Surveys are often not observed [15]. Similar applies to the influence of food insecurity to malnutrition. The prevalence of malnutrition in children increases with food insecurity [17,18], which applies also for the lowest income groups after controlling for wealth status.Wealth status was calculated as a latent variable computed from a composite measure of household assets and amenities [17].

We assumed that malnutrition is more prevalent in food-insecure rural children than in urban ones. This difference demands different strategies for nutrition improvement in the respective settings. The purpose of this study is to describe and compare malnutrition and micronutrient deficiencies linked to dietary intake among urban and rural schoolchildren from food insecure settings in Kenya.

2.Materials and Methods

2.1. Study Design and Sites

In this paper, analyses of the baseline data from a study on the impact of baobab fruit pulp (*Adansonia digitata L.*) consumption on the iron status (*Baobab Nutrition Intervention Study*) are presented. We selected children with low hemoglobin levels at screening because we expected to see greater improvements in their iron status through the intervention with the additional supply of vitamin C through baobab fruit pulp compared to children without anemia. The number of children was calculated for the *Baobab Nutrition Intervention Study*. Sites were selected according to a reported low prevalence of malaria, high levels of food insecurity, and no or little access to baobab fruits. The public pre- and primary schools were purposely selected according to the following criteria: (a) school meal program in place, (b) day school, (c) about 250 children aged 6–12 years, and (d) accessibility by car. For both schools, we only approached schools were the authorities and the head masters were known to be supportive and open for the intervention. The study was implemented in May 2017 and 2018, respectively towards the end of the rainy season. In 2017, the amount of rain was below normal rainfall while it was above normal in 2018. In both settings, school meals were not fortified.

In 2017, the study was conducted in an urban resource poor setting at Heidemarie Primary School in Mathare IV, (Nairobi, Kenya). About 1400 children attended the urban school, which was supported by the school meal program of the World Food Programme of the United Nations. Families had to pay a fee (11 Kenyan shillings per day, which equals 0.10 USD) at the beginning of each trimester for participation in the school meal program. One year later, a second round of the study was implemented at the rural Kakumuti Primary School in Kakumuti, a village in Musengo zone, Kitui-West, Eastern Province of Kenya, approximately 165 km away from Nairobi. About 430 children

attended the school with a self-governed school meal program. At the beginning of the trimester, families had to bring maize and beans, as well as firewood during the trimester.

2.2. Sample Size

The sample size calculation was based on the planned *Baobab Nutrition Intervention Study* of which the baseline data are presented here. With a total of 66 children aged 6–12 years for the urban and rural school, respectively, an assumed dropout of 10%, and a prevalence 5% of homozygote forms of sickle cell and α-thalassemia traits or a coincidence of heterozygote forms, data on 56 children was required for a planned *Baobab Nutrition Intervention Study*.

2.3. Sampling of Study Participants

After receiving official research permits and consent from the representatives of the purposely selected urban and rural schools, locally trained project assistants described the study in Kiswahili (urban setting) and Kikamba (rural setting) to caregivers of the children eligible for screening. Written informed consent (signature or fingerprint) was obtained from children's primary caretakers prior to any data collection. Only children of caretakers who gave consent were invited to the screening. The assistants orally informed these children about the study objective and procedure of the upcoming exercise, and children signed a sheet to document that they have been informed. Children's oral consent and their signature was prerequisite for any further interview and examination. Locally trained nurses and laboratory technicians performed the clinical screening in a separate room and gave all children a deworming remedy (albendazole USP 400). Exclusion criteria are shown in Figure 1.

2.4. Questionnaires and 24h-Recalls

Interviewer with a formal background in nutrition or food science, as well as literate in English and the local languages, were trained on applying standardized semi-structured questionnaires and 24h-recalls with primary caregiver. The questionnaire and 24h-recalls used were translated into the local languages (Kiswahili and Kikamba) and retranslated into English, reviewed during the 6-day interview training, pre-tested and modified to ensure meaning equivalence of the questions. Pre-testing was carried out among households with children not sampled for the study.

With the tablet-based questionnaires developed with Open Data Kit (ODK), information on the history of disease during one month preceding the interview, infant feeding, socioeconomic status, water, sanitation and hygiene, and location of the household with GPS was collected. The linear distance from households to the schools was determined with the software ArcGISPro 2.3 (https://www.esri.com/de-de/arcgis/about-arcgis/overview).

The interviews for the multiple-pass 24h-recalls consisted of (a) listing all foods and drinks consumed the day before the interview, (b) gathering detailed information about each food or recipe for dishes, (c) estimated quantification of the amount of consumed food/drink and used ingredients for the recipes, and (d) reviewing the information with the respondent at the end of the recall. Specially designed photo books for each the urban and rural setting were developed to estimate the quantity of intake of food and drinks. Interviewer also used local measuring tools such as spoons and cups for quantifying portion sizes.

Table 1 shows the recommended dietary allowances for energy, vitamins, and trace elements for school-aged children. Individual energy adequacy ratios were calculated as total energy intake divided by sex, and age-specific energy requirements, based on the recommendations of the FAO/WHO/UNU expert committee on human energy requirements [19]. The nutrient adequacy ratio (NAR) was determined for vitamin C, B12, and A (defined as retinol equivalent), as well as for iron and zinc. Individual NARs were calculated as a total intake of the nutrient divided by the recommended daily allowance (RDA) for that nutrient, based on intakes recommended by the Kenyan Ministry of Health [14].

Table 1. Recommended dietary allowances of energy, vitamin, and trace elements forschool-aged children.

Energy and Nutrients [1]	7–8 Years	9 Years
Energy (kcal)	1694	1916
Vitamin C (mg)	25	45
Vitamin B12 (µg)	1	1.8
RE (µg)	400	600
Iron (mg)	10	8
Zinc (mg)	5	8

[1] Reference values of the Kenya National Micronutrient Survey 2011 [14] except for energy (presented as means for boys/girls aged 7–8 years and 9 years, respectively) [19]; RE: retinol equivalent.

To evaluate the source of nutrients, food and meals consumed were allocated to a modified food group list [20]:

(1) Starchy staple food
    (a) Ugali (stiff cornmeal porridge made from maize flour)
    (b) Chapati (unleavened fried flatbread made from wheat flour)
    (c) Mandazi (African doughnut made from wheat flour)
(2) Mixed dishes with legumes
(3) Vegetables and fruits
    (a) Vitamin A-rich fruits
    (b) Green leafy vegetables
(4) Flesh foods (any kind of meat, organ meat, seafood, insects, and insect eggs)
(5) Dairy products
(6) Eggs
(7) Others (fat, tea, sugar and sweets, spices)

The food group 'others' contains ingredients that could only be quantified for overall nutrient intake and is therefore not included in Table 6. As children in the rural setting consumed different types of beans in one mixed dish, while urban children only consumed kidney beans, a food group-mixed dish with legumes was constructed. In the urban setting, it was common to buy cooked beans and add them to mixed dishes, while in the rural setting dried beans were used and boiled as part of the preparation process. Therefore, the total amount of beans per meal could not be compared. For *chapati* and *mandazi*, only the overall nutrient intake could be evaluated.

Mixed dishes with legumes were commonly consumed at home and at school. Table 2 shows the intake from regular school meals, a mixture of maize and beans. The data presented here are baseline data of the *Baobab Nutrition Intervention Study*. Any intervention-related foods and drinks were not included in the data analysis.

Table 2. Energy and nutrients of one regular portion of school meal (calculated in NutriSurvey©).

Variables	Urban (Portion = 248 g)	Rural (Portion = 234 g)
Energy (kcal)	312	277
Vitamin C (mg)	5	0
Vitamin B12 (µg)	0.0	0.0
RE (µg)	17	0
Iron (mg)	3.6	3.3
Zinc (mg)	1.6	1.7

2.5. Anthropometric Measurements

At screening, registered local nurses received an additional instruction on how to assess the mid-upper arm circumference (MUAC) with a measuring tape that allows an assessment to the nearest 0.1 cm. Moderate undernutrition was defined at MUAC <14.5 cm and <18.5 cm for children aged 6–9 years and 10–12 years, respectively [21]. At baseline, children were checked for edema and weighed without shoes and in light clothing to the nearest 0.1 kg, using a Seca (R) UNICEF scale (mod. 874, SECA, Hamburg, Germany). Body height was measured to the nearest 0.5 cm using a calibrated SECA (R) height scale (SECA 213). Weight and height measurements were repeated twice with a maximum tolerable difference of 0.1 kg for weight and 0.5 cm for height.

The weight-for-age *z*-score (WAZ), body mass index-for-age *z*-score (BAZ), and height-for-age *z*-score (HAZ) were calculated using Anthro Plus, the anthropometric calculator module based on the 2007 WHO reference for children aged 5–19 years [22,23]. Stunting, underweight, and thinness were defined by HAZ, WAZ, and BAZ below −2SD, respectively. The school provided data on the age of the children, which was crosschecked with primary caregivers. If the primary caregiver could not verify the date of birth, WAZ, BAZ, and HAZ were not calculated.

2.6. Blood Sample Collection and Analysis

To minimize any discomfort, a local anesthetic ointment containing lidocaine and prilocaine (EMLA^{TM}) was applied onto the area of skin to be numbed prior to pricking. At screening, capillary blood samples of children were taken for two subsequent hemoglobin measurements using a HemoCue HB 301 photometer device (HemoCue AB, Ängelholm, Sweden). The maximum tolerated difference between the measurements was 0.5 g/dL. The mean value was used to determine hemoglobin levels.

At baseline, local nurses took from each child a non-fasting venous blood sample, which was spun within 30 min to obtain 50–100 µL serum. The serum was pipetted into labelled 0.2 mL Multiply® PCR tubes (Sarstedt 72.737.002). In the field, samples were either stored cool for a maximum of seven days and then put into a freezer or stored in a freezer on the same day [24]. The samples were taken to the laboratory (VitMin Lab, Willstaett, Germany), where serum ferritin, soluble transferrin receptor (sTfR), retinol-biding proteins (RBP), acidic glycoprotein (AGP), and C-reactive protein (CRP) levels were analyzed using a sandwich ELISA [25]. The Wako zinc test was applied to determine serum zinc via colorimetric measurements [26].

Hemoglobin was adjusted for altitude and anemia defined as adjusted hemoglobin < 11.5 g/dL in children aged 7–11 and <12 g/dL in children aged 12 [27]. Iron deficiency was defined by depleted iron stores (adjusted serum ferritin <15 g/L) [28] and tissue iron deficiency by high serum sTfR (>8.3 mg/L) [25]. RBP concentrations were used as a surrogate measure for circulating retinol to evaluate vitamin A status. Vitamin A deficiency (VAD) was defined by adjusted serum RBP < 0.70 mol/L [29].

CRP and AGP were assessed for identifying and classifying inflammation: incubation (CRP levels > 5 mg/L and AGP levels ≤ 1 g/L), early convalescence (CRP levels > 5 mg/L and AGP levels > 1 g/L), late convalescence (CRP levels ≤ 5 mg/L and AGP levels > 1 g/L). Serum ferritin and RBP concentration were adjusted for inflammation stage with correction factors for each inflammation stage [30,31]. Zinc deficiency without clinical signs was identified by serum zinc < 0.65 µg/dL only [32]. Genotyping for sickle cell trait and the common African 3.7 kb a-globin a+-thalassemia deletion was conducted via PCR [33,34] at KEMRI Wellcome Trust laboratories in Kilifi, Kenya.

2.7. Data Management and Statistical Analysis

Data entry and validation via double entry was performed for anthropometry and hemoglobin, as well as for the 24 h-recalls. The country-specific food database for Kenya was read into the NutriSurvey nutrient database. Missing food items were supplemented from the Tanzania Food Composition Tables [35] the FoodData Central of the United States Department of Agriculture [36]. Data management and statistical analysis were executed using SPSS software version 24 (IBM Corp., Armonk, NY, USA).

Normality of distributions was evaluated using the Shapiro–Wilk test. As most continuous variables (anthropometry, micronutrient status, and nutrient intake) had unequal distributions, descriptive statistics for continual variables are therefore represented by the median, interquartile range (IQR), and minimum and maximum values. The non-parametric median test was applied for comparing data from urban and rural children. The strength of association was calculated with Cramer's V, which equals r. Variables were tested for associations with non-parametric Spearman's correlation. A p-value of <0.05 was considered statistically significant.

2.8. Ethics

The institutional review board of the Faculty of Medicine at Justus Liebig University Giessen, Germany (197/16) and the AMREF Ethics and Scientific Review Committee (AMREF—ESRC P313/2017) Kenya, approved the Baobab Nutrition Intervention Study—which is not further reported here—under the Kenyan National Commission for Science, Technology and Innovation research permit (NACOSTI/P/17/60305/15018 and NACOSTI/P/18/60305/20841). The study was registered with the German Clinical Trials Registry (DRKS00011935). The municipal and governmental authorities approved the implementation of the study in both schools. Written informed consent of primary caregiver and schoolchildren via signature or fingerprint was obtained prior to data collection. The ethics committees also approved the consent format prior to data collection.

3. Results

3.1. Enrollment of Screened Participants

Figure 1 shows the number of children who participated in the study in the format of a CONSORT diagram. After the screening, the age distribution of eligible children was centered to 7–9 years in the urban group while equally distributed in the rural group. In order to increase homogeneity of the target groups, age was restricted to 7–9 years in both groups for data analysis except for reported underweight and high hemoglobin levels at screening.

3.2. Child Care

The majority of children enrolled were girls (urban: 75.0%; rural: 51.4%). Except for one urban child, all children were breastfed, and 63.9% of urban and 60.0% of rural children were initially breastfed within the first hour after delivery. The median duration of exclusive breastfeeding was four months in both settings. Among all children, 27.8% of urban and 32.4% of rural children had been exclusively breastfed for six months. Table 3 shows reported sickness of 30 urban and 34 rural children one month preceding the interview.

Table 3. History of diseases among schoolchildren in the month preceding the interview.

Disease	Urban — n = 30		Rural — n = 34	
	n	%	*n*	%
Fever	6	20.0	21	61.8
Diarrhea (watery stools ≥ 3 times/day)	2	6.6	0	0
Stomach upset/aches	11	36.7	13	38.2
Nausea and/or vomiting	4	13.3	8	23.5
Cough	12	40.0	22	64.7
Malaria (positively tested)	0	0	3	8.8
Child took medication, in case of (multiple answers)				
Fever	4	13.3	9	26.5
Diarrhea	3	10.0	1	2.9
Stomach upset/aches	2	6.7	3	8.8
Nausea and/or vomiting	3	10.0	2	5.9
Cough	5	16.7	16	47.1
Malaria	1	3.3	14	41.2

The reported prevalence of diseases was similar in both groups except for the lower prevalence of fever in urban children (20.0%, n = 30) than in rural (61.8%, n = 34) ones. Deworming within the past six months was reported for 73.3% of urban (n = 30) and 67.6% of rural (n = 34) schoolchildren. Five urban and seven rural respondents remembered to provide a supplement to their children, while only vitamin A supplements were recognized, namely by three and four respondents, respectively.

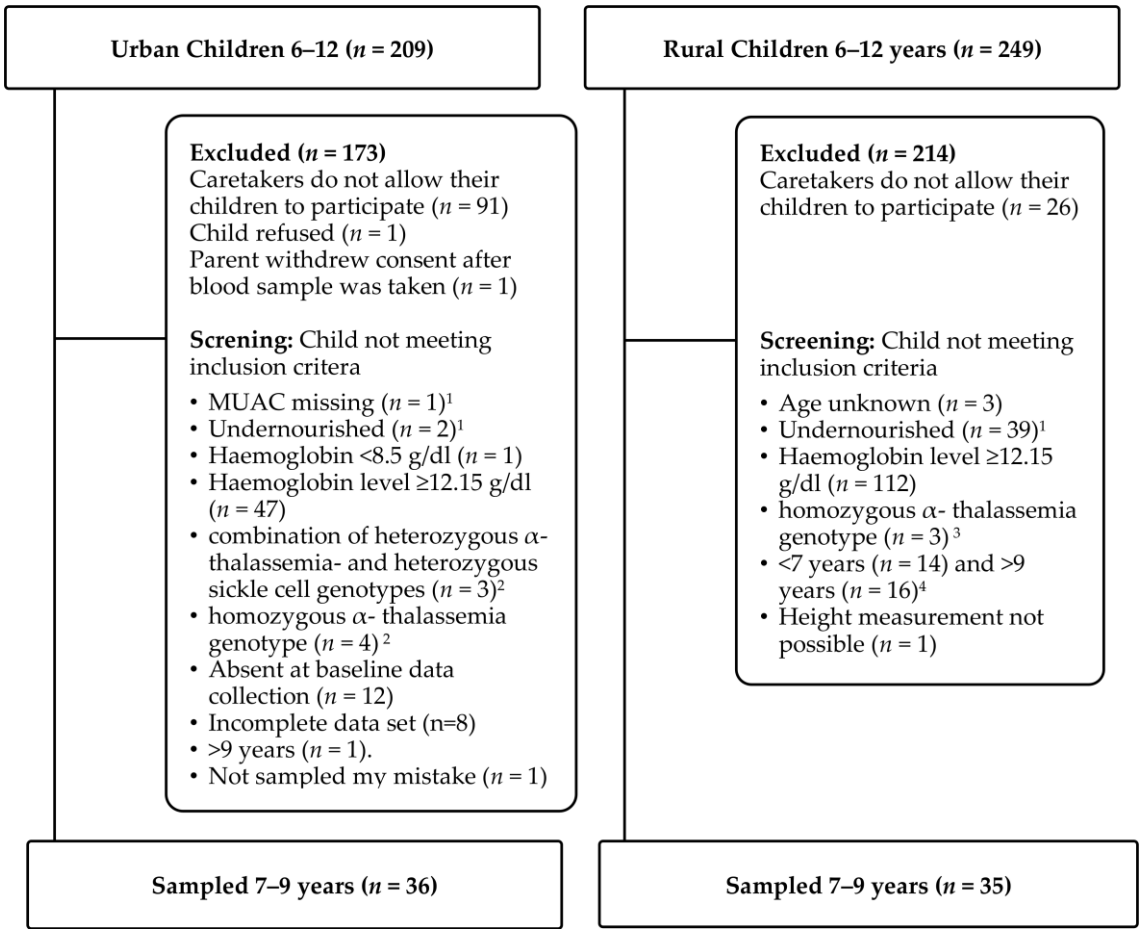


Figure 1. Consort Diagram of the Sampling Procedure. [1] Moderate undernutrition defined as mid-upper arm circumference (MUAC) < 14.5 cm for children aged 5–9 years and <18.5 cm for children aged 10–15 years by National AIDS/STI Control Program [21]; [2] Blood samples for genotyping collected at screening; [3] Blood samples for genotyping collected at baseline; [4] Reference dates: 3 May 2017 and 7 May 2018.

3.3. Nutritional Status

The results of the anthropometric assessment among children are shown in Table 4. At screening, the prevalence of underweight children aged 6–9 years was low in both settings (urban: 0%, n = 110; rural: 0.9%, n = 112), but much higher in the age group 10–12 years (urban: 33.3%, n = 6; rural: 35.2%, n = 108). Medians of weight and height of urban children were 2.7 kg and 5.8 cm higher than those of rural children. These differences were significant (weight: $\chi^2(1) = 6.222$, $p = 0.013$, r = 0.296; height: $\chi^2(1) = 4.079$, $p = 0.043$, r = 0.240). Regarding moderate underweight (WAZ ≤ −2 SD), wasting (BAZ ≤ −2 SD), and stunting (HAZ ≤ −2 SD), the prevalence was lower in urban children than in rural ones, namely 2.8% vs. 23.5%, 0% vs. 11.8%, and 11.1% vs. 17.7%, respectively. One urban child was found to be overweight (BAZ > 2 SD). The differences in median z-scores were significant for underweight and wasting (WAZ: $\chi^2(1) = 14.641$, $p < 0.001$, r = 0.457; BAZ: $\chi^2(1) = 11.209$, $p = 0.001$,r = 0.400).

Table 4. Median and IQR of anthropometry and nutritional status of schoolchildren.

	Urban			Rural			Median Test
Variables	***n***	**Median**	**IQR**	***n***	**Median**	**IQR**	***p***
Screening—MUAC							
6–9 years	111	17.0	16.4–18.0	112	16.6	15.6–17.7	0.200
10–12 years	6	19.0	17.8–20.0	108	19.0	17.7–20.0	-
Missing	1			3			
Sampled children							
7–9 years	36	17.0	16.5–18.0	35	16.5	155–18.0	0.268
Anthropometry							
Weight (kg)	36	24.8	22.8–27.4	35	22.1	19.7–24.2	0.013
Height (cm)	36	129.4	122.8–131.2	35	123.6	118.5–129.0	0.043
Nutritional Status							
WAZ	36	-0.6	-1.2-0.1	34	-1.5	−1.9–−0.8	<0.001
BAZ	36	-0.3	-0.8-0.3	34	-1.0	−1.4–−0.6	0.001
HAZ	36	-0.6	-1.4-0.3	34	-1.1	−1.6–−0.4	0.151

Date of birth was not confirmed for one rural child, and nutritional status was not calculated, BAZ: body mass index-for-age *z*-score; HAZ: height-for-age *z*-score, MUAC: mid-upper arm circumference, WAZ: weight-for-age *z*-score.

3.4. Hemoglobin and Micronutrient Status

Results of the blood analysis are presented in Table 5. At screening, the prevalence of anemia was 28.0% (n = 118) in the urban and 13.0% (n = 223) in the rural setting. Median adjusted hemoglobin levels differed significantly between groups ($\chi^2(1) = 17.643$, $p < 0.001$, r = 0.227). After screening children with the lowest hemoglobin level, the anemia prevalence in the study population increased to 38.9% in urban children and 28.6% in rural ones.

The prevalence of iron deficiency was lower in urban than rural children (2.9% vs. 14.3%). Tissue iron deficiency was present in 11.8% of urban and 8.6% of rural children. The prevalence of VAD was higher in urban (14.7%) than rural (8.6%) children. Zinc deficiency was only found in 34.3% of rural children. Furthermore, significantly higher values for adjusted serum ferritin and zinc were found in urban children than in rural ones (ferritin: $\chi^2(1) = 6.385$, $p = 0.012$, r = 0.304; zinc: $\chi^2(1) = 19.871$, $p < 0.001$, r = 0.537).

The number of children with inflammation stage incubation, early convalescence, and late convalescence was low in both groups, namely 1, 2, and 0 urban children and 1, 0, 3 rural children, respectively. Genotyping for sickle cell and α-thalassemia traits detected two urban children with sickle cell, as well as 12 urban and 13 rural children with α-thalassemia traits. None of these hemoglobinopathies were found among the remaining 22 children in either group.

Table 5. Median and IQR of hemoglobin, iron, vitamin A, zinc status, and subclinical inflammationof schoolchildren.

		Urban			Rural		Median
Variables	***n***	**Median**	**IQR**	***n***	**Median**	**IQR**	**Test**
Screening—Hemoglobin							***p***
Hb in g/dL	118	12.6	11.9–13.1	223	12.7	12.0–13.3	0.212
Hb g/dL, adjusted	118	12.1	11.4–12.6	223	12.5	11.8–13.1	<0.001
Sampled children							
Hb g/dL, adjusted	36	11.7	11.2–12.0	35	11.8	11.4–12.0	0.712
Iron, Vitamin A, Zinc and inflammation marker status							
Iron status							
Ferritin µg/L	34	56.9	39.9–114.5	35	39.2	24.0–67.8	0.041
Ferritin µg/L, adjusted	34	56.9	38.5–96.6	35	39.2	24.0–67.8	0.012
sTfR mg/L	34	5.9	5.1-6.7	35	6.2	5.4–6.8	0.398
Vitamin A status							
RBP µmol/L	34	0.86	0.74–1.08	35	0.91	0.84–1.05	0.398
RBP µmol/L, adjusted	34	0.88	0.76–1.09	35	0.91	0.84–1.05	0.717
Zinc status							
Zinc (µg/dL)	34	84.0	77.3–90.7	35	68.2	63.4–77.7	<0.001
Inflammation							
CRP (in mg/L)	34	0.24	0.14–0.89	35	0.19	0.09–0.49	0.548
AGP (in g/L)	34	0.50	0.43–0.70	35	0.48	0.39–0.64	0.548

AGP: acidic glycoprotein, CRP: C-reactive protein, Hb: hemoglobin, RBP: retinol-binding proteins, sTfR: soluble transferrin receptor.

3.5. Dietary Intake

The frequency of consumption and median nutrient intake through certain food groups are shown in Table 6. High frequency of consumption and highest medium intake of energy, iron, and zinc were reported from mixed dishes with legumes. Green leafy vegetables (GLV) were frequently consumed in both groups and contributed mainly to vitamin C and provitamin A intake. Intake of high caloric snacks and food (*mandazi* and *chapati*) were higher in the urban setting than in the rural one.

Urban children had a lower intake of micronutrients than their rural counterparts, apart from provitamin A and Vitamin B12 (Table 7). Overall, dietary intake was below Kenyan RDAs in urban and rural children, except for zinc in the rural setting. The median adequacy ratio for energy was low, with no significant differences between urban and rural children. Median NAR of vitamin C ($\chi^2(1) = 4.016$, $p = 0.045$, r = 0.250) iron ($\chi^2(1) = 9.035$, $p = 0.003$, r = 0.376) and zinc ($\chi^2(1) = 9.035$, $p < 0.003$, r = 0.376) was significantly lower in urban children than in rural ones.

Table 6. Frequency (n), median (IQR) of intake in gram, energy, and nutrients from selected foodgroups of schoolchildren.

		Food Intake	Energy	Vitamin C	Vitamin B12	RE	Iron	Zinc
	n	(g)	(kcal)	(mg)	(µg)	(µg)	(mg)	(mg)
Urban (*n* = 30)								
Starchy staple food	79	88 (45–181)	192 (117–230)	0 (0–0)	0 (0–0)	0 (0–0)	1.3 (0.3–2.3)	0.8 (0.4–1.2)
Ugali [1]	22	112 (112–112)	162 (129–240)	0 (0–0)	0 (0–0)	0 (0–0)	1.6 (1.2–2.3)	0.9 (0.7–1.3)
Chapati [2]	10	93 (45–135)	219 (110–311)	0 (0–0)	0 (0–0)	0 (0–4)	1.2 (0.8–1.3)	0.8 (0.4–0.9)
Mandazi [3]	20	55 (55–87)	235 (235–370)	0 (0–0)	0 (0–0)	0 (0–0)	0.5 (0.5–0.8)	0.3 (0.3–0.4)
Mixed dishes with legumes	34	248 (103–248)	312 (110–324)	3.1 (0.5–6.3)	0 (0–0)	6 (0–17)	3.6 (1.7–3.8)	1.6 (0.7–1.7)
Vegetables and Fruits	112	13 (6–39)	3 (1–11)	2.2 (0.5–6.1)	0 (0–0)	8 (0–25)	0.1 (0.0–0.3)	0 (0.0–0.1)
Vitamin A rich fruits	2	81.5	23.8	54.7	0	179	0.3	0.05
GLV	22	29 (9–52)	9 (2–15)	7.4 (3.5–20.2)	0.0 (0.0–0.0)	84 (34–305)	0.4 (0.2–0.8)	0.1 (0.0–0.1)
Flesh Food	9	31 (22–56)	94 (45–184)	0.2 (0–5.2)	0.8 (0.1–2.8)	1 (0–7)	0.6 (0.3–2.5)	1.3 (0.5–2.6)
Dairy products	23	60 (46–95)	40 (31–63)	0.6 (0.5–1.0)	0.2 (0.2–0.4)	33 (26–52)	0.1 (0–0.1)	0.2 (0.2–0.4)
Eggs	1	8	12	0	0.1	10	0.1	0.1
Rural (*n* = 34)								
Starchy staple food	126	76 (47–142)	169 (142–247)	0 (0–0)	0 (0–0)	0 (0–0)	1.7 (0.5–1.7)	0.8 (0.3–0.9)
Ugali [1]	23	202 (202–202)	271 (221–348)	0 (0–0)	0 (0–0)	0 (0–0)	2.6 (2.1–3.3)	1.5 (1.2–1.8)
Chapati [2]	3	180	466	0	0	0	1.2	0.9
Mandazi [3]	9	50 (28–66)	214 (120–282)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0.3 (0.1–0.4)
Mixed dishes with legumes	64	2 35 (197–235)	277 (277–277)	0.2 (0.2–2.0)	0 (0–0)	0 (0–0)	3.3 (1.9–3.3)	1.7 (1.7–1.7)
Vegetables and Fruits	130	22 (10–59)	6 (3–14)	1.9 (0.5–7.4)	0 (0–0)	12 (0–46)	0.1 (0.1–0.3)	0 (0.0–0.1)
Vitamin A rich fruits	1	133	17	109.1	0	124	0.6	0.2
GLV	19	88 (69–112)	33 (25–41)	11.6 (8.8–21.1)	0.0 (0.0–0.0)	467 (356–599)	0.7 (0.6–1.4)	1.2 (0.9–1.6)
Flesh Food	2	32	87	0	0.6	0	0.6	1.4
Dairy products	33	44 (22–57)	29 (15–38)	0.4 (0.2–0.6)	0.1 (0.1–0.2)	24 (12–31)	0 (0–0.1)	0.2 (0.1–0.2)
Eggs	0							

[1] *Ugali:* stiff cornmeal porridge, [2]*Chapati*: unleavened fried flatbread, [3]*Mandazi*: African doughnut, GLV: green leafy vegetables.

Table 7. Median and IQR of adequacy ratio of energy and nutrient intake of schoolchildren in percent.

	Urban *n* = 30		**Rural *n* = 34**		
	Percentage the NAR Achieved by the Children				**Median Test**
	Median	**IQR**	**Median**	**IQR**	***p***
Energy	63	45–83	69	56–87	0.316
Vitamin C	49	22–89	65	33–146	0.045
Vitamin B12	25	0–66	10	2–14	0.316
RE	33	10–90	29	17–115	1.000
Iron	77	64–91	96	80–122	0.003
Zinc	71	45–106	109	91–133	0.003

3.6. Characteristics of Households

Table 8 shows descriptive characteristics of 36 urban and 33 rural households. The three most common income sources in urban areas were employment, petty trade, and casual labor (temporary worker), while in the rural area, casual labor (temporary worker), sale of agricultural products, and employment were reported. One urban household did not mention any income. Only 2.8% of urban, but 35.3% of rural households, reported generating income from self-produced agricultural commodities.

Table 8. Descriptive characteristics of households of the schoolchildren enrolled in the study.

	Urban *n* = 36		**Rural *n* = 33**	
Characteristics	***n***	**%**	***n***	**%**
Sex of respondent				
Male	2	5.6	3	9.1
Female	34	94.4	30	90.9
Sex of the head of the household				
Male	26	72.2	28	84.8
Female	10	27.8	5	15.2
Level of education of respondent attended				
No schooling	2	5.6	0	0.0
Some primary	9	25.0	11	33.3
Completed primary (class 8)	12	33.3	17	51.5
Some secondary	2	5.6	3	9.1
Completed secondary (class 12)	10	27.8	2	6.1
More than secondary	1	2.8	0	0.0
Level of education of head of household				
Respondent is head of the HH	1	2.8	8	24.2
No schooling	0	0.0	0	0.0
Some primary	13	36.1	6	24.0
Completed primary (class 8)	5	13.9	10	40.0
Some secondary	4	11.1	6	24.0
Completed secondary (class 12)	12	33.3	2	8.0
More than secondary	1	2.8	1	4.0
Reason for settling in Nairobi/Kitui-West				
Born in this area	2	5.6	7	21.2
Moved here by marriage	7	19.4	26	78.8
Wanted better livelihood	11	30.6	0	0.0
Work in this area	7	19.4	0	0.0
Looking for job opportunity	2	5.6	0	0.0
Other [1]	7	19.4	0	0.0
Three main sources of income (multiple answers)				
Casual labor (temporary worker)	12	33.3	21	61.8
Sale of agricultural products	1	2.8	12	35.3
Employment	19	52.8	3	8.8
Petty trade	15	41.7	5	14.7
Sale of goods/crafts	1	2.8	6	17.6
Remittances from abroad	0	0.0	3	8.8
Other [2]	6	16.7	1	3.0
None	1	2.8	0	0.0

[1] Good infrastructure (*n* = 1), cheap rent (*n* = 5), good security (*n* = 1); [2] Women's group (microfinance) (*n* = 1), washing people's clothes (*n* = 1), not specified (*n* = 5).

All households in the rural setting grew maize and pulses. Pumpkin, cassava, sweet potatoes, and sorghum were grown by 78.8%, 33.3%, 30.3%, and 24.2%, respectively. Only 8.8% of the households were dependent on subsistence cropping throughout the year. None of the urban households reported having a home garden with vegetables, access to fruit trees, and/or livestock. Among rural households, only three grew vegetables, but 24 had access to fruit trees. Both vegetables and fruits were mainly used for their own consumption. Conversely, among 24 rural households keeping livestock, one in four used ASF mainly for their own consumption, another one in four mainly for selling, and one in three for both in approximately equal amounts. One in six used livestock mainly for ploughing, transportation, and manure. Median distances between households and schools were 331 m (IQR 98–497 m, min.

39 m, max. 1059 m) in the urban setting and 820 m (IQR 364–1134 m, min. 108 m, max.2269 m) in the rural setting. The difference was significant ($\chi 2(1) = 10.197$, $p = 0.001$, r = 0.431).

4. Discussion

Among slight to moderate anemic children in Kenya, we found significantly better nutritional, iron and zinc status in urban than rural children. Whereas energy intake was similar in both groups, iron and zinc intake of urban children was even lower than the intake of rural children.

Although the children in this study were selected with a tendency towards lower hemoglobin levels, another study with Kenyan schoolchildren from urban underprivileged areas reported a similar prevalence of underweight, wasting, and stunting [37], and some even came up with higher rates [38,39]. Notably, the latter studies included children in wider age ranges, namely 5–14 years and 6–12 years, respectively. In rural populations, similar negative *z*-scores were observed in children aged 3–8 years [40,41] and 6–14 years [42], while another study found positive *z*-scores in children aged 7–9 years, except for HAZ in children aged 8 and 9 years [43]. In both settings, the sex of the child, years of education of respondent, and distance to school did not correlate significantly with malnutrition. Overall, stunting was similarly reported in both settings. However, in the urban setting stunted height with predominantly appropriate BAZ indicates a sufficient energy intake, but chronic poor-quality nutrition [44]. Conversely, in rural children, insufficient energy intake and chronically poor-quality nutrition are coexistent. Furthermore, the percentage of caregivers who reported their child to have had fever or a cough in the month preceding the interview was higher in the rural setting. Underweight and wasting is mainly due to a recent weigh loss that may not be attributed to lower food intake only, but also to (infectious) diseases.

Similarly to our findings, a low prevalence of anemia was previously found in underprivileged children in Nairobi [38]; however, in this study, the mean children's age was higher, and anemia tend to decrease during childhood [45]. Studies in rural settings in Kenya reported a higher prevalence of anemia [41,42], but those studies were conducted in malaria-endemic zones.

Zinc and iron (serum ferritin) statuses were significantly better in urban than rural children while tissue iron stores (sTfR) did not differ. Other studies confirmed a higher prevalence of iron deficiency in underprivileged urban settings (4.8%) [38] than in rural ones (33%, 15%, 6.3%) [40–42] in Kenya. However, national data indicated 79.9% schoolchildren with low zinc status, with a lower prevalence in urban children than in rural ones [14].

The finding of median RBP levels around the lower limit of the normal range is not surprising as a poor vitamin A status in children screened for lower hemoglobin levels was expected [46]. Already the Kenyan micronutrient study reported a low VAD prevalence of 3.9% in urban and 5.3% in rural settings [14]. Only one third of the children in both settings met the NAR as intake of provitamin A and preformed vitamin A is low. The number of children with elevated inflammation markers was low and associated neither with adjusted ferritin nor RBP levels.

Heterozygous genotype of α-thalassemia was not associated with Hb, ferritin or sTfR levels similar to findings of other investigators, who found this association only for homozygous genotypes in Kenyan preschoolers [41].

The linear distance from households to school was significantly shorter for urban children. Therefore, the physical activity of rural children was likely to be higher, and the higher energy requirement in rural children may contribute to their poorer nutritional status. We did not assess physical activity further; the distance to school was the only estimate. Neither urban nor rural children had access to transportation to and from the school, and in-school physical activity was observed as being very similar.

One food supplementation study over two years found weight and height gain positively predicted by mean daily intake of food energy from ASF containing heme iron, vitamin A, and vitamin B12. Diets high in plant-based foods were associated with poorer growth [47]. Our data supported this observation as intake of flesh foods, dairy products, and eggs was higher in the urban group.

Blood and anthropometric variables indicated a better iron, zinc, and nutritional status in urban children, while rural children had similar NAR for energy, but a higher NAR for iron and zinc than urban children. In both groups, the diets were predominantly based on starchy staple food (mainly maize, wheat, and rice) and legumes (mainly beans) that contributed considerably to the non-heme iron and zinc intake. Several inhibitors (phytate, polyphenols, etc.) reduced the bioavailability of iron and zinc. The higher intake of non-heme iron and zinc in rural children than in urban ones is attributed to a twofold higher intake of mixed dishes with legumes. In the 24 h-recalls, there was no report of the use of soda ash to soften maize and beans or to keep the dark green color of green leafy vegetables during cooking, a method that reduces bioavailability of iron and zinc [48]. One reason for the different data on the Hb and iron status may be that the urban group has more access to flesh foods and consumed fewer vegetables. Thereby, anemia due to deficiencies of B-vitamins might be more common in the urban group.

Consumption of heme iron from flesh food was low in both settings; yet, higher in the urban than in the rural group. In agreement, Farber et al. [49] observed higher consumption of flesh foods in urban than rural South African children under <2 years. In our study, none urban household kept livestock or poultry; therefore they were dependent on the market supply. In Nairobi, beef and poultry expenditure rise with income [9,50] and the price was identified the most important reason for not consuming the animal source food (beef meat, eggs, fish, and yoghurt) [51]. Among 24 rural households keeping livestock, 25% used the animal products commercially, which was not observed for plant-based food. In line with our findings, a study from Western Kenya found that most plant-based food produced in rural households were utilized for home consumption (starchy staple food, vegetables, fruits and pulses), while 29% of ASF was sold in markets [52]. In conclusion, the role of ASF for income generations is greater than for plant-based food, which may contribute to a low consumption of ASF in rural settings. Investigators also reported a low consumption of ASF in rural Kitui county in adults [53]. A higher bioavailability of heme iron and zinc from ASF may explain the significantly better iron and zinc status in the urban group.

Among the few studies on diets consumed in underprivileged areas in Nairobi, low energy intake in children was reported [7,41,54], as well as vitamin A intake below Kenyan RDA combined with adequate iron intake [7]. Studies from rural areas showed a diverse picture ranging from approaching recommendations [43] to low intakes of energy, iron, vitamins C and B12, [10,42,55,56], vitamin A [10,42,56], and zinc [10,42]. In this study, consumption frequency and portion sizes of common high-calorie street foods, namely chapati and mandazi, were higher in urban children than in rural ones. Wheat flour and vegetable oil, the main ingredients for chapati and mandazi, are fortified with iron, zinc, and vitamins B12 and A in Kenya [57]. Therefore, the urban group may have benefitted from this fortification.

Overall, GLVs were the main source of provitamin A, but in the urban setting, GLV portion sizes were too low to meet the RDA. These children also ate and drank dairy products as an additional source of vitamin A. As none of the urban households cultivates vegetables and fruits nor kept livestock, household's food demand was fully dependent on market supply and their economic resources. Among rural children, the amount of GLV consumed was usually high and contributed significantly to meet the RDA. The 24 h-recalls were conducted when green leafy vegetables were in season, which might be aligned with the high quantity of consumption. In line with a higher consumption of GLV in the rural group, indigenous GLV were found to be higher in rural than urban dwellers [58]. In our study, the frequency of consumption of other provitamin A rich vegetables and fruits was low. Median portion size of milk per day was too small to contribute substantially to vitamin A intake.

The nutrient intake from school meals differed marginal between the groups (see Table 2) and resulted in a slightly higher intake for vitamin C and A for the meals in the urban school. Moreover, the food energy of the urban school meal was higher. However, this did not translate into a higher overall food energy intake for the schoolchildren. Therefore, the differences in dietary intake and micronutrient status are attributed to dietary intake outside of the school. The urban school met

the recommendations of the Kenya's national guideline for healthy diets, which states that meals comprise three to four food groups [59]. Cooked maize and beans were the only components of the rural school meal.

Limitations

When conducting the 24 h-recall, information on fortified products was insufficiently recorded. Therefore, we cannot provide more information on the actual intake of fortified food. Since 2012 Kenya has made fortification of wheat and maize flour (including iron, zinc, vitamin B12, and provitamin A) and vegetable oils and fats (provitamin A) mandatory. Notably, the general consumption of fortified wheat flour has increased in recent years, but the rate is higher in the higher income groups [57], and the school meals were prepared from unprocessed maize. Still, the total micronutrient intake may be underestimated. Furthermore, the level of consumption of fortified products outside school may differ between the urban and rural settings.

Another limitation is the small sample size. The number of children enrolled was determined by the sample size calculation of the Baobab Nutrition Intervention Study of which the baseline data are presented here. A full survey including all children might prove the evidence, but as children with Hb> 12.2 g/dL have been excluded in both groups, the comparison appears to be justified.

Overall, the children's and caregivers' availability and willingness to participate in the interviews were lower in the urban setting than in the rural one. Likewise, higher response rates in rural settings were observed in the Kenyan Demographic and Health Survey [13]. Urban underprivileged parents are very busy to generate a small income and do not want to spare time for the interviews from which they do not expect any personal benefit.

5. Conclusions

The nutritional status of underprivileged urban and rural schoolchildren differs. In the urban setting, the low intake of nutrients is of greater concern than nutrient bioavailability and vice versa in the rural setting. Besides poor vitamin A intake and status in both groups, the better iron and zinc status among urban children implies access to food with higher bioavailability of iron and zinc there, although vitamin B12 intake is far below recommendations in both settings.

Strategies that address a higher intake of iron and zinc may include better access to flesh food and promote preparation practices that increase the bioavailability of non-heme iron and zinc. To address the low intake of vitamin A and vitamin B12, interventions should include more meat, fish, dark green leafy vegetables, and other vegetables and fruits rich in provitamin A.

Up to now, guidelines for school meals address the schoolchildren as one homogenous group. Knowing the urban/rural differences in the needs of the pupils may facilitate specifically adapted guidelines, e.g., more vegetables in urban, and more ASF in rural school meals.

Author Contributions: E.C.E., T.-Y.H., and M.B.K. conceived and designed the BAOFOOD nutrition trial; E.C.E. and M.B.K. conducted the field work and managed the data; W.O.O. supported in the preparatory and implementing phase of the study; E.C.E. conducted the data analysis and drafted the initial manuscript; all authors contributed to the review and editing of the manuscript; E.C.E. and M.B.K. had primary responsibility for the final content. All authors have read and approved the final version of the manuscript.

Funding: This research was funded by The German Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food and Agriculture (BLE), grant number FKZ 2813FSNU08. The funders had no role in the design of the study, the collection, analyses, nor in the interpretation of data, in writing the manuscript, or in the decision to publish the results.

Acknowledgments: We thank the school management boards of Heidemarie Primary School and Kakumuti Primary School for their unrestricted help in implementing the study; Yvonne Flammer (Managing Director) and the staff of Baraka Health Centre, and Christi Mawindu (Nurse Coordinator) and the staff at Kauwi Sub-District Hospital, Kitui, for the collaboration during data collection; Juergen Erhardt, VitMin Laboratory, Willstaett, Germany, for the blood nutrient analyses; Tom Williams and Alex Macharia KEMRI/Wellcome Trust Programme Kilifi, Kenya, for hemoglobinopathy screening; the Ministries of Education and Health, Nairobi, Kenya, for their

support; Dilara Celik, Eleonore Kretz and Maike Schnermann, MSc students, for their support in data collection and entry and Anika Reinbott for her friendly review. Johannes Herrmann, PhD, Giessen, Germany, supported the data analysis.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Arimond, M.; Wiesmann, D.; Becquey, E.; Carriquiry, A.; Daniels, M.C.; Deitchler, M.; Fanou-Fogny, N.; Joseph, M.L.; Kennedy, G.; Martin-Prevel, Y.; et al. Simple food group diversity indicators predict micronutrient adequacy of women's diets in 5 diverse, resource-poor settings. *J. Nutr.* **2010**, *140*, 2059S–2069S. [CrossRef] [PubMed]
2. Steyn, N.P.; Nel, J.H.; Nantel, G.; Kennedy, G.; Labadarios, D. Food variety and dietary diversity scores in children: Are they good indicators of dietary adequacy? *Public Health Nutr.* **2006**, *9*, 644–650. [CrossRef] [PubMed]
3. Arimond, M.; Ruel, M.T. Dietary diversity is associated with child nutritional status: Evidence from 11 demographic and health surveys. *J. Nutr.* **2004**, *134*, 2579–2585. [CrossRef] [PubMed]
4. FAO; WFP; IFAD. The State of Food Insecurity in the World: Economic Growth Is Necessary but not Sufficient to Accelerate Reduction of Hunger and Malnutrition. 2019. Available online: http://www.fao.org/3/i3027e/i3027e.pdf (accessed on 17 October 2019).
5. Kimura, A.H. *Hidden Hunger*; Cornell University Press: Ithaca, NY, USA, 2017; ISBN 9780801467691.
6. Hawkes, C.; Harris, J.; Gillespie, S. Changing Diets: Urbanization and the Nutrition Transition. In *2017 Global Food Policy Report*; International Food Policy Research Institute, Ed.; International Food Policy Research Institute: Washington, DC, USA, 2017; pp. 34–41.
7. Van't Riet, H.; den Hartog, A.P.; van Staveren, W.A. Non-home prepared foods: Contribution to energy and nutrient intake of consumers living in two low-income areas in Nairobi. *Public Health Nutr.* **2002**, *5*, 515–522. [CrossRef]
8. Yang, R.Y.; Keding, G.B. Nutritional contributions of important African indigenous vegetables. In *African Indigenous Vegetables in Urban Agriculture*; Shackleton, C.M., Pasquini, M.W., Drescher, A.W., Eds.; Earthscan: London, UK, 2009; pp. 105–143.
9. Ayieko, M.; Tschirley, D.; Mathenge, M. *Fresh Fruit and Vegetable Consumption Patterns and Supply Chain Systems in Urban Kenya: Implications for Policy and Investment Priorities*; Working Paper 19; Tegemeo Institute of Agricultural Policy and Development, Egerton University: Nairobi, Kenya, 2008.
10. M'Kaibi, F.K.; Steyn, N.P.; Ochola, S.; Du Plessis, L. Effects of agricultural biodiversity and seasonal rain on dietary adequacy and household food security in rural areas of Kenya. *BMC Public Health* **2015**, *15*, 422. [CrossRef]
11. Bhutta, Z.A.; Salam, R.A.; Das, J.K. Meeting the challenges of micronutrient malnutrition in the developing world. *Br. Med. Bull.* **2013**, *106*, 7–17. [CrossRef] [PubMed]
12. Paciorek, C.J.; Stevens, G.A.; Finucane, M.M.; Ezzati, M. Children's height and weight in rural and urban populations in low-income and middle-income countries: A systematic analysis of population-representative data. *Lancet Glob. Health* **2013**, *1*, e300–e309. [CrossRef]
13. Kenya National Bureau of Statistics (KNBS) and ICF Macro. Kenya Demographic and Health Survey. 2014. Available online: https://dhsprogram.com/pubs/pdf/fr308/fr308.pdf (accessed on 18 October 2019).
14. Ministry of Health. The Kenya National Micronutrient Survey. 2011. Available online: http://www.nutritionhealth.or.ke/wp-content/uploads/Downloads/The%20Kenya%20National%20Micronutrient%20Survey%202011.pdf (accessed on 18 October 2019).
15. Fotso, J.-C. Urban-rural differentials in child malnutrition: Trends and socioeconomic correlates in sub-Saharan Africa. *Health Place* **2007**, *13*, 205–223. [CrossRef]
16. Haddad, L.; Ruel, M.T.; Garrett, J.L. Are Urban Poverty and Undernutrition Growing? Some Newly Assembled Evidence. *World Dev.* **1999**, *27*, 1891–1904. [CrossRef]
17. Mutisya, M.; Kandala, N.-B.; Ngware, M.W.; Kabiru, C.W. Household food (in) security and nutritional status of urban poor children aged 6 to 23 months in Kenya. *BMC Public Health* **2015**, *15*, 1052. [CrossRef]

18. Bukania, Z.N.; Mwangi, M.; Karanja, R.M.; Mutisya, R.; Kombe, Y.; Kaduka, L.U.; Johns, T. Food Insecurity and Not Dietary Diversity Is a Predictor of Nutrition Status in Children within Semiarid Agro-Ecological Zones in Eastern Kenya. *J. Nutr. Metab.* **2014**, *2014*, 907153. [CrossRef] [PubMed]
19. FAO. *Human Energy Requirements*; Report of a Joint FAO/WHO/UNU Expert Consultation, Food and Nutrition Technical Report Series 1; FAO: Rome, Italy, 2004.
20. FAO; FHI 360. Minimum Dietary Diversity for Women—A Guide to Measurement. Available online: http://www.fao.org/3/a-i5486e.pdf (accessed on 24 October 2019).
21. National AIDS/STI Control Programme (NASCOP). Reference Charts for Nutrition Diagnosis and Protocol for Food by Prescription. 2019. Available online: https://www.fhi360.org/sites/default/files/media/documents/Reference%20Charts%20for%20Nutrition%20Diagnosis%20and%20Protocol%20for%20Food%20by%20Prescription.pdf (accessed on 18 October 2019).
22. WHO. WHO AnthroPlus Software. 2007. Available online: https://www.who.int/growthref/tools/en/ (accessed on 22 November 2018).
23. De Onis, M.; Onyango, A.W.; Borghi, E.; Siyam, A.; Siekmann, J. Development of a WHO growth reference for school-aged children and adolescents. *Bull. World Health Organ.* **2007**, *85*, 660–667. [CrossRef] [PubMed]
24. Erhardt, J.G. Measurement of the Vitamin A and Iron Status in Blood Samples, 3. Measurement of Serum Samples. 2010. Available online: http://www.nutrisurvey.de/blood_samples/index.htm#3 (accessed on 18 October 2019).
25. Erhardt, J.G.; Estes, J.E.; Pfeiffer, C.M.; Biesalski, H.K.; Craft, N.E. Combined measurement of ferritin, soluble transferrin receptor, retinol binding protein, and C-reactive protein by an inexpensive, sensitive, and simple sandwich enzyme-linked immunosorbent assay technique. *J. Nutr.* **2004**, *134*, 3127–3132. [CrossRef]
26. Fujifilm. Zinc Assay. Available online: https://www.wako-chemicals.de/en/product/zinc-assay (accessed on 12 September 2019).
27. WHO. Nutritional Anaemias: Tools for Effective Prevention and Control. 2019. Available online: https://www.who.int/nutrition/publications/micronutrients/anaemias-tools-prevention-control/en/ (accessed on 18 October 2019).
28. WHO. Iron deficiency anaemia assessment, prevention and control: a guide for programme managers. 2001. Available online: https://www.who.int/nutrition/publications/en/ida_assessment_prevention_control.pdf (accessed on 20 June 2018).
29. Gamble, M.V.; Ramakrishnan, R.; Palafox, N.A.; Briand, K.; Berglund, L.; Blaner, W.S. Retinol binding protein as a surrogate measure for serum retinol: Studies in vitamin A-deficient children from the Republic of the Marshall Islands. *Am. J. Clin. Nutr.* **2001**, *73*, 594–601. [CrossRef] [PubMed]
30. Thurnham, D.I.; McCabe, G.P.; Northrop-Clewes, C.A.; Nestel, P. Effects of subclinical infection on plasma retinol concentrations and assessment of prevalence of vitamin A deficiency: Meta-analysis. *Lancet* **2003**, *362*, 2052–2058. [CrossRef]
31. Thurnham, D.I.; McCabe, L.D.; Haldar, S.; Wieringa, F.T.; Northrop-Clewes, C.A.; McCabe, G.P. Adjusting plasma ferritin concentrations to remove the effects of subclinical inflammation in the assessmentof iron deficiency: A meta-analysis. *Am. J. Clin. Nutr.* **2010**, *92*, 546–555. [CrossRef] [PubMed]
32. Brown, K.H.; Rivera, J.A.; Bhutta, Z.; Gibson, R.S.; King, J.C.; Lonnerdal, B.; Ruel, M.T.; Sandtrom, B.; Wasantwisut, E.; Hotz, C. International Zinc Nutrition Consultative Group (IZiNCG) technical document #1. Assessment of the risk of zinc deficiency in populations and options for its control. *Food Nutr. Bull.* **2004**, *25*, S99–S203. [PubMed]
33. Chong, S.S.; Boehm, C.D.; Higgs, D.R.; Cutting, G.R. Single-tube multiplex-PCR screen for common deletional determinants of alpha-thalassemia. *Blood* **2000**, *95*, 360–362. [CrossRef] [PubMed]
34. Waterfall, C.M.; Cobb, B.D. Single tube genotyping of sickle cell anaemia using PCR-based SNP analysis. *Nucleic Acids Res.* **2001**, *29*, e119. [CrossRef]
35. Lukmanji, Z.; Hertzmark, E.; Mlingi, N.; Assey, V.; Ndossi, G.; Fawzi, W. Tanzania Food Composition Tables. 2019. Available online: https://cdn1.sph.harvard.edu/wp-content/uploads/sites/30/2012/10/tanzania-food-composition-tables.pdf (accessed on 18 October 2019).
36. USDA. FoodData Central. 2018. Available online: https://fdc.nal.usda.gov/ (accessed on 8 July 2018).
37. Neervoort, F.; von Rosenstiel, I.; Bongers, K.; Demetriades, M.; Shacola, M.; Wolffers, I. Effect of a school feeding programme on nutritional status and anaemia in an urban slum: A preliminary evaluation in Kenya. *J. Trop. Pediatrics* **2013**, *59*, 165–174. [CrossRef]

38. Suchdev, P.S.; Davis, S.M.; Bartoces, M.; Ruth, L.J.; Worrell, C.M.; Kanyi, H.; Odero, K.; Wiegand, R.E.; Njenga, S.M.; Montgomery, J.M.; et al. Soil-transmitted helminth infection and nutritional status among urban slum children in Kenya. *Am. J. Trop. Med. Hyg.* **2014**, *90*, 299–305. [CrossRef] [PubMed]
39. Chesire, E.J.; Orago, A.S.S.; Oteba, L.P.; Echoka, E. Determinants of under nutrition among school aged children in a Nairobi peri-urban slum. *East Afr. Med. J.* **2008**, *85*, 471–479. [PubMed]
40. Houghton, L.A.; Brown, R.C.; Beaumont, S.; Jennings, S.; Bailey, K.B.; Haszard, J.J.; Erhardt, J.; Daniels, L.; Gibson, R.S. Micronutrient status differs among Maasai and Kamba preschoolers in a supplementary feeding programme in Kenya. *Matern. Child Nutr.* **2019**, e12805. [CrossRef] [PubMed]
41. Andang'O, P.E.A.; Osendarp, S.J.M.; Ayah, R.; West, C.E.; Mwaniki, D.L.; de Wolf, C.A.; Kraaijenhagen, R.; Kok, F.J.; Verhoef, H. Efficacy of iron-fortified whole maize flour on iron status of schoolchildren in Kenya: A randomised controlled trial. *Lancet* **2007**, *369*, 1799–1806. [CrossRef]
42. Neumann, C.G.; Bwibo, N.O.; Murphy, S.P.; Sigman, M.; Whaley, S.; Allen, L.H.; Guthrie, D.; Weiss, R.E.; Demment, M.W. Animal Source Foods Improve Dietary Quality, Micronutrient Status, Growth and Cognitive Function in Kenyan School Children: Background, Study Design and Baseline Findings. *J. Nutr.* **2003**, *133*, 3941S–3949S. [CrossRef]
43. Semproli, S.; Canducci, E.; Ricci, E.; Gualdi-Russo, E. Nutrient intake in 5-17-year-old African boys and girls in a rural district of Kenya. *Nutr. Hosp.* **2011**, *26*, 765–774. [CrossRef]
44. Briend, A.; Khara, T.; Dolan, C. Wasting and stunting—Similarities and differences: Policy and programmatic implications. *Food Nutr. Bull.* **2015**, *36*, S15–S23. [CrossRef]
45. Ngesa, O.; Mwambi, H. Prevalence and risk factors of anaemia among children aged between 6 months and 14 years in Kenya. *PLoS ONE* **2014**, *9*, e113756. [CrossRef]
46. Semba, R.D.; Bloem, M.W. The anemia of vitamin A deficiency: Epidemiology and pathogenesis. *Eur. J. Clin. Nutr.* **2002**, *56*, 271–281. [CrossRef]
47. Grillenberger, M.; Neumann, C.G.; Murphy, S.P.; Bwibo, N.O.; Weiss, R.E.; Jiang, L.; Hautvast, J.G.A.J.; West, C.E. Intake of micronutrients high in animal-source foods is associated with better growth in rural Kenyan school children. *Br. J. Nutr.* **2006**, *95*, 379–390. [CrossRef]
48. Mamiro, P.; Nyagaya, M.; Kimani, P.; Mamiro, D.; Jumbe, T.; Macha, J.; Chove, B. Similarities in functional attributes and nutritional effects of magadi soda and bean debris-ash used in cooking African traditional dishes. *Afr. J. Biotechnol.* **2011**, *10*, 1181–1185.
49. Faber, M.; Laubscher, R.; Berti, C. Poor dietary diversity and low nutrient density of the complementary diet for 6- to 24-month-old children in urban and rural KwaZulu-Natal, South Africa. *Matern. Child Nutr.* **2016**, *12*, 528–545. [CrossRef] [PubMed]
50. Musyoka, M.P.; Lagat, J.K.; Ouma, D.E.; Wambua, T.; Gamba, P. Structure and properties of urban household food demand in Nairobi, Kenya: Implications for urban food security. *Food Secur.* **2010**, *2*, 179–193. [CrossRef]
51. Cornelsen, L.; Alarcon, P.; Häsler, B.; Amendah, D.D.; Ferguson, E.; Fèvre, E.M.; Grace, D.; Dominguez-Salas, P.; Rushton, J. Cross-sectional study of drivers of animal-source food consumption in low-income urban areas of Nairobi, Kenya. *BMC Nutr.* **2016**, *2*, 1715. [CrossRef]
52. Ng'endo, M.; Bhagwat, S.; Keding, G.B. Influence of Seasonal On-Farm Diversity on Dietary Diversity: A Case Study of Smallholder Farming Households in Western Kenya. *Ecol. Food Nutr.* **2016**, *55*, 403–427. [CrossRef]
53. Hansen, A.W.; Christensen, D.L.; Larsson, M.W.; Eis, J.; Christensen, T.; Friis, H.; Mwaniki, D.L.; Kilonzo, B.; Boit, M.K.; Borch-Johnsen, K.; et al. Dietary patterns, food and macronutrient intakes among adults in threeethnic groups in rural Kenya. *Public Health Nutr.* **2011**, *14*, 1671–1679. [CrossRef]
54. Mwaniki, E.W.; Makokha, A.N. Nutrition status and associated factors among children in public primary schools in Dagoretti, Nairobi, Kenya. *Afr. Health Sci.* **2013**, *13*, 39–46. [CrossRef]
55. Ferguson, E.; Chege, P.; Kimiywe, J.; Wiesmann, D.; Hotz, C. Zinc, iron and calcium are major limiting nutrients in the complementary diets of rural Kenyan children. *Matern. Child Nutr.* **2015**, *11*, 6–20. [CrossRef]
56. Shell-Duncan, B.; McDade, T. Cultural and environmental barriers to adequate iron intake among northern Kenyan schoolchildren. *Food Nutr. Bull.* **2005**, *26*, 39–48. [CrossRef]
57. Ministry of Health. Kenya National Food Fortification Strategic Plan: 2018–2022. 2019. Available online: http://www.nutritionhealth.or.ke/wp-content/uploads/Downloads/Food%20Fortification%20Strategic%20Plan%20Final%20Press%20Signed%20-%20Aug%202018.pdf (accessed on 18 October 2019).

58. Gido, E.O.; Ayuya, O.I.; Owuor, G.; Bokelmann, W. Consumption intensity of leafy African indigenous vegetables: Towards enhancing nutritional security in rural and urban dwellers in Kenya. *Agric. Food Econ.* **2017**, *5*, 14. [CrossRef]
59. Ministry of Health. National Guidelines for Healthy Diets and Physical Activity. 2019. Available online: http://nak.or.ke/wp-content/uploads/2017/12/NATIONAL-GUIDELINES-FOR-HEALTHY- DIETS-AND-PHYSICAL-ACTIVITY-2017-NEW-EDIT.pdf (accessed on 18 October 2019).

© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).

3. **Can the supplementary consumption of baobab (*Adansonia digitata L.*) fruit pulp improve the hemoglobin levels and iron status of schoolchildren in Kenya? Findings of a randomized controlled intervention trial**

European Journal of Nutrition
https://doi.org/10.1007/s00394-020-02447-2

ORIGINAL CONTRIBUTION

Can the supplementary consumption of baobab (*Adansonia digitata* L.) fruit pulp improve the hemoglobin levels and iron statusof schoolchildren in Kenya? Findings of a randomized controlled intervention trial

Esther Charlotte Evang[1] · Tsige-Yohannes Habte[1] · Willis Omondi Owino[2] · Michael Bernhardt Krawinkel[1]

Received: 8 May 2020 / Accepted: 6 October 2020
© The Author(s) 2020

Abstract

Purpose In the rural Kenyan diet, the bioavailability of iron is low and predisposes the population to iron deficiency. Fruitpulp of the indigenous baobab tree contains significant amounts of vitamin C, which enhances non-heme iron bioavailability. We studied the impact of baobab fruit pulp (BFP) consumption on the hemoglobin (Hb) and iron status of Kenyan schoolchildren.

Methods The single-blind randomized controlled intervention trial was implemented daily among apparently healthy school-children aged 6–12 years with hemoglobin level < 12.2 g/dl. For 12 weeks, children in the intervention group ($n = 29$) received a drink with BFP, while the control group ($n = 29$) received an isoenergy drink without BFP. At baseline and endline, blood samples were taken.

Results The development of hemoglobin, ferritin (FER) and soluble transferrin receptor (sTfR) did not differ significantly between the intervention and control groups. However, in the intervention group, Hb levels improved slightly (2.2%), while they decreased slightly (1.2%) in the control group. Levels of geometric means of sTfR remained almost unchanged (0.7%) in the intervention group and slightly worsened (2.7%) in the control group. In both the groups, geometric mean of FER levels decreased, yet to a smaller extent in the intervention (17.3%) than in the control (26.0%) group.

Conclusion Even though no significant effects of BFP could be detected in this study, the identification of products such as BFP remains pertinent to help improve non-heme iron absorption in the most vulnerable populations.

Keywords Baobab · Anemia · Bioavailability · Schoolchildren · Kenya

Introduction

In sub-Saharan Africa, anemia is widespread and associated with increased morbidity and mortality [1, 2], and impaired cognitive and behavioral development in children [3]. In Kenya, inadequate food intake is an important driver of anemia in schoolchildren [4], especially a combination of highintake of anti-nutrients [5] and low intake of heme iron [5, 6]. Other drivers of anemia are parasitic infections (*Plasmodium falciparum*, helminths and schistosomiasis) [4] and hemoglobin disorders [7, 8].

The latest representative data in Kenyan children aged 6 months to 12 years show an anemia prevalence of 25% [9], which is classified as moderate public health problem [10]. Nonetheless, a regional heterogeneity in the burden of anemia attributable to different etiological factors [4] has

Michael Bernhardt Krawinkel
michael.krawinkel@uni-giessen.de

Esther Charlotte Evang
esther.c.evang@ernaehrung.uni-giessen.de

Tsige-Yohannes Habte
tsige-yohannes.habte@ernaehrung.uni-giessen.de

Willis Omondi Owino
willis@agr.jkuat.ac.ke

[1] Institute of Nutritional Sciences, Justus Liebig University Giessen, Wilhelmstr. 20, 35392 Giessen, Germany

[2] School of Food and Nutritional Sciences, Jomo Kenyatta University of Agriculture and Technology, Juja 62000-00200, Nairobi, Kenya

Published online: 23 December 2020

been confirmed by several studies. A study on schistosomiasis and soil-transmitted helminths in Kenyan schoolchildren reported 61% with anemia [11]. A recent study on the malaria risk among Kenyan children found 68.8% of the children studied to be anemic, with 23.6% affected by iron deficiency anemia [12]. Other studies representing regional differences reported prevalence of iron deficiency of 33%, 15% and 6.3% [5, 13, 14] and tissue iron deficiency from 20.3 to 70.4% [13].

In a rural population in Kenya, low iron bioavailability was found to be of greater concern than iron intake. Low intake of heme iron and high intake of phytate and polyphenols in the common diet [6] impair iron absorption. Non-heme iron by nature has a low bioavailability because it tends to crystalize in the small intestine; however, vitamin C increases its bioavailability [15]. Therefore, one approach in preventing anemia and iron deficiency is to improve the bioavailability of non-heme iron by increasing intake of vitamin C and other organic acids [16]. Vitamin C prevents the dose-dependent inhibitory effects of polyphenols and phytates on iron absorption [17] and further studies confirmed vitamin C to enhance non-heme iron bioavailability [18–20]. Positive associations between dietary vitamin C intake and hemoglobin (Hb) and ferritin (FER) levels have been found in Mexico, where a traditional beverage (*pulque*) containing 30 mg vitamin C is consumed with a diet based on cereals and beans [21].

The fresh fruit pulp of indigenous baobab trees *(Adansonia digitata* L.*)* contains a high amount (> 200 mg / 100 g) of vitamin C [22, 23], which is unparalleled compared to vegetables and other fruits [24]. In vitro studies on food-to-food fortification of cereal porridge with baobab fruit pulp (BFP) showed enhancement of iron bioaccessibility, probably because it is rich in both vitamin C and other organic acids such as citric acid [25–27]. Nnam et al. [28] studied the effect of vitamin C from BFP on hemoglobin levels and iron status of Nigerian schoolchildren over 3 months. They found a significant improvement in Hb and FER levels of schoolchildren that received a drink with BFP after a meal. Nnam et al. concluded that baobab pulp is a nutritious, natural and inexpensive source of vitamin C with positive implication on the iron status of Nigerian children.

Our *Baobab Nutrition Intervention Study* aimed to define the role of BFP in alleviating iron deficiency problems among schoolchildren in Kenya. The study was part of the BAOFOOD research project that studied the use, processing and market development of underutilized baobab for improved food and nutrition security and rural livelihoods in Kenya. The nutrient composition of BFP has been studied previously, unlike nutrition evaluation of BFP in terms of bioavailability [22]. BFP is locally available within the baobab belt in Kenya (one part in the inland from the Tanzanian border towards the north-east and a second one along the whole coastal region [29]). It is easily accessible to even the poorest communities, thereby offering a sustainable way to prevent micronutrient deficiencies [27]. The objective of the study was to determine the impact of BFP consumptionon the Hb and iron status of Kenyan schoolchildren aged 6–12 years.

Materials and methods

Study design and sites

The study was performed as a single-blind placebo controlled, parallel group study [1:1]. Public primary schools were purposely selected according to the following criteria: (a) school meal program in place, (b) public day school, (c) at least 280 children aged 6–12 years, and (d) accessibility by car. The primary schools with comparatively large number of students in the study area where the authorities and the head teachers expressed support and were open for the intervention were approached.

Under these criteria, Kakumuti Pre- and Primary School was selected in rural Kitui-West (Sub-county), Kitui (County), Eastern Province of Kenya, approximately 165 km away from Nairobi. About 430 children attended the school, which had a self-governed school meal program. Kitui County is of marginal agricultural potential, prone to droughts [30] and the stunting prevalence in children underfive is among the highest in the country [2]. Kitui is considered a low-risk area for malaria transmission [9].

Kitui belongs to the baobab belt [29], except of certain sub-counties such as Kitui-West where the few baobab trees do not produce fruits. Around the school, there were no baobab products identified in the markets, and baobab fruits used for the study were sourced from another area, namely Kyamatu location in Kitui-East Sub-county. The interventionstarted in May 2018, which is generally the end of the long rainy season. The average rainfall was above normal in 2018, and general food security improved during the intervention period, which fell in the postharvest season of staples and pulses.

Sampling study participants

After obtaining official research permits and consent from the school administration, locally trained project assistants described the study in the local Kikamba language to caregivers of the children eligible for screening. Only children whose caregivers provided written informed consent (signature or fingerprint) were invited for the screening. The assistants orally informed these children about the study objective and procedure of the upcoming exercise, and children approved the reception of the information with their

signature. Children's oral consent and their signature were prerequisite for any further interview and examination. Registered nurses and laboratory technicians performed the clinical screening in a separate room and administered a dewormer (Albendazole USP 400) to all children. Thus, intestinal blood loss due to helminth infections was prevented. Eligible participants were apparently healthychildren aged 6–12 years with lowest adjusted Hb level at screening. Exclusion criteria are shown in Fig. 1.

Intervention

The intervention took place daily for a total of 83 days from May to July 2018. In addition to a standardized school meal, study children received either one cup of a drink with BFP or one cup of an isoenergy drink without BFP. The standardized portion of the school meal had an estimated iron content of 7.6 mg per portion, mainly from beans (NutriSurvey2007) (Table 1). BFP is rich in vitamin C [22, 31]; therefore, we expected an improvement in the bioavailability of iron from the school meal.

The preparation of the school meal (mixed beans, maize, iodized salt, and vegetable oil) was standardized. Baobab fruits were delivered from Kyamatu and processed on a daily basis by trained local field assistants. They cleaned the fruits with a wire brush first and with a soft brush afterwards to remove the hair from the outer shell. The fruits were then cracked with a machete, and those with any spots (insects,

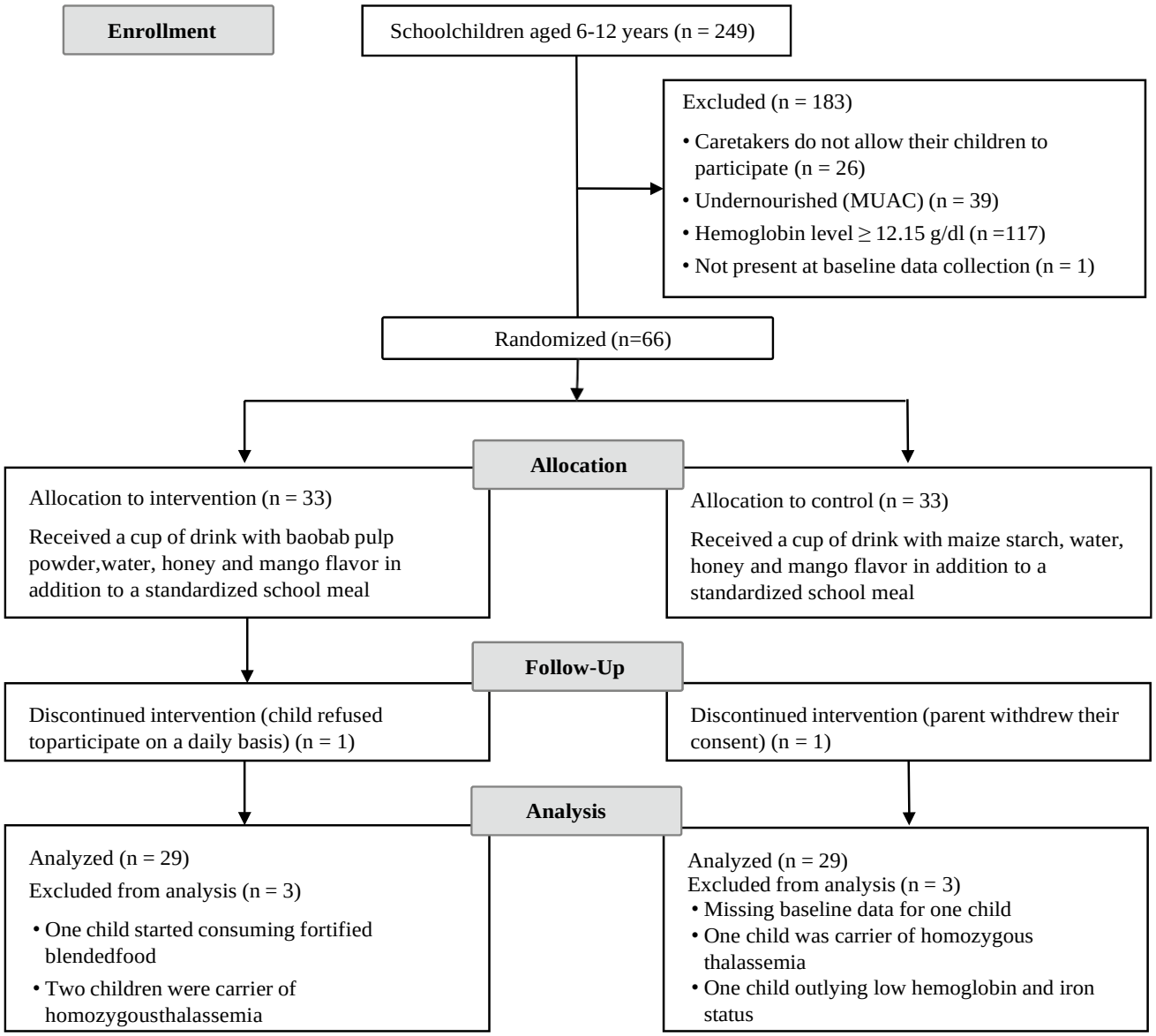


Fig. 1 Consort flow diagram of the *Baobab Nutrition Intervention Study*. MUAC: mid-upper arm circumference

Table 1 Energy and nutrients of one portion of school meal (calculated in NutriSurvey©)

Variables	Portion = 456 g
Energy (kcal)	601
Vitamin C (mg)	1
Iron (mg)	7.6
Zinc (mg)	3.5
Calcium (mg)	105
Magnesium (mg)	201

mold, etc.) inside the fruit were discarded applying the two man rule. The pulp-seed mix was removed from the shell, ground in a mortar to separate pulp (which is in the form of a powder) from the seeds, and the powder sieved twice in succession.

About 20–30 min prior to distributing the drinks to the children, all ingredients for the intervention drink were blended. A weighted cup of intervention drink contained 20 g BFP, 5 g honey, 7 drops of Mango Liquid Flavour Drops (SygLabs, Germany), and 200 ml of bottled water. The isoenergy control drink consisted of 3 g commercially available corn starch, 10 g honey, 5 Mango Liquid Flavour Drops (SygLabs, Germany) and 220 ml of bottled water. The corn starch was boiled in 2 l of bottled water and mixed with the remaining ingredients after cooling. The field assistants weighed 220 g of either baobab drink or control drink in cups. The cups were coded with different colors to differentiate between intervention and control drink. Table 2 shows the nutrient composition of the intervention and control drink. The field assistants observed the children during the consumption to avoid any exchange of food and drinks and recorded the amount of food and drink consumed by each child.

During the intervention, eight BFP samples were taken, stored in the fridge, and protected from light until a laboratory analysis was performed. The vitamin C was determined in triplicate using the method of Vikram et al. [32] with slight modifications. The samples was analyzed using a Shimadzu HPLC (20A Model, Tokyo, Japan), fitted with a ODS-C18 (250 cm × 4.6 mm × 5 µl) column, CTO-10AS VP oven, SPD-M20A diode array detector, DGU-20ASR prominence degassing unit, CBM-20A prominence communications bus module, SIL-20A HT prominence auto sampler and an LC-20AD prominence liquid chromatograph. The mobile phase contained 0.8% metaphosphoric acid at a flowrate of 0.8 ml/min. The injection volume used was 20 µl at a wavelength of 266 nm and oven temperatures of 30 °C. The retention time of pure ascorbic acid was used to identify ascorbic peaks in sample chromatographs. Iron, zinc, calcium, and magnesium were analyzed in duplicate with an inductive coupled plasma-optic emission spectrometer as described by Habte et al. [33]. Table 3 shows the BFP composition.

Table 2 Energy and nutrition composition of 220 ml intervention and control drink (calculated in NutriSurvey©)

Variables	Intervention	Control
Energy (kcal)	40.2	41.2
Vitamin C (mg)	33.3	0
Iron (mg)	0.9	0.1
Zinc (mg)	0.6	0.1
Calcium (mg)	91.9	11.6
Magnesium (mg)	48.3	2.3

Allocation into the intervention and control groups

The allocation of participating children into either the intervention or control group was done using the stratified random sampling in SPSS. Participants were stratified according to sex (30 male and 36 female), Hb level above and below median for male (md = 11.9 g/dl) and female (md = 11.8 g/dl), respectively, resulting in four blocks. Among each block, a random allocation in intervention and control group was performed with the Mersenne Twister random number generator conducted in SPSS (V 24) according to age in years.

Sample size

A total of 33 children were allocated into each group, with an assumed dropout of 10%, and a prevalence of homozygote and mixed forms of sickle cell disease and α-thalassemia of 6% (own data), and 76% of the children with Hb-levels > 11.5 g/dl [9]; we aimed to have data of 56 children be available at the endpoint. Given this sample size, we expected to detect medium to strong effects (Cohen's d = 0.76) with alpha = 0.05 and power = 80, two-sided. The number of probands was expected to translate to 15% decrease in mean sTfR in the intervention group with an unchanged mean sTfR in the control group (mean baseline and control groups: 8.48; mean intervention group at endline: 7.208) with a standard deviation of 1.32 at both

Table 3 Baobab fruit pulp composition

Variables (mg / 100 g)	Mean ± SD (n = 8)
Vitamin C	166 ± 71
Iron	4.1 ± 1.3
Zinc	2.7 ± 1.0
Calcium	408 ± 68
Magnesium	232 ± 77

time points and a correlation of 0.25 between time points. These values were copied from Perignon et al. [34] as we did not have our own data when the study was planned; the assumed correlation of 0.25 is a conservative assumption. We initially aimed to screen a total of 273 children but found 249 children aged 6–12 years only, of whom 223 children participated in the screening. When we selected the school, we were only given the numbers of children per class. Information on child age was provided only after the school had been selected.

Blood sample collection and analysis

To minimize any discomfort, a local anesthetic ointment containing lidocaine and prilocaine (EMLA™, AstraZeneca, Cambridge, UK) was applied onto the area of skin to be numbed prior to pricking. During screening, capillary blood samples of children were taken for two subsequent Hb measurements using a HemoCue HB 301 photometer device (HemoCue AB, Ängelholm, Sweden). The maximum tolerated difference between the measurements was 0.5 g/dl.The mean value was used to determine individual Hb levelsat screening.

At baseline, registered nurses took from each child a non-fasting venous blood sample, which was spun within 30 min to obtain 50–100 µl serum. The serum was pipetted into labeled 0.2 ml Multiply® PCR tubes (Sarstedt Inc., US). In the field, samples were either stored at low temperature fora maximum of 7 days and then put into a freezer or stored in a freezer on the same day [35]. The samples were analyzed for serum ferritin (FER), soluble transferrin receptor (sTfR), acidic glycoprotein (AGP), and C-reactive protein (CRP) levels using a Sandwich ELISA at the VitMin Lab, Willstaett, Germany, [36]. Hb concentrations were measure dimmediately after phlebotomy using a HemoCue HB 301 photometer device (HemoCue AB, Ängelholm, Sweden).

Hb was adjusted for altitude and anemia, which is defined as adjusted Hb < 11.5 g/dl in children aged 7–11 years and < 12 g/dl in children aged 12 years [37]. Iron deficiency was defined by depleted iron stores (adjusted FER < 15 µg/L) [38] and tissue iron deficiency by high serum sTfR(> 8.3 mg/L) [36].

CRP and AGP were assessed for the identification and classification of inflammation: incubation (CRP levels > 5 mg/L and AGP levels ≤ 1 g/L), early convalescence(CRP levels > 5 mg/L and AGP levels > 1 g/L), and late convalescence (CRP levels ≤ 5 mg/L and AGP levels > 1 g/L). FER was adjusted for inflammation stage with correction factors for each inflammation stage [39].

Genotyping for sickle cell trait and the 3.7 kb α-globin deletion that most commonly causes α^{+}-thalassemia in African populations was conducted by PCR [40, 41] at the KEMRI-Wellcome Trust Research Laboratories in Kilifi, Kenya, as described in detail previously.

Anthropometric measurements

At screening, nurses received an additional instruction on how to assess the mid-upper arm circumference (MUAC) with a measuring tape that allows for an assessment to the nearest 0.1 cm. Moderate undernutrition was defined at MUAC < 14.5 cm and < 18.5 cm for children aged 6–9 yearsand 10–12 years, respectively [42].

To control for a potential influence of anthropometric developments from baseline to endline, we assessed weight and height at baseline and endline. Children were checked for edema and weighed without shoes and in light clothing to the nearest 0.1 kg, using a Seca® UNICEF scale (SECA 874, Hamburg, Germany). Body height was measured to the nearest 0.5 cm using a calibrated SECA® height scale (SECA 213, Hamburg, Germany). Weight and height measurements were repeated twice with a maximum tolerable difference of 0.1 kg for weight and 0.5 cm for height.

The weight-for-age z-score (WAZ), body mass index-for-age z-score (BAZ), and height-for-age z-score (HAZ) were calculated using Anthro Plus, the anthropometric calculator module based on the 2007 WHO reference for children aged 5–19 years [43, 44]. Stunting, underweight, and thinness were defined by HAZ, WAZ, and BAZ below − 2 SD,respectively. The school provided data on the age of the children, which was crosschecked with primary caregivers. If the primary caregiver could not verify the date of birth, WAZ, BAZ, and HAZ were not calculated.

Assessment of nutrient intake

To control dietary intake outside the study setting, we conducted 24 h-recalls during the 1st (t1), 5th (t2) and 11th (t3) weeks. Interviewers with a formal qualification in nutrition or food science, as well as literate in English and the local language, were trained on applying standardized 24 h-recalls with primary caregivers. The questionnaire and 24 h-recalls were translated into the local Kikamba language and retranslated into English, reviewed during the 6-day interviewer training, pre-tested, and modified to ensure meaning equivalence of the questions. Pre-testing was carried out among households with children not involved in the study.

The interviews for the multiple pass 24 h-recalls consisted of (a) listing all foods and drinks consumed the day before the interview, (b) gathering detailed information about eachfood or recipe for dishes, (c) estimated quantification of the amount of consumed food/drink and used ingredients for therecipes, and (d) reviewing the information with the respondent at the end of the recall. Specially designed photo books

were developed to estimate the quantity of intake of food and drinks. The interviewer also used local measuring tools such as spoons and cups for quantifying portion sizes.

Table 4 shows the recommended dietary allowances for energy, vitamins, and trace elements for school-aged children. Individual energy adequacy ratios were calculated as total energy intake divided by sex, and age-specific energy requirements, based on the recommendations of the FAO/WHO/UNU expert committee on human energy requirements [45]. The nutrient adequacy ratio (NAR) was determined for vitamins C, iron, zinc, calcium, and magnesium. Individual NARs were calculated as a total intake of the nutrient divided by the recommended daily allowance (RDA) for that nutrient, based on intakes recommended by the Kenyan Ministry of Health [46]. Table 1 shows the energy and nutrients of one portion of school meal that was provided on a daily basis in addition to the drink.

Data management and statistical analysis

Data entry and validation via double entry was performed for anthropometry and Hb, as well as for the 24 h-recalls. The country-specific food database for Kenya was loaded into the NutriSurvey nutrient database. Missing food items were supplemented from the Tanzania Food Composition Tables [47] and the Food Data Central of the United States Department of Agriculture [48].

Data management and statistical analysis were performed using SPSS software (Version 24, IBM Corp., Armonk, NY, USA).

The mean intake of energy and nutrients, determined through NutriSurvey, at time points t1, t2, and t3 was calculated for each child. Normality of distributions was evaluated using the Shapiro–Wilk test. As most continuous variables (micronutrient status and energy and nutrient intake) had heavily skewed distributions, descriptive statistics for continual variables are presented in the median and interquartile range (IQR). For this data, a non-parametric median test was applied for comparing data from intervention and control groups at baseline (blood parameters and anthropometric data) and at t1, t2, and t3 (mean energy and nutrient intake). The strength of association was calculated with Cramer's V, which equals r. For approximately normally distributed data, means and standard deviations are presented, and the independent *t* test was applied.

Outliers in development (baseline to endline) of Hb, FER,and sTfR were identified as described by Tukey [49] and excluded from the analysis (outliers: n(Hb) = 0; n(FER-intervention) = 3, n(FER-control) = 1; n(sTfR-intervention) = 0, n(sTfR-control) = 1).

The baseline and endline data on FER and sTfR were log transformed and used to calculate the development between baseline and endline to apply the independent samples' *t* test for differences between groups and the paired *t* test for development within the group. The effect size for the independent *t* test was not calculated (differences not significant) and paired *t* test was calculated using Cohen's d.

Friedman's ANOVA was conducted to test differences in dietary intake between t1, t2, and t3 (related samples and pairwise comparison). The general linear model was used to evaluate the effects of time (baseline/endline), group (intervention/control), age (in years at baseline), change in weight (endline—baseline), sex (male/female), and genotype (heterozygote carrier of α-thalassemia/non-carrier) on Hb, LN(FER), and LN(sTfR) and the interaction of time with each variable, respectively. For Hb, we also analyzed the interaction time*group*genotype. Variables were tested for associations with non-parametric Spearman's correlation. A p value of < 0.05 was considered statistically significant.

Table 4 Recommended dietary allowances of energy, vitamin, minerals, and elements for school-aged children

Energy and nutrients[a]	6–8 years	9–12 years
Energy (kcal)	1694	1916
Vitamin C (mg)	25	45
Iron (mg)	10	8
Zinc (mg)	5	8
Calcium (mg)	800	1300
Magnesium (mg)	130	240

[a]Reference values of the Kenyan National Micronutrient Survey 2011 [2] except for energy (presented as means for boys/girls aged 6–8 years and 9–12 year, respectively) [30]

Ethical approval

The institutional review board of the Faculty of Medicine at Justus Liebig University Giessen, Germany (197/16) and the AMREF Ethics and Scientific Review Committee (AMREF-ESRC P313/2017) Kenya approved the *Baobab Nutrition Intervention Study* under the Kenyan National Commission for Science, Technology, and Innovation research permit (NACOSTI/P/18/60305/20841). The study was registered with the German Clinical Trials Registry (DRKS00011935). Official permission and approval from Kenya government authorities was obtained, and the municipal and governmental authorities in Kenya approved for the implementation of the study.

Written informed consent of primary caregivers and schoolchildren via signature or fingerprint was obtained prior to data collection. The ethics committees also approved the consent format prior to data collection. The management school board comprising the parent's representative, representatives from the Kenya National Union of Teachers,

church and local leaders were informed about the study and gave their verbal consent after participating in a stakeholder meeting to create awareness on the study.

Results

Of the 249 eligible schoolchildren aged 6–12 years, a total of 223 were screened. After randomization, allocation, and follow-up, data of 58 children was available for the analyses. To include the required number of children, a cut-off for low Hb levels was set at 12.15 g/dl, i.e., 6% above the normal cut-off at 11.5 g/dl used in Kenyan schoolchildren otherwise. The intervention lasted for 83 days, and the median days of participation was 82 in both groups (IQR intervention: 78–82.5 and IQR control: 79–83).

In both groups, 55.2% of participants were girls, and 37.9–41.1% of participants were heterozygous carriers of α-thalassemia in the intervention and control group, respectively. None of the participants were carriers of sickle cell trait.

Baseline characteristics

At baseline, median test did not show significant differences between intervention and control group in terms of median Hb (non-adjusted and adjusted), FER (non- adjusted and adjusted), sTfR, CRP and AGP. Elevated inflammation markers were only present in the control group, with prevalence of 1, 1, and 3 children in incubation, early convalescence, and late convalescence, respectively. Prevalence of low Hb-, FER-, and sTfR levels are presented in Table 5.

Table 6 Mean (SD) of anthropometric characteristics for interventionand control group at baseline

	n	Intervention Mean ± SD	*n*	Control Mean ± SD	*p* (*t* test)
Age					
Age in years	29	8.2 ± 1.8	29	8.7 ± 1.9	0.259
Nutritional status					
Weight (kg)	29	22.8 ± 5.5	29	24.8 ± 5.7	0.177
Height (cm)	29	123.0 ± 10.0	29	126.8 ± 11.1	0.174
WAZ	26	– 1.4 ± 0.9	29	– 1.3 ± 0.8	0.611
HAZ	26	– 1.2 ± 1.0	29	– 1.1 ± 0.8	0.739
BAZ	26	– 0.9 ± 0.7	29	– 0.7 ± 0.8	0.549

Date of birth was not confirmed for three rural children and nutritional status was not calculated

WAZ weight-for-age z-score, *HAZ*: height-for-age z-score, *BAZ* body mass index-for-age z-score

Table 6 shows the baseline characteristic of the anthropometric measurements. In the intervention group, the prevalence of underweight, stunting, and thinness was 21.7%, 23.1%, and 3.8% and in the control group the prevalence was 15.0%, 13.4%, and 3.4%, respectively.

Dietary intake of meals in and outside the school

Median energy and nutrient intake (calculated from mean individual intakes at t1, t2, and t3 for each child) are presented in Table 7, as well as median adequacy ratio. Median vitamin C and calcium intake was significantly higher in the intervention group with a medium effect size (vitamin C: $\chi^2(1) = 11.655$, $p = 0.001$, $r = 0.448$; calcium: $\chi^2(1) = 8.345$, $p = 0.004$, $r = 0.379$). A critical nutrient was calcium because the actual intake was far below recommendations, with a

Table 5 Hemoglobin level and iron status at baseline and endline

	Intervention		Control	
	Baseline	Endline	Baseline	Endline
Hemoglobin (g/dl)	***n* = 29**		***n* = 29**	
Hb, mean ± SD	12.6 ± 0.72	12.9 ± 0.87	13.0 ± 0.69	12.9 ± 0.10
Hb, adj[a] mean ± SD	12.4 ± 0.72	12.7 ± 0.87	12.8 ± 0.69	12.7 ± 0.10
Hb, adj[a] < 11.5, *n* (%)	2 (6.9)	2 (6.9)	1 (3.4)	2 (6.9)
FER (µg/L)	***n* = 26**		***n* = 28**	
FER, geometric mean ± SD	36.7 ± 1.89	30.7 ± 1.96	42.7 ± 1.96	30.2 ± 1.78
FER adj[b], geometric mean ± SD	36.7 ± 1.89	30.4 ± 1.96	40.1 ± 1.90	29.7 ± 1.78
FER adj[b], < 15 µg/L, *n* (%)	4 (15.4)	3 (10.3)	4 (14.3)	3 (10.7)
sTfR (mg/L)	***n* = 29**		***n* = 28**	
sTfR geometric mean ± SD	6.27 ± 1.21	6.31 ± 1.19	6.16 ± 1.21	6.33 ± 1.21
sTfR > 8.3, *n*(%)	2 (6.9)	3 (10.3)	2 (7.1)	3 (10.7)

Hb hemoglobin, *FER* Ferritin, *sTfR* soluble transferrin receptor

[a]Hemoglobin adjusted for altitude

[b]Ferritin adjusted for inflammation stage

Table 7 Median (IQR) daily intake and median adequacy ratio for intervention and control group

	Median (IQR) daily		*p* (median test)	Median adequacy ratio	
	Intervention ($n=29$)	Control ($n=29$)		Intervention ($n=29$)	Control ($n=29$)
Energy (kcal)	1633 (1403–1829)	1524 (1359–1721)	0.189	92%	83%
Vitamin C (mg)	73 (57–96)	40 (27–63)	0.001	211%	122%
Iron (mg)	15 (13–17)	14 (13–15)	0.066	165%	157%
Zinc (mg)	10 (8–11)	8 (8–9)	0.066	156%	128%
Calcium (mg)	396 (344–477)	318 (276–364)	0.004	39%	30%
Magnesium (mg)	513 (435–581)	437 (402–492)	0.066	310%	242%

median adequacy ratio of 39% (IQR = 32–52%) in the intervention and 30% (IQR = 25–35%) in the control group.

During the intervention, the dietary intake changed (Fig. 2). At t2, vitamin C intake increased, while energy and iron intake decreased compared to t1. At all times, median adequacy ratio for iron was met in both groups (≥ 140%) and the intake of vitamin C and iron at t1, t2, t3 did not differ significantly in the intervention and control group, respectively. Only the energy intake in the control group differed significantly between t1 and t2 with a small effect size ($p = 0.026$, $r = 0.128$). Noteworthy, the energy intake was always higher in the intervention than in the control group.

Impact of baobab intake on hemoglobin and ironstatus

Table 5 shows the baseline and endline data of the intervention and control group without outliers in development. Figure 3 presents the changes in Hb, FER, and sTfR in both groups. Between baseline and endline, developments showed a better tendency of Hb (mean), FER (geometric mean), andsTfR (geometric mean) in the intervention than in the control group. Hb and sTfR within each group did not significantly change between baseline and endline. Mean Hb levels improved by 2.2% in the intervention and worsened by 1.2%in the control group. Geometric mean of sTfR level in the intervention group levels remained almost unchanged (0.7%) while it worsened by 2.7% in the control group. The numberof children with tissue iron deficiency increased by one in each group between baseline and endline.

Geometric mean of FER levels worsened by 17.3% in the intervention and 26.0% in the control group. Mean LN(FER) values within the intervention ($t(27) = 3.820$, $p = 0.001$, $r = 0.675$) and control ($t(25) = 3.444$, $p = 0.002$, $r = 0.722$) group significantly worsened. Even though the mean FER level significantly decreased in both groups, the number of children with iron deficiency decreased by one child in each group.

The general linear model including age, change of weight, group, gender, carrier of heterozygous α-thalassemia and did not show a significant influence on changes in Hb, LN(FER), or LN(sTfR). Nonetheless, we found small evidence of an effect on the group on Hb (part. $Eta^2 = 0.067$; $p = 0.062$).

Discussion

Positive associations between dietary vitamin C intake and Hb levels, as well as iron status, have been found in Mexico, where a traditional beverage (*pulque)* containing 30 mg vitamin C is consumed with a diet based on cereals and beans. Although the diet was high in phytate and phenolic compounds, similar to the diet in the *Baobab Nutrition Intervention Study*, a higher vitamin C intakes predicted a lower risk of anemia [21]. A study on the impact of BFP intake of Nigerian schoolchildren found that a BFP-drink can significantly improve Hb-levels and iron stores. The amount of vitamin C from the BFP in the intervention drinkwas twice as high as in the *Baobab Nutrition Intervention Study* (60 mg vs. 33 mg vitamin C), the control group did not receive any placebo and the prevalence of anemia and iron deficiency was much higher in the Nigerian study [28].The intervention drink was consumed before the meal with similar ingredients (cereal/legume/vegetable-based meal) as in the *Baobab Nutrition Intervention Study*, but detailed information on food are not provided. Furthermore, information on randomization, blinding, food composition or dietary intake of participants are missing. Though, in vitro studies with BFP are in line with the Nigerian study as they found significant improvements iron bioaccessibility, probably due to the rich vitamin C content and other organic acids such as citric acid [25–27].

The observations from this study can be summarized as follows: mean Hb levels slightly increased in intervention group and slightly went down in controls, but both changes were not statistically significant. The geometric mean of FER levels went significantly down in both groups, but to a lower extent in the intervention than in the control group. Geometric mean of sTfR levels increased in the intervention group and more markedly increased in controls. Overall, the whole study population experienced a general tendency towards worsening iron stores. The tendencies of changes are

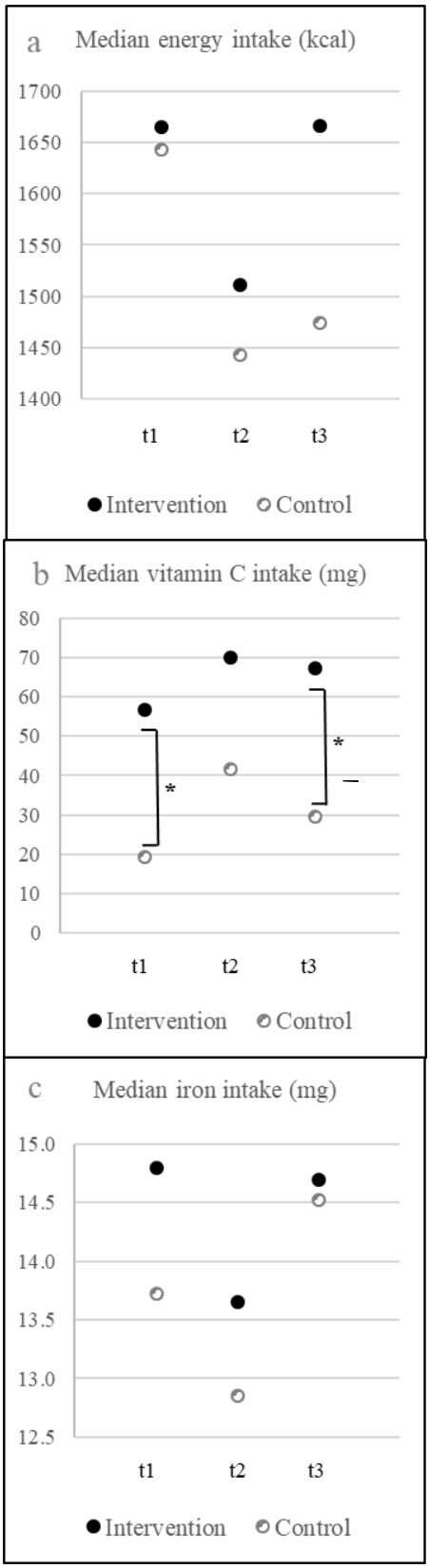


Fig. 2 Median intake of energy, vitamin C and iron at t1, t2 and t3 of the intervention (n = 29) and control (n = 29) group. *Significant differences between intervention and control group ($p < 0.05$)

concordant and point towards a beneficial effect of BFP on iron absorption as summarized in Table 8. Since the study population had an unexpectedly low prevalence of anemia and iron depletion, significant effects could not be demonstrated within the chosen study design. Although the school-children were selected for low to low normal Hb levels, the observed change—brought about the consumption of BFP as a supplement to the school meal—were not as high as expected.

To control for the intervention effect, we selected a low-risk area for malaria transmission, we provided albendazole to children at screening, and determined the two most common Hb-disorders, sickle cell and α-thalassemia trait. Therefore, it is unlikely that worsening of FER levels can be attributed to helminth infections. Moreover, α-thalassemia trait was neither associated with baseline Hb, FER, and sTfR levels nor with their development, while sickle cell trait was not present in the studied population.

The expected intervention effect of vitamin C on improved iron bioavailability might have been mitigated by inhibitory compounds of the school meal and the BFP itself. To allow for a significant enhancing effect of iron absorption, Teucher et al. [15] suggest a molar ratio of 2:1 and of 4:1 of vitamin C to iron for meals with low medium and high levels of inhibitors, respectively. In the *Baobab Nutrition Intervention Study* the calculated molar ratio for the vitamin C rich BFP and iron of the phytate rich school meal was lower, namely 1.3:1. Besides compounds in BFP that promote the iron bioavailability, BFP also contains phenolic compounds that are generally known to inhibit iron absorption. However, of the total phenolics, 21.5% were identified as catechin [26], a strong promoter of iron bioavailability [50]. But, other phenolics found in BFP are iron-chelating compounds, in particular tannins [25, 26, 31]. The tannin content may be caused by contamination of the fruit pulp with seed fragments, which themselves are high in tannins [51]. The BFP in our study was processed by mechanical separation of the pulp from the seeds using a mortar. Even though we sieved the BFP two times, we cannot exclude contamination with seed fragments.

During the intervention, the experienced food security improved (data not presented here) as the intervention started at the end of the rainy season. This was reflected in a change of dietary intake, towards a higher intake of vitamin C and lower energy and iron intake towards the middle of the intervention (t2) (Fig. 2). Due to the positive dose-depended relationship of vitamin C intake and iron bioavailability [52], the lower iron intake could have been compensated by higher iron bio-availability. However, FER levels decreased in both groups during the intervention even though the iron intakes above the recommended intake. Notably, presented changes of vitamin C intake were attributed to dietary patterns at household level, because the composition of the intervention drink and

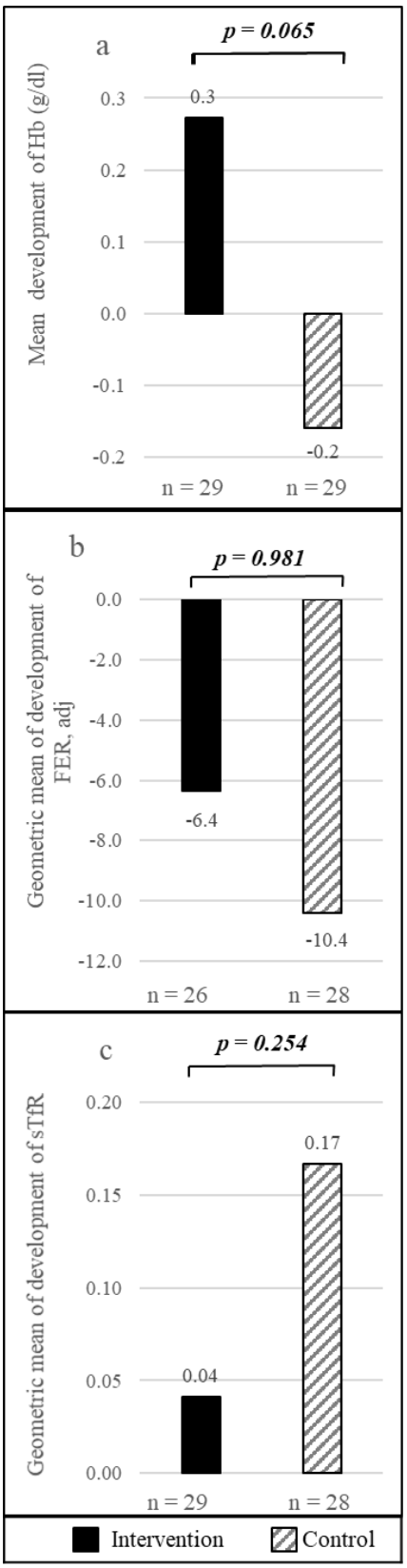


Fig. 3 Changes in hemoglobin and iron status from baseline to end-line; independent *t* test was applied for difference in development between intervention and control group. **a** Mean development of Hb with non-significant differences between intervention and control group. **b** Development of geometric mean of FER and with non- significant difference of LN(FER) development between intervention and control group. **c** Development of geometric mean of sTfR with non-significant difference between development of LN(sTfR) in the intervention and control group. Hb: hemoglobin, FER: ferritin adjusted, sTfR: soluble transferrin receptor

school meal remained unchanged throughout the intervention. However, the impact of BFP on improved bioavailability of iron might have varied widely, according to the natural variations of the vitamin C content of the BFP ranging from 80 to 266 mg/100 g. In conclusion, the low iron bioavailability may have been the limiting factor for iron utilization in this study.

Apart from the tested intervention effect, we found significantly higher vitamin C and calcium intake in the intervention than in the control group. In BFP, both nutrients are particularly high (mean vitamin C and calcium intake through BFP: 33 mg and 81 mg, respectively). An inhibitory effect of calcium on iron absorption has been discussed in several studies; yet, a review on long-term calcium supplementation concluded that there is no adverse effect on iron status [53]. Moreover, a 1-month calcium supplementation did not result in a reduction of iron bioavailability [54].

BFP contains iron, zinc, magnesium, and phosphorous; however, the higher intake of these nutrients in the intervention than in the control group was not at a significant scale ($p = 0.066$, respectively). However, the energy intake in the intervention groups was higher, yet not significantly, scale, which may also partly explain the higher intake of nutrients. Nevertheless, the energy intake from intervention and control drink was equivalent (Table 2).

Limitations

The prevalence of anemia and iron deficiency in this study population of Kenyan schoolchildren was much lower than expected; therefore, the intervention effect was also lower. The study was conducted in a non-malaria-endemic zone, which might partly explain the lower anemia prevalence compared to other studies [5, 11, 12, 14]. As the sample size was calculated on the assumption of a higher prevalence of iron deficiency, the actual sample size was too small to show significant effects on anemia and iron status.

The measured vitamin C content of the raw BFP for the intervention drink varied widely (Table 3). Therefore, the impact of BFP on the bioavailability of iron might have varied from day to day.

Table 8 Tendencies of the findings in both groups

	Intervention	Control
Hb	↑	↓
FER	↓	↓↓
sTfR	↑	↑↑

Conclusion

In vitro studies showed an increased bioaccessibility of iron from cereals in the presence of the comparable amounts of BFP that were used in our study. However, in vivo, we detected a BFP-driven tendency towards better iron uptake from plant foods, but a significantly improved iron status brought about by supplementation with BFP could not be detected. We conclude that the promoting effect on iron bioavailability from BFP might not have overcome the inhibitory effect of phytate and polyphenols from the school meal. Adverse effects of BFP consumption have not been observed. The identification of products such as BFP remains pertinent to help improve non-heme iron absorption in the populations most vulnerable for iron deficiency. This is particularly relevant for food insecure areas where baobab is native, available, and affordable. Thus, school meal programs that include iron-rich foods as well as components promoting iron uptake are a reasonable approach to prevent childhood anemia.

We suggest to conduct a similar study in a setting with higher prevalence of anemia and to provide a fermented iron-rich cereal porridge (sorghum, etc.) mixed with BFP as the present study did not exclude the expected benefits.

Acknowledgements We thank the school management board Kakumuti Primary School and the field assistants for their unrestricted help in implementing the study; George Kinyua Kaimba, MSc. for his supportin providing contacts with local baobab fruit traders; Joyce Chepngeno,MSc, for conducting the vitamin C analyses; the nurses and lab technician at Kauwi Sub-District Hospital, Kitui, for the collaboration during data collection; Juergen Erhardt, VitMin Laboratory, Willstaett, Germany, for the blood nutrient analyses; Thomas N Williams and Alexander Macharia KEMRI-Wellcome Trust Research Programme, Kilifi, Kenya, for hemoglobinopathy screening; the Ministries of Education and Health, Nairobi, Kenya, for their support; Maike Schnermann, BSc, for her support in data collection. Johannes Herrmann, Ph.D., Giessen, Germany, supported the data analysis.

Author contribution E.C.E., T.-Y.H., and M.B.K. conceived and designed the BAOFOOD nutrition trial; E.C.E. and M.B.K. conductedthe field work and managed the data; W.O.O. supported in the preparatory and implementing phase of the study; E.C.E. conducted the data analysis and drafted the initial manuscript; all the authors contributed to the review and editing of the manuscript; E.C.E. and M.B.K. had primary responsibility for the final content. All the authors read and approved the final version of the manuscript.

Funding Open Access funding enabled and organized by Projekt DEAL. This research was funded by The German Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament ofthe Federal Republic of Germany via the Federal Office for Agricultureand Food and Agriculture (BLE), grant number FKZ 2813FSNU08. The funders had no role in the design of the study, the collection, analyses, nor in the interpretation of data, in writing the manuscript,or in the decision to publish the results.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you willneed to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

1. Black RE, Victora CG, Walker SP et al (2013) Maternal and child undernutrition and overweight in low-income and middle-income countries. Lancet 382:427–451. https://doi.org/10.1016/S0140-6736(13)60937-X
2. Scott SP, Chen-Edinboro LP, Caulfield LE et al (2014) The impact of anemia on child mortality: an updated review. Nutrients 6:5915–5932. https://doi.org/10.3390/nu6125915
3. Walker SP, Wachs TD, Meeks Gardner J et al (2007) Child development: risk factors for adverse outcomes in developing countries. Lancet 369:145–157. https://doi.org/10.1016/S0140-6736(07)60076-2
4. Pullan RL, Gitonga C, Mwandawiro C et al (2013) Estimating the relative contribution of parasitic infections and nutrition for anaemia among school-aged children in Kenya: a subnational geostatistical analysis. BMJ Open. https://doi.org/10.1136/bmjopen-2012-001936
5. Neumann CG, Bwibo NO, Murphy SP et al (2003) Animal source foods improve dietary quality, micronutrient status, growth and cognitive function in Kenyan school children: background, study design and baseline findings. J Nutr 133:3941S-3949S
6. Evang EC, Habte T-Y, Owino WO et al (2020) The nutritional and micronutrient status of urban schoolchildren with moderate anemia is better than in a rural area in Kenya. Nutrients. https://doi.org/10.3390/nu12010207
7. Wambua S, Mwangi TW, Kortok M et al (2006) The effect of alpha+-thalassaemia on the incidence of malaria and other diseases in children living on the coast of Kenya. PLoS Med 3:e158. https://doi.org/10.1371/journal.pmed.0030158
8. Suchdev PS, Ruth LJ, Earley M et al (2014) The burden and consequences of inherited blood disorders among young children in western Kenya. Matern Child Nutr 10:135–144. https://doi.org/10.1111/j.1740-8709.2012.00454.x
9. Kenya Ministry of Health (2016) Kenya Malaria Indicator Survey 2015. https://dhsprogram.com/pubs/pdf/MIS22/MIS22.pdf. Accessed 16 Apr 2020

10. WHO (2015) The global prevalence of anaemia in 2011. https://apps.who.int/iris/bitstream/handle/10665/177094/9789241564960_eng.pdf;jsessionid=72ED845B65F92B199ED3A64DD7ED8A4A?sequence=1. Accessed 02 Jul 2020
11. Njaanake KH, Vennervald BJ, Simonsen PE et al (2016) Schistosoma haematobium and soil-transmitted helminths in Tana Delta district of Kenya: infection and morbidity patterns in primary schoolchildren from two isolated villages. BMC Infect Dis 16:57. https://doi.org/10.1186/s12879-016-1387-4
12. Muriuki JM, Mentzer AJ, Kimita W et al (2019) Iron status and associated malaria risk among African children. Clin Infect Dis 68:1807–1814. https://doi.org/10.1093/cid/ciy791
13. Houghton LA, Brown RC, Beaumont S et al (2019) Micronutrient status differs among Maasai and Kamba preschoolers in a supplementary feeding programme in Kenya. Matern Child Nutr. https://doi.org/10.1111/mcn.12805
14. Andang'o PEA, Osendarp SJM, Ayah R et al (2007) Efficacy of iron-fortified whole maize flour on iron status of schoolchildren in Kenya: a randomised controlled trial. Lancet 369:1799–1806. https://doi.org/10.1016/S0140-6736(07)60817-4
15. Teucher B, Olivares M, Cori H (2004) Enhancers of iron absorption: ascorbic acid and other organic acids. Int J Vitam Nutr Res 74:403–419. https://doi.org/10.1024/0300-9831.74.6.403
16. Gibson RS, Perlas L, Hotz C (2006) Improving the bioavailability of nutrients in plant foods at the household level. Proc Nutr Soc 65:160–168. https://doi.org/10.1079/PNS2006489
17. Siegenberg D, Baynes RD, Bothwell TH et al (1991) Ascorbic acid prevents the dose-dependent inhibitory effects of polyphenols and phytates on nonheme-iron absorption. Am J Clin Nutr 53:537–541
18. Davidsson L, Galan P, Kastenmayer P et al (1994) Iron bioavailability studied in infants: the influence of phytic acid and ascorbic acid in infant formulas based on soy isolate. Pediatr Res 36:816–822. https://doi.org/10.1203/00006450-199412000-00024
19. Fidler MC, Davidsson L, Zeder C et al (2003) Iron absorption from ferrous fumarate in adult women is influenced by ascorbic acid but not by Na2EDTA. BJN 90:1081. https://doi.org/10.1079/BJN2003995
20. Balay KS, Hawthorne KM, Hicks PD et al (2010) Orange but not apple juice enhances ferrous fumarate absorption in small children. J Pediatr Gastroenterol Nutr 50:545–550. https://doi.org/10.1097/MPG.0b013e3181b1848f
21. Backstrand JR, Allen LH, Black AK et al (2002) Diet and iron status of nonpregnant women in rural Central Mexico. Am J Clin Nutr 76:156–164. https://doi.org/10.1093/ajcn/76.1.156
22. Chadare FJ, Linnemann AR, Hounhouigan JD et al (2009) Baobab food products: a review on their composition and nutritional value. Crit Rev Food Sci Nutr 49:254–274. https://doi.org/10.1080/10408390701856330
23. Stadlmayr B, Charrondiere UR, Eisenwagen S et al (2013) Nutrient composition of selected indigenous fruits from sub-Saharan Africa. J Sci Food Agric 93:2627–2636. https://doi.org/10.1002/jsfa.6196
24. Habte T-Y, Krawinkel MB (2017) Metaphysical analysis of the nutritional and therapeutic value of baobab (*Adansonia Digitata* L.). J Nutr Health Sci 5:101. https://doi.org/10.15744/2393-9060.5.101
25. Gabaza M, Shumoy H, Muchuweti M et al (2018) Baobab fruit pulp and mopane worm as potential functional ingredients to improve the iron and zinc content and bioaccessibility of fermented cereals. Innov Food Sci Emerg Technol 47:390–398. https://doi.org/10.1016/j.ifset.2018.04.005
26. van der Merwe R, Kruger J, Ferruzzi MG et al (2019) Improving iron and zinc bioaccessibility through food-to-food fortification of pearl millet with tropical plant foodstuffs (moringa leaf powder, roselle calyces and baobab fruit pulp). J Food Sci Technol 56:2244–2256. https://doi.org/10.1007/s13197-019-03711-y
27. Adetola OY, Kruger J, White Z et al (2019) Comparison between food-to-food fortification of pearl millet porridge with moringa leaves and baobab fruit and with adding ascorbic and citric acid on iron, zinc and other mineral bioaccessibility. LWT Food Sci Technol 106:92–97. https://doi.org/10.1016/j.lwt.2019.02.044
28. Nnam NM, Madukwe EU, Udenta EA (2011) Effect of baobab (*Adansonia digitata* L.) fruit pulp drink on iron status of school children. Nig J Nutri Sci. https://doi.org/10.4314/njns.v32i2.71713
29. Gebauer J, Adam YO, Sanchez AC et al (2016) Africa's wooden elephant: the baobab tree (*Adansonia digitata* L.) in Sudan and Kenya: a review. Genet Resour Crop Evol 63:377–399. https://doi.org/10.1007/s10722-015-0360-1
30. FEWS NET (2018) Kenya price bulletin: April 2018. https://fews.net/sites/default/files/documents/reports/Kenya_2018_04_PB.pdf. Accessed 14 Apr 2020
31. Tembo DT, Holmes MJ, Marshall LJ (2017) Effect of thermal treatment and storage on bioactive compounds, organic acids and antioxidant activity of baobab fruit (Adansonia digitata) pulp from Malawi. J Food Compos Anal 58:40–51. https://doi.org/10.1016/j.jfca.2017.01.002
32. Vikram VB, Ramesh MN, Prapulla SG (2005) Thermal degradation kinetics of nutrients in orange juice heated by electromagnetic and conventional methods. J Food Eng 69:31–40. https://doi.org/10.1016/j.jfoodeng.2004.07.013
33. Habte TY, Suleiman I, Adam HE et al (2019) The potential of Baobab (*Adansonia Digitata* L) leaves from North- and West Kordofan in Sudan as mineral complement of common diets. J Nutrition Food Process. https://doi.org/10.31579/2637-8914/015
34. Perignon M, Fiorentino M, Kuong K et al (2014) Stunting, poor iron status and parasite infection are significant risk factors for lower cognitive performance in Cambodian school-aged children. PLoS ONE 9:e112605. https://doi.org/10.1371/journal.pone.0112605
35. Erhardt JG (2010) Measurement of the Vitamin A and Iron Status in Blood Samples, 3. Measurement of Serum Samples. 2010. https://www.nutrisurvey.de/blood_samples/index.htm#3. Accessed 16 Apr 2020
36. Erhardt JG, Estes JE, Pfeiffer CM et al (2004) Combined measurement of ferritin, soluble transferrin receptor, retinol binding protein, and C-reactive protein by an inexpensive, sensitive, and simple sandwich enzyme-linked immunosorbent assay technique. J Nutr 134:3127–3132
37. WHO (2017) Nutritional anaemias: tools for effective prevention and control. https://www.who.int/nutrition/publications/micronutrients/anaemias-tools-prevention-control/en/. Accessed 16 Apr 2020
38. WHO (2001) Iron deficiency anaemia: assessment. Prevention and Control, Version current, p 2001
39. Thurnham DI, McCabe LD, Haldar S et al (2010) Adjusting plasma ferritin concentrations to remove the effects of subclinical inflammation in the assessment of iron deficiency: a meta-analysis. Am J Clin Nutr 92:546–555. https://doi.org/10.3945/ajcn.2010.29284
40. Chong SS, Boehm CD, Higgs et al (2000) Single-tube multiplex-PCR screen for common deletional determinants of alpha-thalassemia. Blood 95:360–362
41. Waterfall CM, Cobb BD (2001) Single tube genotyping of sickle cell anaemia using PCR-based SNP analysis. Nucleic Acids Res 29:e119. https://doi.org/10.1093/nar/29.23.e119
42. National AIDS/STI control programme (2012) Reference charts for nutrition diagnosis and protocol for food by prescription. https://www.fhi360.org/sites/default/files/media/documents/

Reference%20Charts%20for%20Nutrition%20Diagnosis%20and%20Protocol%20for%20Food%20by%20Prescription.pdf. Accessed 16 Apr 2020
43. WHO (2009) WHO AnthroPlus software. https://www.who.int/growthref/tools/en/. Accessed 16 Apr 2020
44. de Onis M, Onyango AW, Borghi E et al (2007) Development of a WHO growth reference for school-aged children and adolescents. Bull World Health Organ 85:660–667. https://doi.org/10.2471/BLT.07.043497
45. FAO (2004) Human energy requirements. Report of a Joint FAO/WHO/UNU Expert consultation, food and nutrition technical report series 1,Rome. http://www.fao.org/3/a-y5686e.pdf. Accessed 16 Apr 2020
46. Kenya ministry of health (2015) The Kenya national micronutrient survey 2011. https://www.nutritionhealth.or.ke/wp-content/uploads/Downloads/The%20Kenya%20National%20Micronutrient%20Survey%202011.pdf. Accessed 16 Apr 2020
47. Lukmanji Z, Hertzmark E, Mlingi N et al. (2008) Tanzania food composition tables. https://cdn1.sph.harvard.edu/wp-content/uploads/sites/30/2012/10/tanzania-food-composition-tables.pdf. Accessed 16 Apr 2020
48. USDA (2018) FoodData Central. https://fdc.nal.usda.gov/. Accessed 16 Apr 2020
49. Tukey JW (1977) Exploratory data analysis. Addison-Wesley, Reeading, MA
50. Hart JJ, Tako E, Kochian LV et al (2015) Identification of black bean (*Phaseolus vulgaris* L.) polyphenols that inhibit and promote iron uptake by Caco-2 Cells. J Agric Food Chem 63:5950–5956.https://doi.org/10.1021/acs.jafc.5b00531
51. Osman MA (2004) Chemical and nutrient analysis of baobab (Adansonia digitata) fruit and seed protein solubility. Plant Foods Hum Nutr 59:29–33. https://doi.org/10.1007/s11130-004-0034-1
52. Lynch SR, Cook JD (1980) Interaction of vitamin C and iron. Ann NY Acad Sci 355:32–44. https ://doi.org/10.1111/j.1749-6632.1980.tb21325.x
53. Lönnerdal B (2010) Calcium and iron absorption–mechanisms and public health relevance. Int J Vitam Nutr Res 80:293–299. https://doi.org/10.1024/0300-9831/a000036
54. Ríos-Castillo I, Olivares M, Brito A et al (2014) One-month of calcium supplementation does not affect iron bioavailability: a randomized controlled trial. Nutrition 30:44–48. https://doi.org/10.1016/j.nut.2013.06.007

4. Discussion

This chapter links the results of the papers presented in Chapters 2 and 3 and augments the findings with results of the first round of the *Baobab Nutrition Intervention Study* presented in Chapter 1, as well as of similar studies.

4.1 Synopsis of the main findings

The results in Chapter 2 show that rural children have higher nutrient intakes but worse nutritional status than their urban counterparts. Median adequacy ratio for iron was 96% in rural and 77% in urban children. Critically low intakes of vitamin C, B12, and retinol equivalent were observed in both groups. Intake of heme iron was low in both groups, but higher in urban than in rural children. Comparison of the baseline data from the urban and rural contexts concluded that bioavailability of non-heme iron is of greater concern in the rural setting.

The evaluation of the *Baobab Nutrition Intervention Study* in Chapter 3 showed a BFP-driven tendency towards better iron uptake from plant foods, but significantly improved Hb levels and iron status brought about by supplementation with BFP were not observed. Factors limiting the beneficial effects on iron bioavailability might include the inhibitory effects of phytate and polyphenols from the school meal and phytochemicals from BFP itself that did not overcome the beneficial effects of BFP. Consuming the baobab drink with 20 g of fresh BFP significantly increased the intake of vitamin C and calcium. Through the intervention, the intake of vitamin C significantly increased ($p = 0.001$, $r = 0.448$), and the median vitamin C adequacy ratio was enhanced from 122% to 211%. Furthermore, the median calcium adequacy ratio increased from 30% to 39%. Even though the difference was of statistical significance ($p = 0.004$, $r = 0.379$), the intake of calcium in the intervention group was still far below the Kenyan recommended dietary allowances.

4.2 Nutritional intake and status of urban and rural schoolchildren

The urban and rural differences described in Chapter 2 conclude that the additional vitamin C from BFP are of greater benefit in the rural setting to increase Hb levels and iron status as intestinal iron absorption is higher in people with low iron status [1, 2].

In terms of the most limited identified nutrients in the urban and rural diets [3], BFP can only contribute vitamin C to the diet, but not provitamin A or vitamin B12. Vitamin B12 intake was higher in urban children that may have also benefited more from mandatory fortification of flour with B12 and iron, folate, and vegetable oil with provitamin A than their rural counterparts. The prevalence of iron and zinc deficiency is reflected by the higher prevalence

of underweight (defined by mid-upper arm circumference (MUAC)), stunting, and wasting in rural than urban children [3]. This observation is supported by the Kenyan Demographic and Health Survey that shows the highest stunting prevalence in Kitui within Kenya [4].

The results of the anthropometric assessment further indicate a decrease in nutritional status with increasing age [3] in both settings. To reverse this trend, investments in school meal programs represent an important strategy for capitalizing on a second window of opportunity to support the prevention of malnutrition [5]. Benefits of school meals on children include decreasing micronutrient deficiency and anemia, improving school enrollment and attendance, increasing cognitive and academic performance, and contributing to gender equity in access to education [6–9].

Nonetheless, the quality of the school meal is an important driver to achieve the full potential of a school meal program. The urban school meal alone contributed four out of ten food groups, recommended by the Kenyan School Nutrition and Meal Strategy [10] . The non-modified rural meal only contributed two food groups, indicating a lower likelihood for scoring minimum dietary diversity—a proxy for nutrient adequacy of the diet of individuals [11, 12]—than their urban counterparts. Notably, the addition of BFP to a meal would increase food group consumption by one group in both settings. Incorporating BFP or other indigenous fruits and vegetable into existing national school meal programs and nutrition education activities, including school gardens, can further increase the diversification of school meals [13] while following the Kenyan School Nutrition and Meal Strategy [10].

4.3 Contribution of baobab fruit pulp to nutrient intake

Furthermore, the findings from Chapter 3 indicate that BFP significantly contributed vitamin C and calcium to the diet, yet it is critical to take into account the actual amount of BFP consumed in the general population. The potential of FtFF with BFP can easily be overestimated when only looking at the high nutrient composition per 100 g without taking into account the amount of BFP that is likely to be consumed. The pulp itself only contains around 10% water [14], which accounts partly for its high nutrient density. Other local fruits such as passion fruit, oranges, mangoes, and pawpaw consist of 76%, 88%, 83% and 90% water, respectively [15], which results in a lower nutrient density per 100 g. The evaluation of the baobab drink composition between 2017 (containing 30 g BFP) and 2018 (containing 20 g) indicated a higher acceptance of the lower BFP density drink (data not presented).

In the cross-sectional urban/rural comparison, the vitamin C adequacy ratio was only 49% in the urban and 65% in the rural group [3], indicating that BFP can contribute vitamin C to the

diet in order to reach the recommended dietary allowance. However, in the longitudinal *Baobab Nutrition Intervention Study*, the intake of vitamin C was above the nutrient adequacy ratio with 211% and 122% in the intervention and control group, respectively. Here, the dietary intake is presented as the median intake of the first (t1), fifth (t2), and eleventh (t3) week. From t1 to t2, vitamin C intake increased in both groups, yet slightly decreased in eleventh (t3) week [16]. This is in line with improvements in the experienced food security (data not presented) as the intervention started at the end of the rainy season when food insecurity was low with limited access and availability of vitamin C-rich fruits and vegetables. Even though the vitamin C adequacy ratio was met without the additional vitamin C from the BFP, vitamin C from the BFP is attributed to a tendency towards better iron uptake in the intervention group. The facilitating effect of vitamin C is not observed when vitamin C is administered several hours before the consumption of an iron-rich meal [17]. Furthermore, the facilitating effect of vitamin C on iron absorption from a complete diet is far less pronounced than that from single meals [18].

To evaluate the contribution of baobab to the diet, the studies in Chapters 2 and 3 followed the Kenyan RDAs, which differ from the recommended dietary intake of the World Health Organization and Food and Agriculture Organization [19] in terms of recommended nutrient intake and age categories. Furthermore, these recommendations account for bioavailable iron. A Kenyan study—with similar dietary patterns to the *Baobab Nutrition Intervention Study*—estimated a low iron bioavailability in schoolchildren's diets between 4.3% and 7.7% [20]. Under the assumption of low iron bioavailability at 5%, the iron intake of the children from the intervention and control group of the *Baobab Nutrition Intervention Study* is 83% and 72%, respectively. When applying the Kenyan RDAs, the nutrient adequacy ratio of iron may have been overestimated.

Furthermore, the rural school meal was modified to increase iron content to 7.6 mg / portion, as compared to the usual school meal (3.3 mg / portion). This was done by increasing the proportion of beans to maize and increasing the portion size of the school meal, resulting in an adequacy ratio of 165% in the intervention and 157% in the control group. The intake of iron without the modified school meal was lower but still adequate, with an iron nutrient adequacy ratio of 96% [3].

4.4 Limited evidence for an effect of baobab fruit pulp on iron status

The findings in Chapter 3 did not indicate a significantly improved iron status brought about by FtFF with BFP in the study population. The intervention's effect is likely moderated by the

high phytate content of the school meal and by a low prevalence of anemia and iron deficiency at baseline in combination with the small sample size. However, FtFF with BFP remains to be considered for increasing iron bioavailability due to its high content of vitamin C and other promoters and by contributing nutrients to the diet.

The role of phytate, a potent inhibitor of iron absorption [21], is critical for estimating the overall iron bioavailability of complex foods, such as *githeri*, the school meal provided in the *Baobab Nutrition Intervention Study*. Notwithstanding the phytate content, *in vitro* FtFF studies found beneficial effects of BFP on iron bioaccessibility of fermented porridges [22], pearl millet [23], and pearl millet porridge [24], probably because it is rich in citric acid and vitamin C. Grases et al. [25] concluded that the inhibitory effects of phytate on iron absorption are low in well-balanced diets, and little evidence exists from nutritional surveys that dietary phytate affects the iron status in well-nourished populations. In contrast, under non-balanced dietary conditions, phytate may affect the bioavailability and hence the status. In the *Baobab Nutrition Intervention Study*, the dietary intake was high in phytate, but at the same time, the intake of iron and other minerals exceeded dietary recommendations, apart from calcium. Undernourished children (defined by MUAC) were excluded from the study, yet the prevalence of underweight, stunting, and thinness was high [16]. Therefore, the high phytate content of the school meal might likely have reduced the impact of the *Baobab Nutrition Intervention Study*.

A promising approach is the investigation of BFP added onto an iron-rich but phytate-low meal such as milled, fermented, soaked cereals with activation of endogenous phytase [26]. Overall, the effect of polyphenols on iron bioaccessibility and bioavailability remain unclear [27], and investigations into phytochemicals in BFP are still at an early stage. Therefore, the role of phytochemicals from BFP on non-heme iron bioavailability cannot be convincingly clarified.

The effect of vitamin C on Hb levels and iron status has been investigated in several studies. Several isotope studies confirmed the distinct enhancing effects of added vitamin C on the absorption of non-heme iron when foods or drinks rich in or supplemented with vitamin C were tested [28–30]. Furthermore, an intervention trial carried out in India found a significant improvement in Hb levels in young working women who received gooseberry juice (containing 20 mg vitamin C) with their lunch at the workplace for 6 months [31]. Randomized controlled trials investigated the impact of vitamin C on Hb levels and iron status with mixed results. The consumption of a lime-flavored drink (containing 25 mg of vitamin C) twice per day over eight months did not improve the iron status of iron-deficient women in rural Mexico [32]. In contrast, the consumption of a vitamin C rich kiwifruit with an Fe-fortified breakfast cereal

meal for 16 weeks improved irons status in women with low iron stores [33]. Similar to the results of the *Baobab Nutrition Intervention Study*, the consumption of guava juice (containing 200 mg of vitamin C) in addition to the school meal over 10 weeks had a marginal effect on the Hb level and FER status in children consuming high-phytate diets fortified with iron [34]. All three aforementioned studies only enrolled iron-deficient participants (defined as low FER levels) in contrast to the *Baobab Nutrition Intervention Study*, which included children with a Hb $\leq$ 12.2 g/dl. Although the sample size in the *Baobab Nutrition Intervention Study* was higher, the number of iron-deficient participants was lower than in the aforementioned studies. This indicates a smaller intervention effect on Hb levels and iron status in the *Baobab Nutrition Intervention Study* than in the other studies.

Validity of the Baobab Nutrition Intervention Study

One overall strength was the randomized controlled study design and the application of the CONSORT reporting guidelines [35]. Up to a certain degree, the external validity of the study was warranted through the trial's setting [36]. The intervention was performed under usual conditions expect for the modification of the school meal [16], although the school meal ingredients were bought locally and prepared by the school's chef. Furthermore, the baobab fruits came from a neighboring sub-county, and the BFP was processed with traditional tools. Hence, the study was performed under live conditions in a rural setting in a Kenyan school. Compliance was high among participating children and their primary caretakers.

The study did not aim to be representative for schoolchildren in urban or rural Kenya as the researchers opted to include children with low Hb levels and without undernourished children based on MUAC. This was done because the intervention effect was expected to be higher among children with low Hb levels.

The internal validity was ensured through a translation of questionnaires from English into the local languages Kiswahili (Nairobi) and Kikamba (Kitui) and back translation to English by an independent person. Furthermore, intensive enumerator training was conducted, followed by a pretest of the questionnaire and its adaptation afterwards. During the interviews, the interviewers directly entered the answers into electronic devices. The paper-based 24 h-recalls, blood, and anthropometric data were double entered to reduce transcription errors. The researchers monitored the clinical data collection. Only trained and registered health professional performed the clinical assessment [3].

After the first round of the study in 2017, the study design was adjusted according to local conditions and preliminary findings (Table 4, page 12). Unfortunately, the blood samples of FER, sTfR, CRP, and AGP could not be analyzed prior to the second round because local authorities in Kenya did not provide official approval for sample shipment to Germany before the intervention study started in 2018. Accordingly, the researchers could not adjust the study designs that were based on findings of FER and in particular sTfR—the indicator used to determine the sample size. There was no data on sTfR levels of schoolchildren from Kenya. For this reason, data from Cambodia [37] was used to calculate the sample size. Only after analyzing the first round of data did it become evident that the tissue iron stores, determined by sTfR, were higher than assumed.

Due to the adjustments of the study design, it was not possible to merge the data in a meaningful way. However, the results and trends of the individual studies allowed a comparison to further evaluate the external validity and reliability of the study. A comparison of the baseline data from 2017 and 2018 is presented and discussed in Chapter 2.

In both years the results point towards a beneficial effect of BFP on iron absorption as the change in accessed blood iron parameters tended to be better in the intervention than in the control group (Table 5).

Table 5: Change in development between baseline and endline data of hemoglobin level and iron status in 2017 and 2018

Change in %	**2017**		**2018**	
	Intervention	Controls	Intervention	Controls
Mean Hb (g/dl)	-0.4 (26)	-2.7 (23)	2.2 (29)	-1.2 (29)
Geometric mean FER	6.9 (24)	-7.3 (21)	-17.3 (26)	-26.6 (28)
Geometric mean sTfR	1.1 (24)	0.6 (21)	0.7 (29)	2.7 (28)

In both years, many participants were carriers of α-thalassemia traits, which is associated with higher sTfR levels, reflecting lower tissue iron stores than non-carriers. The carriers of sickle cell or α-thalassemia traits were equally distributed among intervention and control groups in both years and sTfR levels.

Table 6: Strengths and limitations of the *Baobab Nutrition Intervention Study* in 2018

	Strengths	**Limitations**
Study design	- Randomized controlled trial - First round allowed adjustments in the study protocol.	Not double blind
Setting	- Selected for low prevalence of confounders that negatively impact Hb levels and iron status (malaria, genotypes of sickle cell trait and α-thalassemia) - Monocentred study	- Purposely selected school
Subjects	- 90% of the approached population participated in the screening. - Apparently healthy participants with low to median Hb levels	- No representative study sample
Inclusion criteria	Homogenous study population: - Screening for low Hb levels - Clinical screening prior to enrollment - Age 6-12 years	
Sample size	- Calculated sample sized based on bio-medical indicator	- Sample size calculation based on secondary data from another context
Intervention	- School meal under real conditions (only modification of the recipe) - School meal ingredients bought via local structures - Standardized preparation of the school meal - Field assistance instructed to serve constant portion sizes; if any, leftovers were recorded - High acceptance/compliance among participants (median participation 82 out of 83 days)	- High inhibitors for iron bioavailability in food that was offered in addition to the intervention and control drinks - Modification of school meal in addition to BFP intervention
Data collection	- Wide range of assessments - Estimation of nutritional intake - High acceptance among primary caretakers for interviews (100%)	- No data collection on folic acid and B12 status - No data collection on parasitic infection
Outcome	- The outcomes of the first and second round of the intervention point in the same direction.	- The outcome variable used to determine the sample size nearly did not change. - Age data is missing for some participants.
Analysis		- Data on the main outcome variable from the first round was analyzed only after the intervention in 2018 had started
Ethical considerations	- Results of the screening were provided to primary caretakers. - If children were undernourished, they were referred to the health facility in the village for nutrition counseling. - Results of the intervention trial were presented one year after the study was completed. - Anonymity of the collected data was assured.	

Nonetheless, the difference in the change in all three parameters between intervention and control group was not significant. A remarkable distinction between 2017 and 2018 is the development of adjusted FER. While the development of the geometric mean improved in 2017 in the intervention group and worsened in the control group, the geometric mean significantly worsened in both groups in 2018. However, the prevalence of iron deficiency remained almost unchanged in both years and did not reflect a significant decrease of FER levels in 2018. The influence of inflammation on elevated FER was limited as the prevalence of inflammation was low in both years. An overview of strengths and limitation of the study is presented in Table 6 (page 56).

Conclusion

In the rural Kenyan diet, the bioavailability of iron is low and predisposes the population to iron deficiency—the major dietary deficiency in Kenyan children. Therefore, strategies for improving children's nutrition and iron status need to be developed. The *Baobab Nutrition Intervention Study* evaluated the impact of baobab fruit pulp (BFP) consumption on the hemoglobin (Hb) levels and iron status of Kenyan schoolchildren. BFP was chosen as the indigenous baobab tree contains significant amounts of vitamin C, which enhances non-heme iron bioavailability. In the randomized controlled intervention trial, apparently healthy schoolchildren aged 6-12 received either a drink with BFP (intervention group) or a drink without BFP (control group).

The *Baobab Nutrition Intervention Study* was conducted in urban and rural school settings. A comparison between the resource-poor settings concluded that iron bioavailability was of greater concern in the rural setting, where the intake of non-heme iron was higher, yet iron status was lower than in urban children. The better iron status among urban children implies access to food with higher bioavailability of iron. Hence, the benefits of addressing iron bioavailability were higher in the rural than in the urban setting.

The implementation of the *Baobab Nutrition Intervention Study* in the rural setting showed slightly positive—yet not significant—benefits with regard to selected Hb levels. The whole study population experienced a general tendency towards worsening iron stores. However, the tendencies in change are concordant and point towards a beneficial effect of BFP on non-heme iron absorption. The intervention effect was moderated by the high phytate content of the school meal and the low prevalence of anemia and iron deficiency at baseline in combination with the small sample size.

In conclusion, the results indicated that food-to-food fortification (FtFF) with the vitamin C-rich BFP, in addition to non-heme iron-rich food, is an approach to be considered to address anemia and iron deficiency in food-insecure settings with access to baobab products. Integration of BFP into homegrown school meal programs would provide an opportunity to do just that.

Recommendations for subsequent research projects

The paucity of data on the efficacy of increasing dietary vitamin C intake to improve Hb levels and iron status underlines the need for better controlled field trials. Careful consideration should be given to the phytate content of non-heme iron-rich food or drinks that are provided in addition to BFP. Furthermore, enrollment of anemic and/or iron-deficient study populations is recommended.

The beneficial effect of vitamin C (and other organic acids) from BFP on iron absorption might not have overcome the inhibitory effects of the phytate-rich school meal to the expected extent. Applying culturally accepted processing techniques to reduce phytate and other inhibitors of iron absorption could further strengthen the effect of BFP on non-heme iron bioavailability.

Investigations of phytochemicals in BFP are still at an early stage, and future findings could provide conclusions on the overall role of BFP in iron bioavailability, apart from only looking at vitamin C and other organic acids. Moreover, the investigation of phytochemicals may help estimate the potential health benefits of BFP consumption per se.

It is also recommended to carry out further scientific research into the potential health risks of consuming baobab products, in particular to define maximum intake levels. Furthermore, a quantification of the contribution of BFP and products to the supply of minerals and trace elements has not yet been investigated, but it would help estimate the role of baobab in food security.

Recommendations for public health stakeholders

It is recommended to raise further awareness of the outstanding nutritional properties of BFP through nutrition education interventions. These interventions may integrate BFP into local dishes with good amounts of non-heme iron, optimally into those that are low in phytate. Notably, the development of recipes and BFP products must account for fresh BFP (short duration between cracking the baobab fruits and their consumption or processing) and should exclude heat processing to maintain the vitamin C or other heat-instable components.

References

1. Hunt JR (2005) Dietary and physiological factors that affect the absorption and bioavailability of iron. Int J Vitam Nutr Res 75:375–384. https://doi.org/10.1024/0300-9831.75.6.375
2. Andang'o PEA, Osendarp SJM, Ayah R et al. (2007) Efficacy of iron-fortified whole maize flour on iron status of schoolchildren in Kenya: A randomised controlled trial. The Lancet 369:1799–1806. https://doi.org/10.1016/S0140-6736(07)60817-4
3. Evang EC, Habte T-Y, Owino WO et al. (2020) The Nutritional and Micronutrient Status of Urban Schoolchildren with Moderate Anemia is Better than in a Rural Area in Kenya. Nutrients 12. https://doi.org/10.3390/nu12010207
4. Kenya National Bureau of Statistics (KNBS) and ICF Macro (2015) Kenya Demographic and Health Survey 2014. https://dhsprogram.com/pubs/pdf/fr308/fr308.pdf. Accessed 18 Oct 2019
5. Bundy DAP, Silva N de, Horton S et al. (2017) Child and Adolescent Health and Development: Child and Adolescent Health and Development: Realizing Neglected Potential, 3rd, Washington (DC)
6. Adelman S, Gilligan D (2008) How effective are food for education programs?: A critical assessment of the evidence from developing countries. International Food Policy Research Institute, Washington, DC
7. Bundy DAP (2009) Rethinking School Feeding: Social Safety Nets, Child Development, and the Education Sector (Directions in development. Human development). The World Bank Group
8. Aliyar R, Gelli A, Hamdani SH (2015) A Review of Nutritional Guidelines and Menu Compositions for School Feeding Programs in 12 Countries. Front Public Health 3:148. https://doi.org/10.3389/fpubh.2015.00148
9. Drake L, Fernandes M, Aurino E et al. (2017) School Feeding Programs in Middle Childhood and Adolescence. In: Bundy DAP, Silva N de, Horton S et al. (eds) Child and Adolescent Health and Development: Child and Adolescent Health and Development: Realizing Neglected Potential, 3rd, Washington (DC)
10. Ministry of Education, Science and Technology, Ministry of Health, and Ministry of Agriculture, Livestock and Fisheries (2016) School Nutrition and Meals Strategy for Kenya. https://extranet.who.int/nutrition/gina/sites/default/files/KEN_school%20nutrition%20and%20meals%20strategy%20for%20Kenya.pdf. Accessed 25 Nov 2018
11. Kennedy G, Ballard T, Dop MC (2011) Guidelines for Measuring Household and Individual Dietary Diversity
12. Martin-Prevel Y, Arimond M, Allemand P et al. (2017) Development of a Dichotomous Indicator for Population-Level Assessment of Dietary Diversity in Women of Reproductive Age. Current Developments in Nutrition 1. https://doi.org/10.3945/cdn.117.001701
13. Hunter D, Borelli T, Beltrame DMO et al. (2019) The potential of neglected and underutilized species for improving diets and nutrition. Planta 250:709–729. https://doi.org/10.1007/s00425-019-03169-4
14. Stadlmayr B, Wanangwe J, Waruhiu CG et al. (2020) Nutritional composition of baobab (Adansonia digitata L.) fruit pulp sampled at different geographical locations in Kenya. Journal of Food Composition and Analysis 94:103617. https://doi.org/10.1016/j.jfca.2020.103617
15. FAO/Government of Kenya (2018) Kenya Food Composition Tables. http://www.kilimo.go.ke/wp-content/uploads/2018/10/KENYA-FOOD-COMPOSITION-TABLES-2018.pdf

16. Evang EC, Habte T-Y, Owino WO et al. (2020) Can the supplementary consumption of baobab (Adansonia digitata L.) fruit pulp improve the hemoglobin levels and iron status of schoolchildren in Kenya? Findings of a randomized controlled intervention trial. Eur J Nutr. https://doi.org/10.1007/s00394-020-02447-2
17. Cook JD, Monsen ER (1977) Vitamin C, the common cold, and iron absorption. Am J Clin Nutr 30:235–241
18. Cook JD, Reddy MB (2001) Effect of ascorbic acid intake on nonheme-iron absorption from a complete diet. Am J Clin Nutr 73:93–98. https://doi.org/10.1093/ajcn/73.1.93
19. WHO/FA0 (2004) Vitamin and mineral requirements in human nutrition: Second Edition, Geneva
20. Shell-Duncan B, McDade T (2005) Cultural and environmental barriers to adequate iron intake among northern Kenyan schoolchildren. Food Nutr Bull 26:39–48
21. Gibson RS (2007) The role of diet- and host-related factors in nutrient bioavailability and thus in nutrient-based dietary requirement estimates. Food Nutr Bull 28:S77-100. https://doi.org/10.1177/15648265070281S108
22. Gabaza M, Shumoy H, Muchuweti M et al. (2018) Baobab fruit pulp and mopane worm as potential functional ingredients to improve the iron and zinc content and bioaccessibility of fermented cereals. Innovative Food Science & Emerging Technologies 47:390–398. https://doi.org/10.1016/j.ifset.2018.04.005
23. van der Merwe R, Kruger J, Ferruzzi MG et al. (2019) Improving iron and zinc bioaccessibility through food-to-food fortification of pearl millet with tropical plant foodstuffs (moringa leaf powder, roselle calyces and baobab fruit pulp). J Food Sci Technol 56:2244–2256. https://doi.org/10.1007/s13197-019-03711-y
24. Adetola OY, Kruger J, White Z et al. (2019) Comparison between food-to-food fortification of pearl millet porridge with moringa leaves and baobab fruit and with adding ascorbic and citric acid on iron, zinc and other mineral bioaccessibility. LWT - Food Science and Technology 106:92–97. https://doi.org/10.1016/j.lwt.2019.02.044
25. Grases F, Prieto RM, Costa-Bauza A (2017) Dietary Phytate and Interactions with Mineral Nutrients. In: Gutiérrez, OM Kalantar-Zadeh, K, Mehrotra R (eds) Clinical Aspects of Natural and Added Phosphorus in Foods, Nutrition and Health, pp 175–183
26. Ruel MT (2001) Can Food-Based Strategies Help to Reduce Vitamin A and Iron Deficiencies ?: A review of Recen evidence, Washington, DC
27. Ferruzzi MG, Kruger J, Mohamedshah Z et al. (2020) Insights from in vitro exploration of factors influencing iron, zinc and provitamin A carotenoid bioaccessibility and intestinal absorption from cereals. Journal of Cereal Science 96:103126. https://doi.org/10.1016/j.jcs.2020.103126
28. Davidsson L, Walczyk T, Zavaleta N et al. (2001) Improving iron absorption from a Peruvian school breakfast meal by adding ascorbic acid or Na2EDTA. Am J Clin Nutr 73:283–287. https://doi.org/10.1093/ajcn/73.2.283
29. Balay KS, Hawthorne KM, Hicks PD et al. (2010) Orange but not apple juice enhances ferrous fumarate absorption in small children. J Pediatr Gastroenterol Nutr 50:545–550. https://doi.org/10.1097/MPG.0b013e3181b1848f
30. Reddy MB, Hurrell RF, Cook JD (2000) Estimation of nonheme-iron bioavailability from meal composition. Am J Clin Nutr 71:937–943. https://doi.org/10.1093/ajcn/71.4.937
31. Gopaldas T (2002) Iron-deficiency anemia in young working women can be reduced by increasing the consumption of cereal-based fermented foods or gooseberry juice at the workplace. Food Nutr Bull 23:94–105. https://doi.org/10.1177/156482650202300113
32. Garcia OP, Diaz M, Rosado JL et al. (2003) Ascorbic acid from lime juice does not improve the iron status of iron-deficient women in rural Mexico. Am J Clin Nutr 78:267–273. https://doi.org/10.1093/ajcn/78.2.267

33. Beck K, Conlon CA, Kruger R et al. (2011) Gold kiwifruit consumed with an iron-fortified breakfast cereal meal improves iron status in women with low iron stores: a 16-week randomised controlled trial. Br J Nutr 105:101–109. https://doi.org/10.1017/S0007114510003144
34. Monárrez-Espino J, López-Alarcón M, Greiner T (2011) Randomized placebo-controlled trial of guava juice as a source of ascorbic acid to reduce iron deficiency in Tarahumara indigenous schoolchildren of northern Mexico. J Am Coll Nutr 30:191–200. https://doi.org/10.1080/07315724.2011.10719960
35. Schulz KF, Altman DG, Moher D (2010) CONSORT 2010 statement: updated guidelines for reporting parallel group randomised trials. PLoS Med 7:e1000251. https://doi.org/10.1371/journal.pmed.1000251
36. Rothwell PM (2006) Factors that can affect the external validity of randomised controlled trials. PLoS Clin Trials 1:e9. https://doi.org/10.1371/journal.pctr.0010009
37. Perignon M, Fiorentino M, Kuong K et al. (2014) Stunting, poor iron status and parasite infection are significant risk factors for lower cognitive performance in Cambodian school-aged children. PLoS One 9:e112605. https://doi.org/10.1371/journal.pone.0112605

Summary

In the Kenyan diet, bioavailability of iron is low and predisposes the population to iron deficiency. Prevalence of anemia and iron deficiency is high, particularly in food-insecure settings. Fruit pulp of the indigenous baobab is rich in minerals and trace elements and contains significant amounts of vitamin C, which enhances non-heme iron bioavailability.

The objectives of this dissertation were 1) to compare malnutrition and micronutrient deficiencies linked to dietary intake among urban and rural Kenyan schoolchildren to identify the population that benefits most from a nutrition intervention with baobab fruit pulp (BFP) and 2) to determine the impact of BFP consumption on the hemoglobin (Hb) levels and iron status of schoolchildren.

Among school children aged 6-12 years in an urban resource-poor setting in Nairobi (in 2017) and in a rural setting in Kiuti (in 2018), apparently healthy children were screened to exclude undernourished children (according to mid-upper arm circumference) and include children with lowest Hb levels. The single-blind randomized controlled *Baobab Nutrition Intervention Study* was implemented over a period of 12 weeks in the rural setting. Children in the intervention group received a drink with BFP, while the control group received an isoenergy drink without BFP. Both groups consumed the drinks in addition to a modified school meal (local dish, mix of maize and beans).

At baseline and endline, registered nurses took blood samples to determine Hb, ferritin (FER), soluble transferrin receptor (sTfR), zinc, C-reactive protein (CRP), and acidic glycoprotein (AGP). Furthermore, the children's weight and height were assessed. Hb disorders (sickle cell and α-thalassemia trait) were assessed only at baseline. During the 12-week intervention, dietary intake was accessed with 24 h –recalls in the first (t1), fifth (t2), and eleventh (t3) week. Nutrient intake was estimated with the software NutriSurvey, and nutrient adequacy ratio was determined by applying the Kenyan recommended dietary allowance.

For urban/rural comparison, baseline data of a subgroup of children aged 7-9 years was used. Among the 36 urban and 35 rural children, the prevalence of moderate underweight, wasting, and stunting were lower in urban than in rural children, with significant differences in median z-scores for underweight ($p < 0.001$) and wasting ($p = 0.001$). Significantly higher values for serum ferritin ($p = 0.012$) and zinc ($p < 0.001$) were found in urban children. Nonetheless, the median nutrient adequacy ratios were higher for vitamin C ($p = 0.045$), iron ($p = 0.003$), and zinc ($p = 0.003$) in rural than in urban children. In conclusion, the low intake of nutrients in the urban setting was of greater concern than nutrient bioavailability, and vice versa in the rural

setting. Hence, the benefits of addressing iron bioavailability were higher in the rural than in the urban setting.

In the *Baobab Nutrition Intervention Study*, 223 rural children participated in the screening, among them 66 were allocated in either intervention or control group and data of 29 children in each group were used for analyses. During the intervention, the intake of vitamin C and calcium were significantly higher in the intervention than in the control group. The development of hemoglobin, FER, and sTfR did not differ significantly between the intervention and control groups. However, in the intervention group, Hb levels improved slightly (2.2%), while they decreased slightly (1.2%) in the control group. Levels of the geometric mean of sTfR remained almost unchanged (0.7%) in the intervention group, and slightly worsened (2.7%) in the control group. In both groups, geometric mean of FER levels decreased, yet to a smaller extent in the intervention (17.3%) than in the control (26.0%) group. Limiting factors on the promoting effect on iron bioavailability might be the inhibitory effect of phytate and polyphenols from the school meal and phytochemicals from BFP itself that did not overcome the promoting effect of BFP.

Even though no significant effects of BFP on Hb levels or iron status could be detected in this study, the tendencies of changes are concordant and point towards a beneficial effect of BFP on non-heme iron absorption. The identification of products such as BFP remains pertinent to help improve non-heme iron absorption in the most vulnerable populations, and particularly in food-insecure areas where the indigenous baobab tree and its nutritious fruits are available and affordable. Therefore, integrating BFP into existing national homegrown school meal programs and nutrition education activities are reasonable approaches to prevent childhood anemia and to create awareness for the nutritional benefits of indigenous fruits.

Declaration

„Ich erkläre: Ich habe die vorgelegte Dissertation selbständig und ohne unerlaubte fremde Hilfe und nur mit den Hilfen angefertigt, die ich in der Dissertation angegeben habe. Alle Textstellen, die wörtlich oder sinngemäß aus veröffentlichten Schriften entnommen sind, und alle Angaben, die auf mündlichen Auskünften beruhen, sind als solche kenntlich gemacht.

Bei den von mir durchgeführten und in der Dissertation erwähnten Untersuchungen habe ich die Grundsätze guter wissenschaftlicher Praxis, wie sie in der „Satzung der Justus-Liebig-Universität Gießen zur Sicherung guter wissenschaftlicher Praxis“ niedergelegt sind, eingehalten.“