January 2015

Protein Intake And Hookworm Infection Susceptibility In Rural Ghanaian School- Age Children: A Mixed-Methods Study

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Background: Because human and animal cross-sectional studies cannot assess temporality, a longitudinal study is needed to assess the association between protein intake and hookworm infection susceptibility. Cultural preferences and belief systems have been noted to affect the intake of food in some African societies but extensive research has not been conducted to characterize them.

Methods: Children (n=274) 7-13 years of age who attended school in the Kintampo North Municipality of Ghana were screened for participation in a longitudinal study assessing the relationship between protein intake and hookworm infection susceptibility. Protein intake was calculated by 24-hour recalls. A qualitative sub-study recruited mothers (n=32), whose children were part of the longitudinal study, to participate in 45-60 minute in-depth semi-structured interviews and assess their beliefs, attitudes, and behaviors around child nutrition, particularly protein-rich foods. Three focus groups were also conducted (two with mothers and one with children).

Principal Findings: After adjusting for covariates, no significant association was found between weight-adjusted protein intake in January 2013 and hookworm infection status in June 2013 (OR: 0.93, 95% CI 0.78, 1.12; $P = 0.455$). The sub-study showed that staple foods were the most important and nutritious foods to give to children in this community. Contributions of food from friends and family were also noted.

Conclusion: Higher protein intake levels were not observed to protect against future hookworm infection in children with adequate protein intake, although more research is warranted. The qualitative findings were consistent with previous qualitative work in other African communities, but future research is needed to determine similarities between belief systems and behaviors in

other African populations, especially those burdening with protein deficiency, and identify sociocultural mechanisms that explain protein intake in school-age children.

Acknowledgements

I am deeply grateful for the invaluable guidance from both my thesis advisor Dr. Debbie Humphries and close colleague Sena Seddoh. They have been nothing short of supportive and extremely patient in every step of the research process. Additionally, I would like to thank the Downs International Health Student Travel Fellowship for funding this study and providing me with the opportunity to conduct an independent research project abroad and immerse myself in a new culture.

I would also like to extend my appreciation to all the team members involved with collecting these data, including Foster Gyan, Sena Seddoh, and Ramseyer Asuah. Their strong work ethic ensured proper data collection and quality. More importantly, their kindness and openness allowed for effective communication, speedy completion, and enjoyment of the work.

The Noguchi Memorial Institute for Medical Research, the Kintampo Municipal Hospital, and the Kintampo Health Research Center considerably contributed to the success of this study. Joseph Otchere and Sena Seddoh should be recognized for their exceptional leadership in the field, as well as the many research personnel involved in the longitudinal project. I also want to give special recognition to my study participants, since this study could not have been made possible without their contributions and experiences.

Finally, I am unconditionally grateful for the constant support, love, and understanding my family and friends have shown me. I would not be at the Yale School of Public Health without the sacrifices my family had to make to ensure a better life for their children in the United States. Their sacrifices have become the flames that fuel my passion for health disparities and global nutrition-related research.

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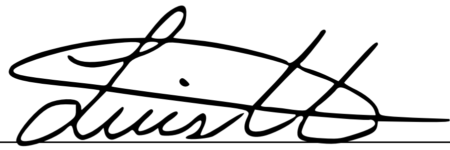

Luis E. Maldonado

April 30, 2015

Date

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Introduction

Worldwide, 576 million people and 156 million children are estimated to be infected with hookworm,[1] most of which are caused by the species *Ancylostoma duodenale, A. ceylanicum,* and *Necator americanus.*[2] Sub-Saharan Africa has particularly high rates of hookworm infection.[1] Other infectious diseases and malnutrition are also prevalent in the same area, thereby compounding negative clinical effects.[3] School-age children often endure high-intensity infections that increase anemia risk and cause detrimental effects on iron status, growth, and cognition.[4-6]

Because malnutrition has been noted to increase rates of morbidity and mortality from infectious diseases,[7] compounding malnutrition with hookworm may further impede the growth and development of children and place them at higher risk of other infections, which may further affect development.[8] Lower dietary intake leads to increased parasitic infection, which, in turn, leads to increased loss of nutrients and susceptibility to hookworm infection and re-infection.[9]

Although different factors may contribute to hookworm susceptibility and re-infection, poor nutritional status may influence risk of infection. In animal models, poor nutritional status has been found to increase susceptibility to helminth infections.[10] In humans, cross-sectional studies have demonstrated an association between poor nutritional status and intestinal helminth infections, although it is not clear which occurs first.[11,12] There is strong evidence showing that treatment for intestinal helminths can improve nutritional status among children, particularly in areas with high prevalence and intensity of infection,[8,13] supporting the notion of the pathway from soil transmitted helminths to malnutrition.

There is evidence to show that clinical malnutrition and macronutrient deficiencies such as protein malnutrition influence immunity to parasitic infection. Mice on protein-restricted diets have been observed to have higher fecal egg counts and worm burdens (*H. polygyrus*) compared to those fed adequate protein.[14,15] In another study, nematode-infected pregnant mice with protein-deficient intake were also found to have significantly lowered fetal length and kidney mass compared to those with sufficient protein intake.[16] A study focusing on ewes also found that ewes supplemented with protein had significant reduction in fecal egg counts compared to those that were not.[17] Protein deficiency has been noted to decrease the adaptive response, particularly the proliferation of T cells[18] and antibodies,[19] thereby increasing the host's susceptibility to infection. A diet with insufficient amount of macronutrients, such as protein, may limit the individual's abilities to combat parasitic infections.

In school-age children, a cross-sectional study also found a significant inverse correlation between intake of protein-rich food groups and hookworm infection status in areas with prevalent hookworm infections,[20] suggesting that protein-rich foods may offer some protection against infection. Although numerous cross-sectional studies have confirmed an association between poor nutritional status and intestinal helminth infections,[21,22] there has not been a longitudinal study assessing the association between protein intake and hookworm infection susceptibility. The present study, therefore, tests the hypothesis that higher levels of protein intake will reduce susceptibility to hookworm infection in rural Ghanaian school-age children.

Maternal Perceptions and Behaviors around Dietary Quality in Children

Designing effective public health interventions to address dietary risks that influence hookworm infections will require a solid understanding of different determinants of the observed dietary patterns, including attitudes, beliefs, and behaviors around protein regional availability, sources, and cultural dietary preferences.

While 75% of the protein in the Ghanaian diet comes from plant sources, fish is the predominant source of animal-based protein.[23] According to a technical report by the Programme for Integrated Development of Artisanal Fisheries in West Africa, much of the fish sold in North Kintampo District comes from the extending rivers of Lake Volta, but effective distribution of fish and fish products may be hindered by poor infrastructure.[24] While animal source foods (ASF) are important sources of dietary protein and micronutrients,[25] data on availability to fish and other protein-rich foods is limited in the Kintampo population.

Cultural preferences and belief systems have been noted to affect the intake of food in some West African societies and how they understand nutrition. A qualitative study conducted in rural Gambia using eight focus groups with 63 women found that women reported men as receiving the largest, best, and first share of the meals compared to children because they were the heads of their households. [26] Another qualitative study in Kenya using ten focus groups comprising between eight and ten mothers each found that although women had a good basic knowledge of foods and what is needed for a nutritionally balanced diet or "good diet" for children, ASF were not mentioned or emphasized as much.[7] A quantitative study conducted in Ghana involving 55 malnourished and 55 well-nourished children (age 10-36 months) and their mothers found a significant association between knowing the causes of kwashiorkor and good nutritional status.[27] Other studies have found that in some cultures, mothers associate malnutrition with an animistic or evil spirit, not lack of food.[7, 26-28] Because these studies involved African and Ghanaian populations, cultural preferences and belief systems related to nutrition found in these studies may be similar to those of nearby populations such as those living in the Kintampo communities and may be important in understanding the protein intake sources and eating behaviors of school-age children in the Kintampo region.

Availability of protein-rich foods may facilitate protein intake patterns and malnutrition among Ghanaian children. The North District Kintampo area is primarily low income, with most producing their own food products; about 71% of the households in the district are engaged in agriculture as their main economic activity.[29] In most African communities, people rely on one or two staple crops.[30] Staple foods are complemented with legumes or ASF rich in protein and fats/oils for a balanced diet.[30] These staple foods (e.g., cassava and yam), however, contain limited protein[30,31]; cassava contains 1.16 grams of protein, while yam contains 1.5 grams.[32] Availability of protein sources to human populations in Africa has not been greatly studied,[33] and mechanisms such as availability to protein-rich foods may play a role in the dietary intake patterns of Ghanaian school-age children.

Individual access and behaviors such as those of the mother have a strong influence on a child's nutritional status in Africa.[7,30] Several studies have demonstrated that in the midst of poverty and childhood malnutrition, some mothers successfully raise well-nourished children.[27,34,35] These mothers may be practicing behaviors not widely used in their communities (also known as positive deviance),[27] thereby leading to better nourished children. Lapping et al. implemented a positive deviance inquiry study in which observations and interviews with Afghan refugee family members identified several feeding, caring, and health-seeking behaviors associated with good nutrition in children under 5 years of age that were not widely practiced, such as intention to breastfeed for 2 years, increasing breastfeeding during diarrhea, and fed more during and after illness.[36] These findings suggest that better understanding of maternal perspectives is important to characterize the key influences on intake of protein-rich foods, especially since mothers are the primary care providers for children in Ghana.[24]

Characterizing maternal attitudes, beliefs, and behaviors provides insight to a mother's role in influencing nutritional status and protein intake among their children. There is growing evidence that the attitudes, beliefs, and behaviors related to childcare and the way limited resources are utilized, as much as economic status, distinguish between children who become malnourished and those who do not.[24,27] Additionally, underlying influences on nutritional status have not yet been clearly characterized, especially in the Kintampo region. Therefore, the qualitative portion of the present study explores and characterizes influential sociocultural mechanisms of protein intake among school-age children in rural Ghana.

Methods

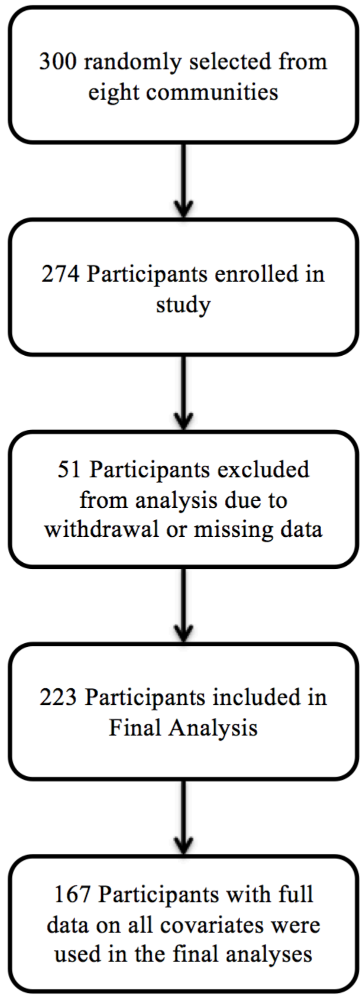
Setting

In January 2013, a two-year longitudinal epidemiological field study began among 7-13 year old school children in Kintampo North Municipality of the Brong Ahafo Region of Ghana. The Republic of Ghana has endemic infections of the two major human hookworm species, *Ancylostoma duodenale* and *Necator americanus*.[37,38] The study is investigating response to albendazole treatment and susceptibility to hookworm infection and re-infection.

Subjects

Inclusion criteria required study participants to be enrolled in a Kintampo North Municipality school, between 7-13 years of age, and residing within the Kintampo North District. School-age children (n=300) were randomly selected from eight communities previously identified as having high hookworm infection prevalence (with an extra 10% included at the initial selection to account for nonparticipation). A sample size of 240 children was calculated to provide 80% power to detect a difference in re-infection of at least 40% in children with low dietary protein, as compared with a 25% re-infection rate in children with high dietary protein based on published data.[39] A census, previously conducted by the Noguchi Memorial Institute for Medical Research, reporting the ages of children in the eight communities was used to randomly select participants between the ages of 7 and 13 from each community. The study enrolled 274 school age children, ages 7-13, in January 2013. Due to withdrawing and missing data, the final study sample reduced to 223 participants (Figure 1).

Figure 1. Flowchart of Longitudinal Study Participants



Dietary Protein Assessment

Dietary protein and energy intake were measured by 24-hour recalls (n=250).[40] Recalls were done together with both mother and child at baseline.[41] For a subset of the sample (n=88), a second 24-hour dietary recall was performed for each child. The two days for the duplicate recalls were randomly selected, with one weekend day and one week day, spaced two to three weeks apart. Although a single 24-hour recall is not sufficient to describe an individual's day-to-day variations of dietary intake of food, using two 24-hour recalls on two non-consecutive days over a period of four weeks enhances capturing usual intake.[40] No significant difference was observed with regards to within-subject variability between the recalls after performing a repeated-measures test (data not shown). Therefore, usual protein intake was calculated by averaging the intake of the two duplicate recalls. Confidence intervals of the weight-adjusted protein intake mean from all sources were adjusted for the coefficient of variation (38%) found between duplicate recalls.

A Uganda food composition table (FCT)[32] in combination with a Ghana FCT[42] were used to identify protein composition of foods eaten and to calculate protein intake. Protein intakes for ASF-only and plant-based only foods were also calculated to observe the separate effects of protein by source on hookworm infection. Protein adequacy was determined by whether a child met the World Health Organization (WHO) per kg body weight protein requirements for children in their age group.[43]

Hookworm Infection Assessment

In January 2013 and June 2013, stool cups were distributed to the children and/or their guardians. Students who agreed to participate were asked to provide a fecal sample. Samples were collected each morning from participants, placed in a cooler for preservation, and driven to a laboratory for processing and analysis. Microscopy and the Kato-Katz method were used to identify parasite eggs, as outlined by the WHO.[44] Children diagnosed with hookworm were referred to local health staff for oral treatment (albendazole 400 mg). Hookworm infection susceptibility was measured by infection status (yes/no) in June 2013.

Household Questionnaire

The survey was adapted from the Demographic and Health Surveys, the Household Dietary Diversity Score for Measurement of Household Food Access: Indicator Guide, and the Household Food Insecurity Access Scale (HFIAS) for Measurement of Food Access: Indicator Guide.[45] All survey materials were translated into the local language (Twi) and back-translated into English by native speakers to ensure accuracy. Questions included birth date, household socioeconomic characteristics, water and sanitation access, access to health care and vaccinations, bed net use, food insecurity, and dietary diversity. Trained local interviewers administered the surveys in the local language. Household assets were used to construct an absolute wealth index[46] based on the number of assets, including a tile floor, use of advanced cooking fuel (not charcoal, straw or wood), electricity, radio, TV, phone, refrigerator, bike, car or motorcycle, land, cow, horse or donkey, goat or sheep, pig, poultry, bank or savings account, improved water source, improved toilet). The wealth index was used as a continuous variable.

Determination of Anthropometric Status

Participant birth dates were confirmed with a household member as part of the household survey, and a local calendar was used to identify season and year, when necessary. Height was measured using a stadiometer, and weight was defined by taking the average of two digital scale measurements for each child. Body-Mass-Index-for-Age (BAZ) was used because it is most closely connected to susceptibility to infections[47] and was included in the analysis as a continuous variable. Height-for-Age (HAZ) was also used as a continuous variable.

Dietary diversity and household food insecurity

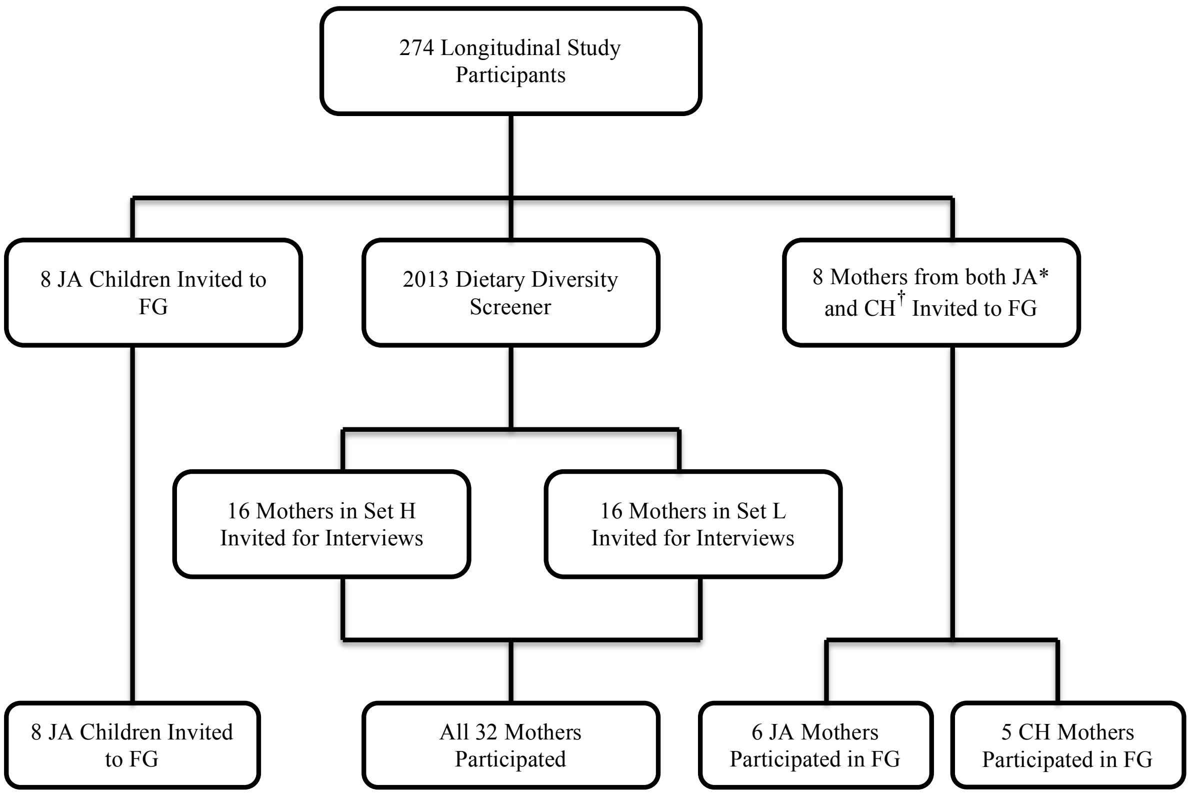
Children and caregivers were asked about consumption of foods the previous day and the previous week. Eleven nutrient-rich food groups (excluding condiments and sugar) were transformed into a binary dietary diversity variable, with participants either above or below the group mean. Weekly dietary diversity scores were used to better capture usual intake. The Household Food Insecurity Access Scale was categorized according to standard methods by the Fanta Project's Food and Nutrition Technical Assistance.[48]

Qualitative Interviews

The mothers of participants enrolled in the longitudinal study were recruited. Respondents were selected to achieve variation in the variables of interest, namely dietary protein. Recent studies have found measures of dietary diversity to be valid indicators of dietary nutrient quality.[49-51] As such, a separate co-investigator used the dietary diversity screener results from January 2013 to identify two households tha reported above-average consumption of protein-rich food groups (Set H) and two households that reported below-average consumption of protein-rich food groups (Set L) in each of the eight communities, for a total of 32 households. Above- and below-average protein intake status was calculated by determining whether a child was above or below a calculated mean using the sum of the number of protein-rich food groups consumed by each child, as indicated by a validated dietary diversity scale. The interviewers were blinded to the protein intake status of the children whose mothers were invited to interview. An online randomizer (randomizer.org) was used to create sequences and recruit participants in a systematic manner.

Mothers from the longitudinal study who were not included in the in-depth interviews were invited to participate in one of two focus groups, while children participating in the longitudinal study whose mothers were not interviewed were also invited to participate in a third focus group. One focus group was conducted in Jato-Akuraa (n=6) while the second focus group was held in Cheranda (n=5). The third focus group only consisted of school-age children (n=8) and was conducted in Jato-Akuraa (Figure 2).

Figure 2. Flowchart of Qualitative Study Participants



*JA: Jato-Akurra
†CH: Cheranda

Households were visited and the mother or caregiver whose child completed the selected dietary diversity screeners was invited to participate in an in-depth interview. All recruitment, consent, and interviews took place in the local language (Twi), through the help of an interpreter. Interviews and focus groups were audiotaped, and field notes were kept after each interview. Thirty-two 45-60 minute in-depth semi-structured interviews and three focus groups were conducted. The focus groups took place in Jato-Akuraa and Cheranda, two neighboring communities in the southern part of the study area that have previously demonstrated different patterns of health and hygiene behaviors and different responses to anthelmintic treatment.

Mothers were asked about child eating practices, nutrition, hookworm infection, protein intake, access, and sources, and household involvement and nutrition knowledge. An abridged version of the individual semi-structure interview questionnaire was used for the focus groups involving mothers. The children in the third focus group were asked questions about eating practices at home, school, and elsewhere, foods that they often consume, where and with whom they eat, food preferences, and foods regularly consumed by their friends and family.

Interview and focus group guides were reviewed by local partners and community representatives to assure clarity and cultural appropriateness of the language and the questions. Questions were translated into Twi by an independent community representative and translated back into English by another. Questions were also piloted with three mothers to ensure accurate interpretation of the questions and address any confusion.

Ethical Approval

These studies were approved by the Yale University Human Investigations Committee and the Institutional Review Boards at the Noguchi Memorial Institute for Medical Research, the Ghana Health Service, and the Scientific Review Committee and the Institutional Ethics Committee at the Kintampo Health Research Center.

Statistical Analysis

All data generated from this study were stored in Microsoft excel (2007) and Microsoft word (2007) (Redmond, WA). Descriptive statistics were performed to characterize the sample. A repeated-measures test was performed to detect significant differences between repeated recall means. Bivariate and adjusted analyses were also performed to determine associations between sample characteristics and hookworm infection. Initial variables tested were derived from a theoretical framework of factors supported by the literature to influence hookworm infection. Important variables from the bivariate analysis were used in a logistic regression analysis by applying logistic regression analyses within generalized estimating equation algorithms. Final models included control variables and bivariate variables that demonstrated a p-value equal to or less than 0.10 with the hookworm infection in June 2013. Data were analyzed using SPSS software (version 22) and SAS version 9.3. Participants with complete data for all covariates (n=167) were used in the final analysis.

For the qualitative interviews, a coding structure was developed using grounded theory analysis, and the constant comparison method was used to fully capture themes.[52] All codes and themes were generated from the translated transcripts. To compare interpretations and reduce bias, coders met after several transcripts, agreed upon thematic categories, and addressed coding concerns. Three independent co-investigators reviewed codes and transcripts for accuracy and agreement of interpretations. Once a coding structure was developed, ATLAS.ti version 1.0.16 (82) was used to analyze all interview and focus group transcripts.

Results

Quantitative Results

The distribution of weight-adjusted protein intake in the sample is skewed (Figure 3). The graph suggests that children are receiving their recommended protein requirements. When weight-adjusted protein is separated by source, the children in this sample received most of their protein from ASF (mean = 2.24) than from plant-only sources (mean = 1.22) (Figure 4). Moreover, when weight-adjusted protein is separated by age and gender, girls received more protein as compared to boys (5.4 ± 4.2 and 3.3 ± 1.6) (Figures 5 and 6).

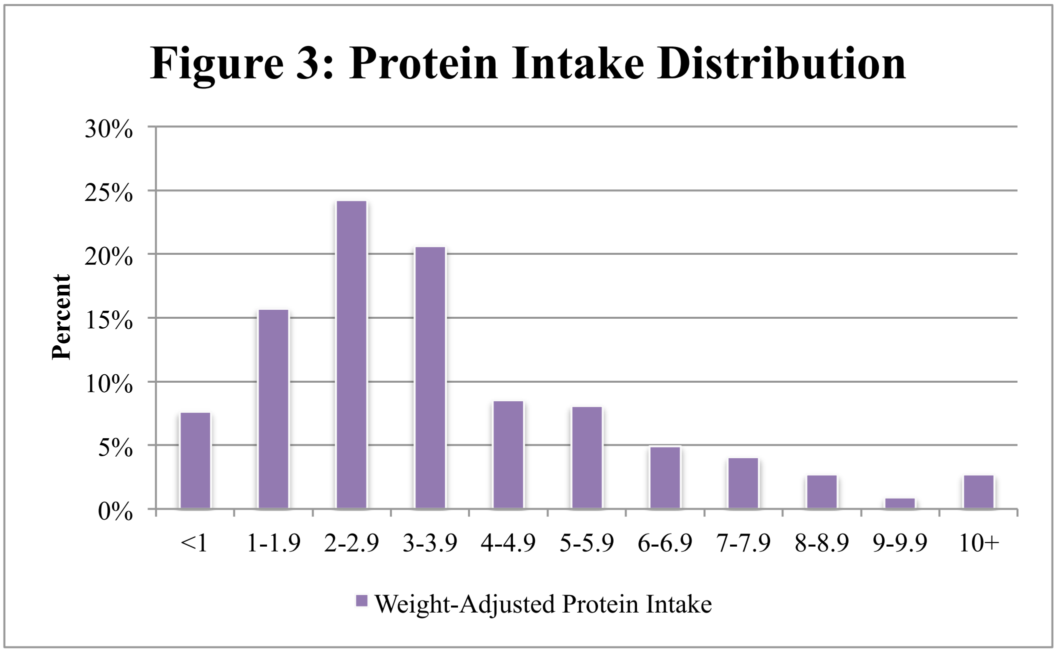


Figure 3: Protein Intake Distribution
Percent
30%
25%
20%
15%
10%
5%
0%
<1
1-1.9
2-2.9
3-3.9
4-4.9
5-5.9
6-6.9
7-7.9
8-8.9
9-9.9
10+
Weight-Adjusted Protein Intake

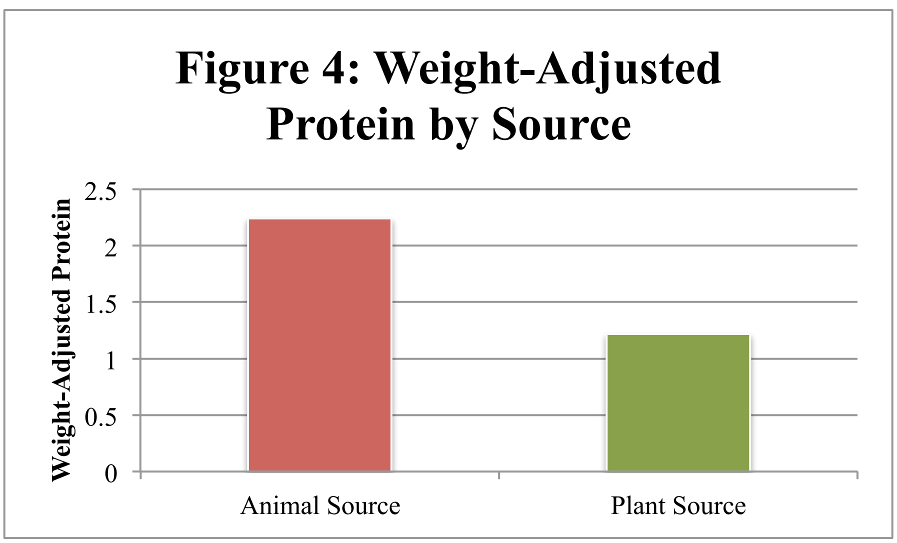


Figure 4: Weight-Adjusted Protein by Source
Weight-Adjusted Protein
2.5
2
1.5
1
0.5
0
Animal Source
Plant Source

Figure 5: Protein Intake Among Girls

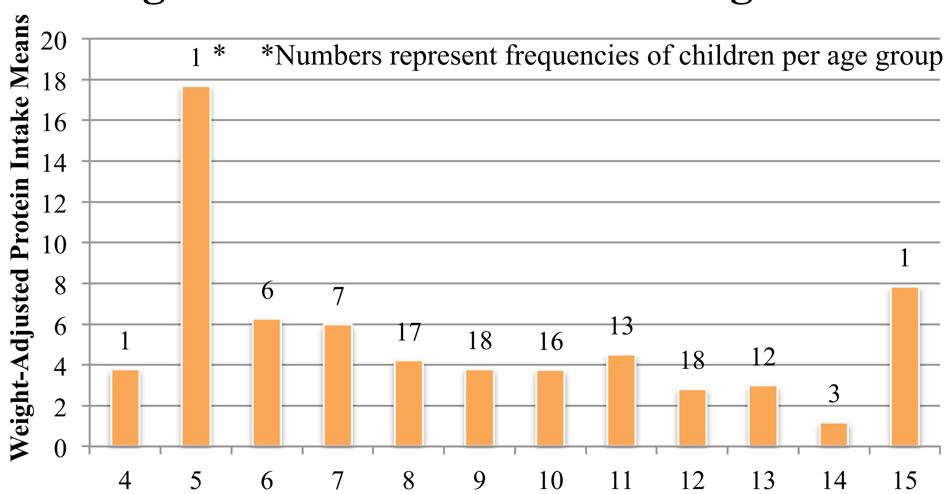
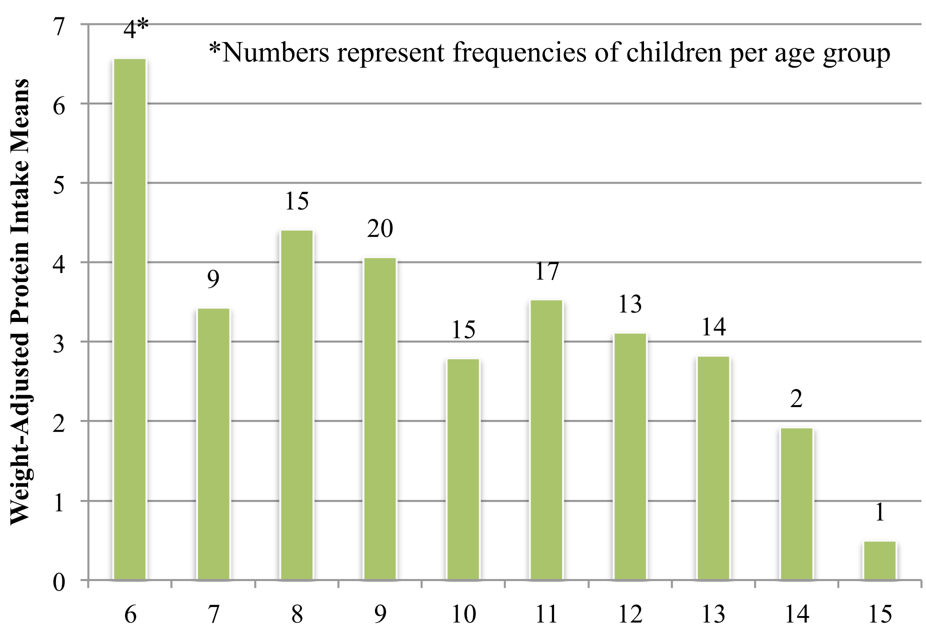


Figure 6: Protein Intake Among Boys



The mean protein intake from all sources was 3.8 ± 3.9, while the means for only animal sources and only plant sources were 2.2 ± 2.01 and 1.2 ± 1.31, respectively (Table 1). Of the 223 school-age children, 21.6% were infected in June 2013, 21.1% were infected in January 2013, 50.7% were female, 25.8% used a latrine, 67.6% were food insecure, 45.4% had improved sanitation, 49.7% had seen a healthcare worker within the last year while 50.3% had not seen one for more than one year or never, and 95.5% were protein adequate. The mean age was 10.0 ± 2.17. The mean for body-mass-index-for-age z scores (BAZ) was -0.5 ± 0.98 and the mean height-for-age (HAZ) was -1.1 ± 1.51. The mean wealth index score was 7.2 ± 2.52, while the mean dietary diversity score was 7.0 ± 2.67. 95.

Table 1. Associations between characteristics by hookworm infection status in June 2013 (N = 208)*

Characteristic	All (N = 223)	Hookworm Positive (N = 45)	Hookworm Negative (N = 163)	*P*
Protein intake,[‡] mean ± SD				
All sources	3.8 ± 3.9	3.3 ± 2.9	4.0 ± 4.2	0.114
Animal sources only	2.2 ± 2.01	2.0 ± 1.6	2.3 ± 2.2	0.433
Plant sources only	1.2 ± 1.31	1.0 ± 1.2	1.3 ± 1.4	0.133
Hookworm Infected at January, % (n)	21.1 (46)	40.0 (18)	15.3 (25)	<0.001
Female, % (n)	50.7 (113)	33.3 (15)	54.0 (88)	0.014
Age, mean ± SD	10.0 ± 2.17	10.6 ± 2.1	9.9 ± 2.1	0.057
BAZ,[a] mean ± SD	-0.5 ± 0.98	-0.5 ± 1.0	-0.6 ± 1.0	0.513
Wealth index, mean ± SD	7.2 ± 2.52	7.4 ± 2.1	7.3 ± 2.5	0.823
Household size, mean ± SD	8.2 ± 4.12	8.7 ± 5.0	8.1 ± 3.9	0.528
No. children ≤ 5 years of age, mean ± SD	2.0 ± 1.04	1.8 ± 0.8	2.0 ± 1.1	0.310
Dietary Diversity, mean ± SD	7.0 ± 2.67	7.3 ± 2.7	7.1 ± 2.6	0.623
Use of a latrine, % (n)	25.8 (56)	24.4 (11)	26.9 (43)	0.744
HAZ[b], mean ± SD	-1.1 ± 1.5	-1.1 ± 1.6	-1.1 ± 1.5	0.662
Food Insecurity, % (n)				0.661
Yes	67.6 (146)	64.4 (29)	67.9 (108)	
No	32.4 (70)	35.6 (16)	32.1 (51)	
Sanitation, % (n)				0.507
Improved	45.4 (98)	50.0 (22)	44.4 (71)	
Unimproved	54.6 (118)	50.0 (22)	55.6 (89)	
Child health care access, % (n)				0.038
Within the last year	49.7 (88)	34.4 (11)	54.8 (74)	
More than one year/never	50.3 (89)	65.6 (21)	45.2 (61)	
Protein Adequate, % (n)	95.5 (211)	93.3 (42)	95.7 (154)	0.458

* Numbers may not sum to 223 due to missing data, and percentages may not sum to 100% due to rounding. Values are % (no.) for
categorical variables and mean ± SD for continuous variables.
‡ Weight-adjusted protein intake = protein intake (g) / body weight (kg)
a BAZ = body-mass-index-for-age Z scores
b HAZ = height-for-age Z scores

Bivariate analyses showed baseline hookworm infection, gender, and visiting a doctor within the last year to be significantly associated with infection status in June 2013 (all $P < 0.05$) (Table 1). Unadjusted and adjusted multivariate logistic models were performed to assess independent effects of each protein source on risk of infection in June 2013 (Table 2). An unadjusted model showed no significant association between weight-adjusted protein intake from all sources and hookworm infection (OR: 0.91, 95% CI 0.80, 1.05; $P = 0.207$). After accounting for age, female, and baseline hookworm infection, the effect diminished further (OR: 0.92, 95% CI 0.80, 1.07; $P = 0.302$). The fully adjusted model also showed no significant association between weight-adjusted protein intake from all sources and hookworm infection status in June 2013 (OR: 0.93, 95% CI 0.78, 1.12; $P = 0.455$). An unadjusted model demonstrated no significant association between weight-adjusted protein intake from only ASF and hookworm infection (OR: 0.94, 95% CI 0.79, 1.13; $P = 0.502$). After accounting for all covariates, no significant association was found (OR: 0.92, 95% CI 0.70, 1.20; $P = 0.526$). Finally, an unadjusted model also showed no significant association between weight-adjusted protein intake from only plant sources and hookworm infection (OR: 0.80, 95% CI 0.59, 1.08; $P = 0.139$). After adjusting for all covariates, no significant association was found (OR: 0.95, 95% CI 0.69, 1.30; $P = 0.742$).

Table 2. Unadjusted and adjusted odds ratios (OR) and 95% confidence intervals (CI) for hookworm infection in June 2013 by protein source (N = 167)

Models	**OR (95% CI)**	***P***
All protein sources*		
Model 1: Unadjusted odds	0.91 (0.80, 1.05)	0.207
Model 2: Model 1 + Age + Female	0.95 (0.82, 1.10)	0.516
Model 3: Models 1-2‡ + Baseline hookworm	0.92 (0.80, 1.07)	0.302
Model 4: Models 1-3 + Use of latrine	0.92 (0.79, 1.07)	0.292
Model 5: Models 1-4 + HAZ + BAZ	0.93 (0.79, 1.09)	0.359
Model 6: Models 1-5 + Wealth index	0.93 (0.80, 1.09)	0.360
Adjusted model: Models 1-6 + Health care access	0.93 (0.78, 1.12)	0.455
Weight-adjusted animal sources only		
Model 1: Unadjusted odds	0.94 (0.79, 1.13)	0.502
Model 2: Models 1+ Age + Female	1.00 (0.83, 1.21)	0.995
Model 3: Models 1-2 + Baseline hookworm	0.94 (0.76, 1.15)	0.541
Model 4: Models 1-3 + Use of latrine	0.94 (0.76, 1.15)	0.536
Model 5: Models 1-4 + HAZ + BAZ	0.95 (0.77, 1.18)	0.654
Model 6: Models 1-5 + Wealth index	0.95 (0.77, 1.18)	0.654
Adjusted model: Models 1-6 + Health care access	0.92 (0.70, 1.20)	0.526
Weight-adjusted plant sources only		
Model 1: Unadjusted odds	0.80 (0.59, 1.08)	0.139
Model 2: Model 1 + Age + Female	0.84 (0.62, 1.14)	0.262
Model 3: Models 1-2 + Baseline hookworm	0.86 (0.64, 1.14)	0.293
Model 4: Models 1-3 + Use of latrine	0.85 (0.63, 1.14)	0.277
Model 5: Models 1-4 + HAZ + BAZ	0.86 (0.64, 1.16)	0.319
Model 6: Models 1-5 + Wealth index	0.86 (0.64, 1.16)	0.320
Adjusted model: Models 1-6 + Health care access	0.95 (0.69, 1.30)	0.742

* Weight-adjusted protein intake = protein intake (g) / body weight (kg)
‡ Model 1-2 = Model 1 + Model 2

Unadjusted and fully adjusted odds ratios and 95% confidence intervals for each covariate used in the final model were also calculated (Table 3). Although female, baseline hookworm infection, and health care access within the last year were significantly associated with hookworm infection status in June 2013, the fully adjusted model only showed baseline hookworm infection to be a significant predictor (OR: 3.51, 95% CI 1.35, 9.15; *P* = 0.010).

Table 3. Unadjusted and fully adjusted odds ratios (OR) and 95% confidence intervals (CI) for each covariate*

Covariates	Unadjusted OR (95% CI)	*P*	Adjusted** OR (95% CI)	*P*
Protein from all sources	1.09 (0.95, 1.26)	0.207	0.93 (0.78, 1.12)	0.455
Age	0.86 (0.73, 1.01)	0.059	1.11 (0.90, 1.39)	0.333
Female	2.35 (1.18, 4.69)	0.016	0.45 (0.19, 1.06)	0.068
Baseline hookworm infection	0.27 (0.13, 0.57)	<0.001	3.51 (1.35, 9.15)	0.010
Use of latrine	1.14 (0.53, 2.44)	0.744	1.18 (0.45, 3.14)	0.736
HAZ	0.81 (0.38, 1.72)	0.579	1.76 (0.64, 4.89)	0.275
BAZ	0.89 (0.62, 1.26)	0.511	1.22 (0.76, 1.97)	0.414
Wealth index	0.98 (0.86, 1.13)	0.822	0.99 (0.82, 1.20)	0.946
Child health care within one year	2.32 (1.04, 5.18)	0.041	0.53 (0.22, 1.24)	0.143

*Unadjusted (N=208) and Adjusted (N=167).
**Adjusted for all covariates shown

Qualitative Results

All mothers who were invited for an interview participated (n=32). Of the 8 Jato-Akuraa mothers invited to a focus group, 6 participated. In Cheranda, 5 out of the 8 mothers invited to a focus group participated. Additionally, all the Cheranda children invited to a focus group participated (n=8). All participants were recruited from the longitudinal study sample. Figure 2 shows the flowchart of subjects who participated in the qualitative interviews. The mean age of the children whose mothers were in set H was 9.5 ± 2.0, while that of the children whose mothers were in set L was 9.4 ± 2.2. Overall, the mean age of the children in both groups was 9.4 ± 2.0. Recurrent themes that emerged from the data are described below. Additionally, local foods mentioned in these interviews can be found in Table 4, along with their respective descriptions.

Table 4. Foods mentioned in the qualitative interviews

Name	**Description**
TZ (Tuo Zaafi)	Boiled mixture of cassava flour (tuber) and maize flour
Banku	Boiled maize flour
Fufu	Boiled mixture of cassava and yam
Ayoyo soup	Green leaves and soup stock
Groundnut soup	Recipe: Boiled, groundnuts, groundnut paste, tomatoes, and onions
Palmnut soup	Recipe: Boiled, tomatoes, onions, garlic cloves, chili powder, bouillon cube (maggi), fresh chilies, salt
Dawadawa	Fermented baoba seed, used as a spice, in small quantities, strong
Garden eggs	Small egg plant-like fruit classified as a vegetable
Coco yam leaves (Kontomire)	Spinach
Agushie or Egusi	Fat- and protein-rich seeds of cucurbitaceous (squash, melon, gourd) plants.

Cooked Usually

The food that is usually cooked at home was noted to be staple foods such as TZ (tuo zaafi), banku, and fufu. All the women in both individual interviews and focus groups reported cooking these foods often when asked to describe what is usually cooked at home.

> **Interviewer:** "Could you please describe what you usually cook for your child at home?"
>
> **Jato-Akuraa Mother:** "You know how we villagers eat, it's always TZ."

Mothers from the Cheranda focus group further reinforced the frequency to which these staples are cooked:

> **Mother 1:** "In the morning you have to know that he/she is a child and you need to provide food for them… For instance, if we cooked TZ yesterday, we eat the left overs in the morning"
>
> **Interviewer:** "What about the afternoon"?
>
> **Mother 2:** "We can cook either Ampesi or rice"
>
> **Interviewer:** "And in the evening?"
>
> **Mother 3:** "We can either cook fufu or TZ"

When asked what foods were important to feed children, 24 out of the 32 mothers described staples such as fufu, TZ, banku, and rice as important foods to cook for their children.

> **Interviewer:** "What are the foods that you think are important to cook for your child?"
>
> **Atta-Akuraa Mother:** "I think it's TZ because it's heavy."

The child focus group in Jato-Akuraa also confirmed these findings. When asked what was usually eaten in the morning, afternoon, and evening, four children from the Cheranda focus group reported eating TZ during all meals of the day. Other children reported eating other staples.

> **Interviewer:** "What do you usually eat in the morning, afternoon, and evening?"
>
> **Child 1:** "I eat TZ in the morning, TZ in the afternoon, and TZ at night or evening."
>
> **Child 2:** "In the morning I eat TZ, at school I eat rice, and in the evening I also eat TZ."

Good Feeding

When asked about what contributes to good feeding in a child, differences were noted between women in Set H and Set L. Women in Set L, for instance, reported staples such as banku, fufu, and rice to be good sources of nutrition. They also expressed that they consider children eating favorite foods as receiving good nutrition. Most (15/16) of the women in Set L reported their children's favorite foods to be the staple foods they usually cook (e.g., banku, TZ, rice, fufu). Examples from individual interviews are as follows:

> **Interviewer:** "What do you believe contributes to good nutrition in a child?"
>
> **Kawampe Mother 1:** "he is able to eat… if [the child] gets fufu or TZ, he will eat it."
>
> **Kawampe Mother 2:** "it's because if we cook banku, fufu, and TZ, that's what the child eats or likes most."

Atta-Akuraa Mother also reported similarly: “When [the child] eats his or her favorite food like banku, fufu, then it means [they] are eating well.”

No mother in this set mentioned meats, fish, eggs, and legumes/nuts as contributors to good nutrition. On the other hand, almost half of the women (5/12) in Set H who answered the question mentioned that receiving meats, fish, eggs, and/or beans contributes to good nutrition in a child.

Interviewer: “What do you believe contributes to good nutrition in a child?”

Cheranda Mother: "foods like meat, fish, groundnut soup, green vegetables like ayoyo, [and] palmnut soup."

Kadelso Mother: “Fufu and fresh or smoked meat or fish.”

Cheranda Mother 2: “Banku with groundnut or palmnut soup with smoked fish or beef. Also TZ with more maize flour and ayoyo soup with dawadawa and fish is a good meal for the child. You can also prepare stew with fish for the child.”

General food intake, especially staple foods, was a priority in both groups. Some women in Set H believed a child’s eating habits contributes to good nutrition in a child. Women from Tahiru and Jato-Akuraa said,

Tahiru Mother: “For my children – when I prepare food, they eat to my expectation. The day that I will see that they did not eat as expected, it means maybe they are sick.”

Jato-Akuraa Mother: “If the stomach is filled with food and looks like a pot belly, then the child eats well.”

These responses suggest that mothers in this population prioritize staple foods or “favorite” foods over others, especially since staple foods tend to be liked and eaten better by their children, are “filling,” and “heavy.”

Protein Sources

Because protein is not a word in Twi, we used "grow fast and strong" as a means to assess participants' beliefs around protein sources. The participants in this sample (22/30) who answered the question asking what foods help children grow fast and strong, generally believed that staple foods such as TZ, banku, fufu, and rice helped children grow fast and strong.

> **Interviewer: "**What are the foods that help children grow fast and strong?"
>
> **Cheranda focus group** agreed**:** "TZ and vegetables [helps a child] grow faster."

An Atta-Akuraa Mother also believed that "fufu and TZ" provides a strong foundation for growth. She also said, "you know here the children like fufu and TZ," which further supports staple foods as being well liked compared to other foods by children in this community.

Food Access: Family and Friends

Out of the 32 women who answered the question asking whether friends and family contributed to buying nutritious foods, 18 women reported having received food from family or friends. The women in Set H (12/16), however, reported receiving food from family or friends more than the women in Set L (6/16). Although probing was not enforced in this question, many mothers mentioned receiving protein-rich foods (e.g., fish and beef) and staple foods (e.g., cassava and yam) from family or friends.

> **Interviewer:** "Do your friends and family contribute to buying nutritious foods?"
>
> **Set H Kawampe Mother:** "We get food from friends… they bring cassava and yam… even yesterday they gave us meat."
>
> **Set H Cheranda Mother:** " [I get food] from my children… they normally give me yam and beef."

Family and friends aiding with food may be a mechanism by which mothers in Set H are feeding their children more protein-rich foods compared to mothers in Set L.

Portion Size: Protein

Of the 30 mothers who responded to the question, "Who receives the largest portion of meats," 25 reported that either the father, head of the household, or eldest member in the household received the largest portion. The remaining 5 women reported sharing these foods equally with their husbands and children.

The 25 women also reported receiving less protein-rich foods compared to their husbands, while the children received the least amount:

> **Interviewer:** "Who gets the bigger portion of meats like beef, chicken, or fish?"
>
> **All Cheranda Focus Group Mothers:** "My husband."
>
> **Interviewer:** "Who gets the second biggest?"
>
> **Cheranda Focus Group Mother 1:** said, "The woman."
>
> **Interviewer:** "And the least?"
>
> **Cheranda Focus Group Mother 2:** "The children."

Furthermore, when asked, "When you share your meat, how much do the children consume?" the mothers from the Cheranda focus group, in agreement, also said, "[The children] get one piece each," while mothers "also [receive] one piece," or sometimes receive more than the children but less than their husbands. A woman from Kadelso said, "Kids and adults can't eat equally." All mothers in both focus groups said that their husbands would take the largest portion of meats, since "he is head of the household." Additionally, most of the mothers said their husband or eldest son received the largest portion of food in general.

Clinic/Hospital Nutrition Advice

When asked about what type of advice they had received from the Kintampo clinic/hospital regarding child feeding practices, 8 out of the 17 mothers who answered the question mentioned being advised to feed their children fruits such as banana, orange, and pineapples and vegetables, often leaving out protein-rich foods in general such as meats, beans, agushie, legumes/nuts, etc. The following are example quotations from individual interviews:

> **Interviewer:** "Did they tell you anything about foods that you [should] give to your child?"
>
> **Gulumpe Mother:** "[The nurses] told us to give the child watermelon, oranges, and bananas."
>
> **Jato-Akuraa Mother:** "When we go to the hospital [the nurses] advise us to give [the children] kontonmire (cocoyam leaves), herrings, palm oil, garden eggs."

Also, some mothers talk about receiving nutrition advice during pregnancy, but with little to no advice on child feeding practices.

> **Interviewer:** "Do you remember going to the hospital for antenatal care during pregnancy? What did they teach you about child health?"
>
> **Kadelso Mother:** "They only taught us about how to care for ourselves and not the children."

Although healthcare professionals provide mothers with nutrition advice for school-age children, most protein-rich animal-source foods such as meats were not emphasized. The following conversation was with mothers from the Jato-Akuraa focus group:

> **Interviewer:** "Did [the healthcare professionals] advise on the kind of food to give to your child from five years and above"
>
> **Mother 1:** "Yes, they do"
>
> **Interviewer:** "what did they say?"

Mother 2: "They told us to prepare stews like kontomire, beans, and palm oil for [the children] so that if you cook rice or yam, they can eat it with it"

Discussion

In general, mothers believe that staple foods such as TZ, banku, fufu, and rice are the most important and nutritious foods to give to children. Mothers often associated good nutrition with eating a food "well," or entirely, and because these types of foods are "heavy" and "favor[ed]" by their children, it partly explains why mothers may cook them often and view them to be important and nutritious. Staple foods tend to taste well and suppress hunger because they take longer to digest. Another reason why these foods may be consumed frequently is because they are traditional foods (e.g., cassava and yam); they are, as one woman from Jato-Akuraa put it, "village" foods.

When mothers were asked about their beliefs surrounding sources of growth and strength, they reported staple foods as sources for "growth." Because these foods are well-liked by their children, are "heavy," and are often cultivated on their farms, they are often cooked and consumed by their children. Staple foods may, therefore, be frequently cooked and consumed due to traditional use and generational perpetuation of these foods as being sources for growth.

In the household, the largest portion of ASF such as meat, including chicken, beef, and fish are often given to the "head of the household," typically the participants' husbands. Mothers would also either receive the second largest portion size of protein-rich ASF or equal amounts to those of their children. With regards to food portions in general, most mothers also reported that the head of the household, their husbands, or eldest son received the largest portion compared to everyone else in the household. Thus, increasing plant-based protein in the diet may increase protein intake in both mothers and children without affecting cultural norms.

Almost half the sample of mothers who responded to questions about nutrition advice from the clinic/hospital said that they were often provided with advice related to fruit and vegetable consumption, but with little to no focus on protein-rich foods in general such as meats, beans, agushie, legumes/nuts, etc. Also, they were not provided with much advice on early child feeding practices. One mother mentioned that she was provided with maternal nutrition advice but no advice for her child's nutritional needs. Although some mothers did mention being advised on child feeding practices, protein-rich ASF such as meats were not emphasized. Therefore, education of nutritional needs in school-age children should be integrated in curricula being currently used at the clinic/hospital to better help mothers understand the need of protein intake and encourage the cooking and consumption of protein-rich foods among school-age children.

Protein Intake Group Discrepancies

There were differences in reporting protein-rich foods as sources of good nutrition between women in Set L and Set H. When asked, "what do you believe contributes to good nutrition in a child," no mother in Set L mentioned meats, fish, or eggs as contributors to good nutrition, whereas almost half of the mothers in Set H did. Mothers in Set L often reported staple foods such as banku, TZ, fufu, and rice as sources of good nutrition, especially since these foods were well favored by their children. To the mothers in Set L, good nutrition was not about a particular food, but rather about their children's liking to a food and its entire consumption. They believed that if their children consumed most, if not all, the food provided to them, those foods were sources of good nutrition.

There were also differences between Set L and Set H related to egg intake among children. On average, children in Set H were provided 0.5-2 more eggs per week compared to those in Set L. More research is needed to determine specific factors associated with feeding children eggs in this population. These differences in egg intake may partly explain the differences between children who had above- and below-average consumption of protein-rich foods based on the dietary diversity screener. Nonetheless, the clinic/hospital may be a means to promote the consumption of eggs among school-age children. Training community health volunteers in basic nutrition can be another mechanism through which nutrition information can reach mothers in this population.

There were also differences in contribution of nutritious foods by family and friends between mothers in Set L and Set H. More mothers in Set H reported receiving food from family or friends compared to the women in Set L. Food access through family and friends may be a mechanism by which children may be receiving more protein-rich foods. Because probing about the type of food received from family or friends was not enforced, there are remaining questions about the extent to which protein-rich plant-based and/or ASF were provided and other mechanisms related to the availability of these foods.

Hookworm Susceptibility

There was no significant association found between weight-adjusted protein intake, regardless of source, and hookworm infection in June 2013. Although insignificant, all three protein sources showed slightly protective effects.

Because this population is protein-adequate, however, these results suggest that there may not be a benefit against hookworm infection beyond the WHO recommended dietary protein requirements for children. Higher levels of protein intake, regardless of protein source, in this study show no significant protective effect against susceptibility to hookworm infection in rural Ghanaian school-age children. This study is the first to longitudinally assess the relationship between protein intake and hookworm infection susceptibility; as such, more research is warranted. Many studies assessing the relationship between protein intake and hookworm infection have primarily focused on protein-deficient animal models.[14-17] One study used a dietary diversity scale and found a significant cross-sectional association between below-average ASF and hookworm infection compared to above-average ASF in the Kintampo school-age population.[20] Similar results, however, were not found in the present study. Thus, more research is needed before any recommendation can be made about protein consumption as a preventive measure against hookworm infection susceptibility in rural school-age populations.

Perceptions and behaviors in the Kintampo community regarding staple foods and protein intake may be similar in other Ghanaian or African communities. These qualitative findings are consistent with those found in a study conducted in Gambia, wherein women reported that men received the largest, best, and first share of the meals compared to children because they were the heads of their households.[26] Like the women in the Kenya study, the women in this study did not mention protein-rich foods such as chicken, fish, and beef as being good sources of growth and strength.[7] Most African communities rely on one or two staple crops, commonly maize, teff, cassava, yam, sweet potato, plantain, and enset,[30, 53] all of which are limited in protein. Therefore, these findings provide insight into why African communities frequently use certain foods such as staples in food preparation for their children and may be relevant to other African populations.

Protein malnutrition remains prevalent in Ghana,[54] and low intake of animal-source foods, which are rich in protein and micronutrients, is a risk factor for stunting.[55] Thus, further understanding the sociocultural and behavior determinants of protein malnutrition in rural African populations provides potential areas at which to intervene, especially since food is local-specific in rural farming areas where it is produced.[30] In order for an intervention to be successful, its content and approach must address the need for variability, determined by individual and local needs, concerns, attitudes, and beliefs.[7] Qualitative research, however, is limited in this area and is needed to determine consistency of these belief systems and behaviors in other Ghanaian and African communities, especially those burdening with high protein deficiency.

Limitations

A limitation of this study is its longitudinal nature, which fails to fully account for all confounding variables. Although these recalls may not have covered all day-to-day variations of dietary intake, using non-consecutive days better estimates usual intake in children who had duplicate recalls. The coefficient of variation used to adjust the confidence intervals of the sample protein intake mean, however, was relatively high. We also used a single measure to capture diet, which could vary significantly from day-to-day. Also, we may not have had adequate statistical power to observe an effect of weight-adjusted protein intake on infection status in June 2013.

A challenge of qualitative research is that perceived social desirability of specific responses may cause mothers to become reluctant in sharing their attitudes, beliefs, and behaviors around the themes of interest. Nutrition in children may be a sensitive topic, particularly if there is a concern about malnutrition. To address this, however, the study began with questions about good nutrition rather than malnutrition. A local interpreter who has been previously involved in field studies conducted by Noguchi established rapport with mothers to ensure a comfortable and confidential space before the interview began. To minimize bias associated with informing participants about study objectives and goals, confidentiality protocols and accurate responses were clearly and strongly emphasized.

Conclusion

In summary, weight-adjust protein intake in January 2013 was not significantly associated with risk of hookworm reinfection in June 2013, after adjusting for several covariates. Staple foods are the most important and nutritious for children in the Kintampo community, especially because they are well liked and consumed often by children. Although foods are sometimes received from family and friends, more research is warranted to assess the type of foods provided and whether similar behaviors and belief systems around nutrition found in this study exist in other African communities, especially those with high protein deficiency.

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