Impact of Biscuit-Type Feeding Supplementation on the Neurodevelopment of Children 0-4 Years of Age Born to HIV-Infected Mothers: A Randomized, Double-Blind, Controlled Feeding Intervention

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Abstract

Background: Children in HIV-affected families living in resource-poor areas are vulnerable to malnutrition and have at-risk developmental status. This study examines the impact of nutritional supplementation on the neurodevelopmental outcomes of young children prenatally exposed to HIV in Kenya.

Methods: A total of 101 children 0-4 years of age born to HIV-infected mothers were enrolled in a randomized, double-blind, controlled feeding intervention trial in rural western Kenya. The feeding intervention consisted of one of three isocaloric biscuit-type supplements – soy, wheat, or beef over 18 months. Children were assessed using Bayley Scales of Infant and Toddler Development, third edition, every 3 months from ages 6 until 11 months, after which the assessment interval was spaced out to every 6 months, from age 12 months onwards until 47 months. Directly observed treatment (DOT) was used to ensure adherence and to quantify intake of the biscuit supplement.

Results: Mixed effects modeling did not reveal significant differences among the three intervention groups for Bayley subscale scores in cognitive development, expressive and receptive language, or fine and gross motor domains.

Conclusion: Provision of animal source food supplementation to HIV-affected children 0-4 years of age does not substantially improve neurodevelopmental outcomes compared to wheat or soy supplementation.

ABBREVIATIONS


INTRODUCTION

Childhood micronutrient deficiencies and protein-energy malnutrition are prevalent in the developing world [1-3]. In children 0-5 years of age, the effects of protein-energy malnutrition and micronutrient deficiencies are particularly...
The HIV virus can have devastating effects on neurodevelopment. Repeated cycles of nutritional deprivation and immunological compromise, with susceptibility to infections, particularly gastrointestinal and respiratory illnesses, may result in growth stunting and failure to thrive [4,5]. Thereby, brain growth and neurodevelopment can be adversely affected [6,7].

In sub-Saharan Africa, the plant-based diet commonly consumed is lacking in animal-source foods, including milk, tends to be low in energy density, is deficient in vitamin B12 and other micronutrients, and has high phytate and fiber content, which diminishes the bioavailability of micronutrients such as iron, calcium and zinc [8-10]. Iron deficiency is associated with microcytic anemia, as well as immune, cognitive and endocrine dysfunction [11]. In early childhood, the risk for iron deficiency is exacerbated by the combination of high iron needs and low-iron diets [12]. Although iron deficiency is the most common cause of anemia in resource-poor areas of the world, women and children are also susceptible to anemia due to other nutrient deficiencies, including low Vitamins B12 and D, zinc, calcium, and iodine [13,14]. Vitamin B12 deficiency results in pernicious anemia, and is known to have adverse effects on brain development [15]. Zinc is essential for adequate growth and neurobehavioral development [6,16]. Zinc also has an essential role in T-cell functioning, and its insufficiency leads to immune compromise, diarrhea and respiratory infections [17-19]. Calcium and Vitamin D are integral to bone metabolism and insufficiency of both remains an important cause of rickets and impaired physical activity [10]. Iodine is important for optimal cognitive development and growth [20].

Among children in sub-Saharan Africa who are already vulnerable to malnutrition due to poor dietary quantity and quality, HIV-positive status of mothers is an additional risk factor for undernourishment in their children [21]. HIV-affected families are often food insecure, which may limit the quality and quantity of food and nutrients the child receives [22,23]. Poverty and the effects of stigmatization and socioeconomic stressors may further deplete caregivers’ abilities to optimize feeding practices and provide developmental stimulation. Caregivers are themselves subject to malnutrition, infection, lethargy/encephalopathy, and mental health morbidity [24-26]. Disordered attachment is more often found in preschool-aged children of mothers with HIV and HIV-related psychosis, compared to mothers without HIV infection and other high risk populations [27]. Children who are HIV-exposed but uninfected may thus be subjected to the effects of HIV on the family and community ecology.

Empirical data on the cognitive functioning of children who were prenatally exposed to HIV (and whose families are HIV-affected) are limited. However, studies have generally indicated adverse cognitive performance of HIV-exposed uninfected children compared to non-HIV exposed children, with children in resource-poor areas being particularly vulnerable. A literature review indicated that in comparison with resource-rich settings, HIV-infected or HIV-affected (exposed but uninfected) infants in resource-poor settings demonstrated greater neurodevelopmental delay compared with HIV-unexposed counterparts [28]. Results from a recent study on HIV-affected children aged 2-5 years old in Uganda revealed that caregiver quality of care as assessed by the Home Observation for the Measurement of the Environment (HOME) was positively associated with Mullen scores of cognitive ability [29]. In a study on the neurodevelopment of preschool-aged children in Kinshasa, Democratic Republic of the Congo, 35 HIV-infected, 35 HIV-affected, and 90 control children aged 18 to 72 months were assessed using the Bayley Scales of Infant Development II, Peabody Developmental Motor Scales, Snijders-Oomen Nonverbal Intelligence Test, and Rossetti Infant-Toddler Language Scale [30].

Several food-based interventions to increase animal source food (ASF) intake have decreased morbidity, including fever, anemia, respiratory and diarrheal illnesses [31-33]. ASF supplementation has also been found to improve growth, physical activity, cognitive and behavioral outcomes in school-aged children [34,35]. ASF of a wide variety provide important macro- and micronutrients, including lysine, complete protein, vitamin B12, and bioavailable iron and zinc, which are important in maintaining normal immune function and lean body mass [36]. Results from the USAID Global Livestock Collaborative Research Support Program (GL-CRSP) Child Nutrition Project (CNP) demonstrated improved cognitive behavior, improved school performance, decreased morbidity, increased arm muscle accretion, near reversal of vitamin B12 deficiency, improved weight gain, and increased physical activity among rural Kenyan school-aged children [37-39]. Data on the effects of ASF supplementation on younger-aged or HIV-exposed children, however, are lacking.

The current study focuses on young children in rural Kenya, who have neurodevelopmental vulnerabilities due to the double threats of undernourishment and being in a HIV-affected care giving environment, wherein the mother is HIV-positive and the target child is HIV-exposed. The intervention consisted of providing one of three isocaloric biscuit-type supplements – soy, wheat, or beef – to the child. To the best of our knowledge, this study is the first randomized, double-blind, controlled intervention trial to assess the effect of animal source food (ASF) supplementation on the neurodevelopmental outcomes of HIV-affected children 0-4 years of age.

**MATERIALS AND METHODS**

**Study design**

This was a 3-arm randomized, double-blind nutrition intervention trial with a sample of 101 children born to HIV-positive drug-naive women in 3 rural locations in western Kenya (Turbo, Soi, and Mautuma) approximately 30 km from Eldoret. Women and their youngest child, between 6 months and 4 years old, were randomly assigned to receive one of three isocaloric intervention food biscuits that contained either beef, soy, or wheat. The wheat biscuit served as a comparison group for this...
study. At enrollment, women did not have CD4 counts that were low enough to qualify for treatment with antiretroviral therapy (ART) under the treatment guidelines in place at the time of the study, and this timing was selected based on the hypothesis that a food intervention may have a positive impact on the women's health and CD4 counts thus delaying the need for ART. The feeding intervention consisted of providing the mother and children with food biscuits 5 days per week for 18 months. After the feeding phase, the mother and child/children were followed up at six months post intervention. Further details on the study design have been published previously [40].

Population

The study population of women received medical care through the Academic Model Providing Access to Healthcare (AMPATH) partnership, which is supported by the United States Agency for International Development (USAID) [41,42]. This partnership operates under the joint direction of Moi Hospital, Moi University and Indiana University Schools of Medicine, and cares for over 160,000 HIV-infected adults and children at over 500 clinical sites throughout western Kenya. Participants were enrolled over a one and a half-year period (December 2008-June 2010). Women were included if they were drug naïve and classified as WHO Stage 1 or 2. During the study period, the recommendations for ART initiation were liberalized in Kenya from a CD4 of 200 cells/µL or less to a CD4 count of 350 cells/µL or less. Therefore, the inclusion criteria regarding baseline CD4 was changed from > 250 cells/µL to > 400 cells/µL. Over the course of the study, and after, it was discovered that some women had received ART and/or were pregnant at baseline and even though they received the intervention, their data and the data of their child were excluded from the intervention effect analyses. A few target children were also found to be HIV-positive and receiving ART at baseline. The data from these children were excluded from the intervention effect analyses. HIV-positive target children who were drug naïve were not excluded. Other exclusion criteria were: having one or more opportunistic infections, being pregnant, allergic to meat, soy or wheat, or not having permission from spouse/family to participate in the study.

Intervention food

The research team developed isocaloric biscuits made with wheat flour as the basic recipe [40, 43]. Dried beef powder or soy flour was added to the basic recipe in amounts to uniformly provide 4.0 total grams protein per 100 kilocalories in the beef and soy biscuits. Dried beef strips provided by a sole supplier in Nairobi were processed into a powder in a commercial blender at the research kitchen in Eldoret, Kenya. Soy flour was purchased through one supplier in Eldoret and then roasted using a consistent method. Refined unfortified wheat flour manufactured in Kenya was an ingredient in all of the biscuit recipes. The biscuits were prepared, weighed, packaged, labeled and stored in the research kitchen in Eldoret, which was specifically designed with standardized mixing, weighing, baking and storage methods that allowed for a reliable, safe and reproducible product. The production kitchen was set up and operated by research project staff trained in quantity food production with oversight for quality control and safety. Food preparation staff was required to wear clean uniforms, aprons and hair nets, and have initial and periodic medical examinations, testing for tuberculosis, and stool examinations for parasites. They were required to wash hands and work with gloves. The kitchen was inspected by the local department of public health for sanitation and cleanliness. Nutrient and bacterial analyses of the developed foods were carried out in a reliable food laboratory (Covance Laboratories, Inc., Madison, Wisconsin, USA), and repeated quarterly for quality control of macro and micronutrient, phytate and fiber content. Biscuit-based soy supplementation has been found to be accepted by pregnant women [44], and palatability of biscuit-type supplements by children in this study was also well accepted. The meat biscuit was $0.25/biscuit or $0.50/day (2 biscuits/day), the soy biscuit was $0.12/biscuit or $0.24/day (2 biscuits/day), and the wheat biscuit was $0.11/biscuit or $0.22/day (2 biscuits/day).

Delivery of biscuits and direct observation of treatment (DOT): Intervention biscuits were delivered to the field twice per week and stored in a project office. Directly observed treatment (DOT) was used to administer biscuits at participants' homes to assure adherence, quantify intake, and avoid the possible stigma associated with a central feeding location outside of the home. A DOT field worker, who used a motorcycle, delivered the biscuits to participants' homes daily, observed consumption of biscuits, and then returned any leftovers to a central location for quantification. Reasons for any leftovers were recorded daily for each of the participants. A known and consistent amount of filtered and boiled water was added to biscuits, on site in the field, to provide a porridge consistency for young children or those with difficulty chewing. Adherence was assessed daily as part of the DOT methodology that has been found to be reliable in the dispensing of medication for treatment of tuberculosis. With this method, the individual dispensing the medication observes the patient take the medication [44].

Socioeconomic status (SES) measure: The SES score adapted for the local population was previously used [45] and was further updated for the study population in 2013. The SES score was comprised of 33 binary items and 3 scaled items: land score, animal score, and possessions score. A higher score would indicate higher SES.

Outcome measures

The cognitive, language and motor scales of the Bayley Scales of Infant and Toddler Development, third edition (BSID-III), were used to assess the developmental functioning of children receiving the nutritional supplement. The BSID-III is a widely used empirical measure of cognitive and motor development in infancy and early childhood. It has been used in various cultural contexts, including sub-Saharan Africa [46-48]. For the purpose of this study, raw scores for the cognitive, language and motor scales were used, since application of standard scores based on U.S. norms would otherwise be invalid [49]. Two psychologists with expertise in developmental testing of children in the local population adapted the BSID-III for the local cultural context. In the receptive and expressive language sections, pictures deemed relatively unfamiliar to Kenyan children were changed (e.g., apple substituted by local fruit; vacuuming replaced by washing clothes). Items which could be confounded by cultural practices and experience (e.g., assembly of an ice cream cone puzzle in the
Cognitive domain; understanding of pronouns in the Receptive Language domain; use of pronouns, possessives and present progressive forms in the Expressive Language domain; skills involving scissors in the Fine Motor domain; stair climbing skills in the gross motor domain) and items for newborns and neonates were omitted (since the youngest age in this cohort was 6 months) – there were 22 such items in the Cognitive section; 16 items in Receptive Language; 12 items in Expressive Language; 20 items in Fine Motor; and 18 items in Gross Motor Subscales. A higher raw score would indicate a higher developmental status in the domain.

The cognitive testing team consisted of a supervisor and three enumerators, who were trained by a clinical and research psychologist (S.D.S.). Each enumerator was assigned a panel of children to follow, so that longitudinal assessments were administered by the same enumerator familiar with the child and family. The supervisor conducted inter-rater reliability checks on 1-2 out of every 10 assessments. Two-thirds of all the reliability evaluations were between supervisor and one enumerator (i.e. enumerator assigned to the child), and one-third involved the whole team (supervisor, 2 observing enumerators, and the administering enumerator concurrently scored the Bayley exam on the same child). Inter-rater agreement was maintained at 95-100% for each subscale (cognitive, language and motor). Oversight for reliability training and quality control were further provided by a Developmental Pediatrician (K.K.L.). The cognitive team members, including training personnel, were blinded to the randomization group, as well as the history of child and family. From 6 months to 11 months of age, children were assessed every three months, and thereafter at every 6 months till the age of 47 months. The children were tested every three months from ages 6 months to 11 months from their initial testing at enrollment. Therefore, if a child was tested for the first time at the age of 11 months, he/she was tested again at 14 months and thereafter every 6 months, until he/she aged out at 47 months.

STATISTICAL ANALYSES

All BSID-III raw scores were adjusted for the infant’s gestational age at birth. Descriptive statistics were used to summarize baseline characteristics of the study population. Frequencies were reported for categorical variables and the mean ± SD was reported for continuous variables.

The data were longitudinal, with repeated measures nested within subjects. Analysis generally followed methods described by Weiss [50]. Data were plotted in profile plots and histograms and summaries were tabulated of covariates by treatment group and location. Linear mixed effects models with random intercepts were used to model the covariance structures of the outcomes. Random intercept and time slope models were also considered but had larger AIC and BIC (Aikake and Bayes Information Criteria), so random intercept models were fitted for all outcomes. Indicators for study location (Mautuma, Soi and Turbo), gender, age and age squared, and the treatment group by time interaction were included. Subjects were randomized to the three treatment groups after baseline so no main effect for treatment group was included to increase power. Data analysis was carried out using the statistical software package Stata/SE 14. Differences in the rates of change of neurocognitive measurements by biscuit types were tested with an F-test. Values of p< 0.05 were considered statistically significant.

Ethics

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the Institutional and Ethics Review Boards at UCLA, Indiana and Moi Universities. Informed and written consents were obtained, by specifically trained staff, from women participants and from parents on behalf of their children.

This study was part of a main study. The main study was registered at ClinicalTrials.gov Identifier: NCT00562874. CONSORT guidelines were followed in the design and reporting of this RCT.

RESULTS

The flow of participants is depicted in Figure 1. There were 101 children enrolled. Thirty-five (35%) children received soya biscuits, 33 (33%) received beef biscuits, and 33 (33%) received wheat biscuits. Forty-four percent were females, and 56% were males. The attrition at each stage of the study is also shown in Figure 1. For example, at 6 months, 7 children in the Soya group dropped out, leaving 28 children from the baseline of 35. There were a total of 324 visits in the study. An average of 2.5 visits per child were carried out during the study, with a minimum of 1 and up to 6 visits.

Table (1) shows the baseline characteristics of age, gender, height, weight, BMI, head circumference, hemoglobin and CD4 count. These parameters did not differ significantly among children in the 3 randomized groups. Mean age of children at enrollment was 24.2, 24.0, and 20.8 months, for the soya, beef and wheat groups respectively.

Maternal characteristics are presented in Table 2. There were 99 mothers in the sample, as 2 families had 2 children enrolled. Maternal age, SES score, CD4 count and hemoglobin were comparable across the three intervention groups. Mothers in the beef group had higher education levels compared to those in the soya and wheat groups, but this difference was not significant (p=0.075).

Table 3 presents the Bayley-III subscale raw scores by biscuit type at baseline. There were no statistically significant differences for the three intervention groups in the cognitive subscale, receptive language, expressive language, fine motor and gross motor raw scores at baseline.

Mixed model results

Table 4 shows the results from the mixed effects models for the five subscale scores on the Bayley-II (n=101). There were no statistical differences in Bayley-III subscale scores by study location. There were significant increases in the developmental subscale scores over time for all three biscuit groups, as would be expected through the developmental maturation of the children. However, the F-tests reveal that these increases over time were not significantly different across biscuit groups for any of the outcomes.
Table 1: Child baseline characteristics by intervention group (n=101).

<table>
<thead>
<tr>
<th>Location</th>
<th>SOYA (n=35)</th>
<th>BEEF (n=33)</th>
<th>WHEAT (n=33)</th>
<th>All (n=101)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Turbo</td>
<td>17 (48.6)</td>
<td>15 (45.5)</td>
<td>17 (51.5)</td>
<td>49 (48.5)</td>
<td>0.829</td>
</tr>
<tr>
<td>Soi</td>
<td>5 (14.3)</td>
<td>5 (15.2)</td>
<td>7 (21.2)</td>
<td>17 (16.8)</td>
<td></td>
</tr>
<tr>
<td>Mautuma</td>
<td>13 (37.1)</td>
<td>13 (39.4)</td>
<td>9 (27.3)</td>
<td>35 (34.7)</td>
<td></td>
</tr>
</tbody>
</table>

Baseline Characteristics

| Males      | 21 (61.8)   | 17 (51.5)   | 18 (54.6)    | 56 (56)     | 0.685   |

| mean (SD)  |             |             |              |             |         |
| Baseline age (months) | 24.2 (10.9) | 24.0 (11.4) | 20.8 (12.6) | 23.2 (11.6) | 0.304   |
| CD4 count (cells/mm3)* | 1669.8 (656.4) | 1939.2 (681.2) | 2116.7 (1081.2) | 1904.6 (830.6) | 0.192   |
| HGB (g/dl)*   | 11.11 (2.0) | 11.2 (1.2)  | 10.77 (1.6)  | 11.04 (1.6) | 0.535   |
| Weight (kg)*  | 10.70 (2.0) | 10.87 (2.7) | 9.98 (2.4)   | 10.52 (2.4) | 0.233   |
| Height (cm)*  | 81.4 (8.4)  | 81.9 (10.7) | 78.16 (10.7) | 80.50 (10.0) | 0.187   |
| BMI (kg/m2)*  | 16.12 (1.8) | 16.05 (1.8) | 16.26 (1.7)  | 16.15 (1.7) | 0.768   |
| Head circumference (cm)* | 47.84 (1.9) | 47.09 (3.0) | 47.09 (2.4)  | 47.45 (2.5) | 0.472   |

*Missing data occurred in 10% of CD4 counts and HGB measures; in 1% of weight, height and BMI measures; and in 4% of head circumference measures.

CD4: Cluster of Differentiation 4; HGB: Haemoglobin; BMI: Body Mass Index
Table 2: Maternal baseline characteristics by intervention group (n=99).

<table>
<thead>
<tr>
<th>Location</th>
<th>SOYA (n=34)</th>
<th>BEEF (n=32)</th>
<th>WHEAT (n=33)</th>
<th>All (n=99)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
</tr>
<tr>
<td>Turbo</td>
<td>17 (50.0)</td>
<td>14 (43.8)</td>
<td>17 (51.5)</td>
<td>48 (48.5)</td>
<td>0.816</td>
</tr>
<tr>
<td>Soi</td>
<td>5 (14.7)</td>
<td>5 (15.6)</td>
<td>7 (21.2)</td>
<td>17 (17.2)</td>
<td></td>
</tr>
<tr>
<td>Mautuma</td>
<td>12 (35.3)</td>
<td>13 (40.6)</td>
<td>9 (27.3)</td>
<td>33 (34.3)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Education*</th>
<th>n (%)</th>
<th>n (%)</th>
<th>n (%)</th>
<th>n (%)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>No education</td>
<td>1 (3.0)</td>
<td>2 (6.3)</td>
<td>1 (3.0)</td>
<td>4 (4.1)</td>
<td>0.075</td>
</tr>
<tr>
<td>Primary education</td>
<td>27 (81.8)</td>
<td>15 (46.9)</td>
<td>22 (66.7)</td>
<td>63 (65.3)</td>
<td></td>
</tr>
<tr>
<td>Secondary education</td>
<td>5 (15.2)</td>
<td>13 (40.6)</td>
<td>10 (30.3)</td>
<td>28 (28.6)</td>
<td></td>
</tr>
<tr>
<td>Middle college</td>
<td>0 (0)</td>
<td>2 (6.3)</td>
<td>0 (0)</td>
<td>2 (2.1)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Location</th>
<th>n (%): mean (SD)</th>
<th>n (%): mean (SD)</th>
<th>n (%): mean (SD)</th>
<th>n (%): mean (SD)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location</td>
<td>n (%): mean (SD)</td>
<td>n (%): mean (SD)</td>
<td>n (%): mean (SD)</td>
<td>n (%): mean (SD)</td>
<td></td>
</tr>
<tr>
<td>Turbo</td>
<td>17 (50.0): 31.82 (7.5)</td>
<td>14 (43.8): 31.36 (6.8)</td>
<td>17 (51.5): 31.81 (6.3)</td>
<td>48 (48.5): 35.68 (7.9)</td>
<td></td>
</tr>
<tr>
<td>Soi</td>
<td>5 (14.7): 12.4 (1.5)</td>
<td>5 (15.6): 12.4 (1.7)</td>
<td>7 (21.2): 12.87 (1.5)</td>
<td>17 (17.2): 12.57 (1.6)</td>
<td></td>
</tr>
<tr>
<td>Mautuma</td>
<td>12 (35.3): 0.23 (0.11)</td>
<td>13 (40.6): 0.28 (0.19)</td>
<td>9 (27.3): 0.25 (0.12)</td>
<td>33 (34.3): 0.25 (0.14)</td>
<td></td>
</tr>
</tbody>
</table>

*Missing data occurred in 2% of SES scores, and in 1% of education entries. SES: Socioeconomic Status

Table 3: Bayley-III subscale raw scores by intervention group (n=101).

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>n (%)</th>
<th>n (%)</th>
<th>n (%)</th>
<th>n (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cognitive Score</td>
<td>53.9 (10.2)</td>
<td>52.2 (13.2)</td>
<td>47.2 (14.0)</td>
<td>51.1 (12.7)</td>
<td>0.106</td>
</tr>
<tr>
<td>Receptive Language Score</td>
<td>22.0 (7.3)</td>
<td>22.0 (7.2)</td>
<td>19.8 (8.2)</td>
<td>21.3 (7.8)</td>
<td>0.258</td>
</tr>
<tr>
<td>Expressive Language Score</td>
<td>23 (9.3)</td>
<td>23.3 (9.2)</td>
<td>20.1 (11.0)</td>
<td>22.1 (9.9)</td>
<td>0.236</td>
</tr>
<tr>
<td>Fine Motor Score</td>
<td>36.5 (9.1)</td>
<td>35.7 (9.4)</td>
<td>33.3 (10.1)</td>
<td>35.2 (9.5)</td>
<td>0.285</td>
</tr>
<tr>
<td>Gross Motor Score</td>
<td>48.8 (8.6)</td>
<td>47.9 (10.8)</td>
<td>43.6 (11.3)</td>
<td>46.8 (10.4)</td>
<td>0.135</td>
</tr>
</tbody>
</table>

Table 4: Mixed effect models for cognitive outcomes (n=101).

<table>
<thead>
<tr>
<th>Cognitive</th>
<th>Receptive Language</th>
<th>Expressive Language</th>
<th>Fine Motor</th>
<th>Gross Motor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Est</td>
<td>SE</td>
<td>P-value</td>
<td>Est</td>
<td>SE</td>
</tr>
<tr>
<td>Study Location</td>
<td>Turbo</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Soy</td>
<td>-1.09</td>
<td>0.97</td>
<td>0.259</td>
<td>0.26</td>
</tr>
<tr>
<td>Mautuma</td>
<td>-0.50</td>
<td>0.76</td>
<td>0.943</td>
<td>-0.35</td>
</tr>
<tr>
<td>Female</td>
<td>-0.49</td>
<td>0.72</td>
<td>0.493</td>
<td>0.56</td>
</tr>
<tr>
<td>Age (in years)</td>
<td>25.5</td>
<td>1.29</td>
<td>0.001</td>
<td>12.0</td>
</tr>
<tr>
<td>Age^2</td>
<td>-3.32</td>
<td>0.37</td>
<td>0.001</td>
<td>-1.19</td>
</tr>
<tr>
<td>Time*Soya</td>
<td>1.17</td>
<td>0.58</td>
<td>0.045</td>
<td>0.51</td>
</tr>
<tr>
<td>Time*Beef</td>
<td>1.30</td>
<td>0.67</td>
<td>0.052</td>
<td>0.31</td>
</tr>
<tr>
<td>Time*Wheat</td>
<td>1.86</td>
<td>0.66</td>
<td>0.004</td>
<td>1.14</td>
</tr>
</tbody>
</table>

Test of interaction | F | P-val | F | P-val | F | P-val | F | P-val | F | P-val | F | P-val | F | P-val |
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<tbody>
<tr>
<td>Time*biscuit group</td>
<td>0.96</td>
<td>0.622</td>
<td>1.91</td>
<td>0.386</td>
<td>1.16</td>
<td>0.561</td>
<td>1.90</td>
<td>0.386</td>
<td>1.42</td>
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DISCUSSION

Children born to HIV-infected mothers are vulnerable to the effects of undernourishment and micronutrient deficiencies, which are known to impair cognitive and neurodevelopmental outcomes. While ASF supplementation, particularly the incorporation of meat, has been found to improve cognitive and behavioral outcomes in school-aged, non HIV-exposed children, the effects of ASF intake on the neurodevelopmental status of young children in HIV-affected families remain obscure. As far as we know, this current study is the first randomized, double-blind, controlled intervention trial to assess the effect of ASF supplementation on the neurodevelopmental outcomes of HIV-affected children 0-4 years of age. Results of this study did not demonstrate significant effects of ASF supplementation on neurodevelopmental outcomes of HIV-affected children 0-4 years of age, compared to wheat or soy supplementation.

The meat and soy intervention biscuits were designed to provide infants with 64% of recommended daily protein intake, and more than 100% for children 1-3 years [38]. In contrast, the wheat biscuit provided a lesser amount (25–42% of recommendation) of protein that was also of poorer quality [51,52]. Infants and children in the meat and soy groups received lysine in amounts that met 80–135% of recommended intakes, while those in the wheat group did not receive lysine from the
Vitamin B12 was provided primarily to those in the meat group in amounts of 0.44 µg/day (infants) and 0.88 µg/day (1-8 years), representing 73-98% of the recommended intake. Higher amounts of absorbable zinc were available to those receiving meat biscuits, and these supplements were also lower in fiber and phytate, thus increasing bioavailability of the micronutrient. Therefore, those who received meat were expected to show more positive study outcomes.

Findings from school breakfast programs [55,56] and the Child Nutrition Project (CNP) in rural Kenya demonstrated statistically significant higher learning and cognitive outcomes in school-aged, non-HIV-exposed children who received ASF supplementation [34,57,58]. CNP was a randomized feeding intervention study which provided three types of school snacks to school children in rural Kenya (plain githeri plus added oil, githeri plus milk, or githeri plus meat) and included a comparison control group. Students who received supplemental food with meat significantly outperformed peers in Milk, Energy or Control (no feeding) groups on the Raven’s Progressive Matrices (RPM), a measure of nonverbal intellectual functioning. Achievement scores in academic and language areas increased as well. Intake of riboflavin, available iron, energy per body weight, zinc, and vitamin B12 were associated with improved school test scores [57]. Greater gains in RPM scores were also associated with intake of available iron [57]. However, these findings on school-aged children may not be generalizable towards the current study on HIV-affected young (6-47 months).

Data from nutritional interventions on neurodevelopmental outcomes of HIV-affected children are scant. A randomized placebo-controlled trial of multivitamin supplementation on a sample of 206 HIV-exposed infants in Tanzania did not detect significant improvement in the cognitive, language, and motor scales of the Bayley Scales of Infant and Toddler Development, third edition, at age 15 months, although there was a trend toward improved fine motor skills among infants randomized to the multivitamin group (difference in mean score = 0.38; 95% CI = -0.01, 0.78, p = 0.16) [47]. Other feeding trials have not focused on neurodevelopmental outcomes, but positive effects on child anthropometry have been noted. Specifically, a concentrated formula resulted in increased weight gain of HIV-exposed infants compared with standard formula [59].

Reasons for the lack of effects of ASF supplementation on neurodevelopmental outcomes of young HIV-affected children in this present study remain to be clarified. It is possible that the timing to intervene and to detect differences may not occur in the 6 to 47-month time period. The window of opportunity to intervene may be further upstream, extending to supplementation in the prenatal and perinatal periods. During gestation and in the first postnatal months, developmental processes may be affected by nutrition in a time-dependent manner [60]. Effects on neuronal migration, differentiation and apoptosis may be critical elements for nutritional supplementation to impact neurodevelopmental outcomes. The transient presence of the cortical subplate, and its virtual dissolution 3-4 months after term also coincides with achievement of major goal-oriented activities of the infant, for example, the replacement of general movements with reaching behavior [61,62]. The earliest age of nutritional supplementation for infants was 6 months in this present study.

Another possibility is that effects of the nutritional intervention in infancy and toddlerhood may not be immediate or short term, and thus the time points for detection of effects may lie further downstream, past 4 years of age. Effects of nutritional supplementation may not be direct, and may be dependent on other mediators. For example, effects on gross motor skills may be mediated by bone growth and muscle mass, and thus these trickle-down effects may not be captured in the preschool ages. Possible pathways through nutritional benefits to mother and siblings, for instance, in improvement of physiological or mental health status and maternal ability to provide developmental or play stimulation, may be expected to percolate with a substantial time lag before differences in developmental outcome measures can be detected among intervention groups.

Limitations of this current study include the following: 1) Although longitudinal assessments were conducted, the overall sample size of 101 children was limited. The final population of children in this study was fewer in number and more diverse in age at baseline than originally intended. Reasons include: a) study participants were children of HIV-infected women who were drug naive and not pregnant at baseline; those with mothers found to be pregnant and/or receiving ARVs at baseline, were removed from the analysis, b) a slow enrollment forced an amendment to the original study design to include a wider age range of children as well as mothers without children. 2) As noted in the tables, missing data occurred in 10% of CD4 counts and hemoglobin measures; in 1% of weight, height and BMI measures; and in 4% of head circumference measures for the child. Missing data for the family characteristics occurred in 2% of SES scores, and in 1% of education entries. 3) While statistically non-significant, maternal education levels at baseline were observed to differ across the three groups in our sample. However, this difference was to the advantage of the beef group. Thus the main finding of a lack of improvement in BSID-III scores despite ASF supplementation in this intervention trial is supported. 4) The dropout rate was high in working in this resource-poor area. Participants’ willingness to be followed up over time could not be ensured. Some families, particularly those who dropped within the first 6 months, had difficulty with the stigma associated with HIV [63]. Field conditions were difficult, including weather conditions; contacting families frequently involved road travel to meet in person. Families might not be present at the appointed day/time (e.g., went to town to run an errand). Another road trip had to be planned to meet with the family under these circumstances. Should a family miss an appointment, other logistical issues arose (e.g., the assessment team might not be able to get to other families on time).

The strengths of this study include the randomized, double-blind controlled design; the use of longitudinal assessments based on age of enrollment; the employment of DOT to assure adherence and to quantify intake; rigorous quality control and safety standards for intervention food production and delivery; reliability in measurement of outcome variables; and access to the networking and collaborative framework afforded by AMPATH partnership in western Kenya.

Future directions for research may include maternal nutritional supplementation in the prenatal period. Restricting the age range of children enrolled to 0-3 years and increasing the
sample size may also increase the power to detect differences in developmental status of children among the randomized groups. The ethical concern that denies enrollment into a nutritional supplementation study, due to inclusion criteria, may be addressed with ongoing concurrent studies that will accommodate all who are interested in study participation; so that no one is turned away. This will require a larger geographic study area with a higher level of logistical support.

CONCLUSION

The current study is a randomized, double-blind, controlled intervention trial to assess the effect of ASF supplementation on the neurodevelopmental outcomes of HIV-infected children 0-4 years of age. Results did not indicate significant improvement in neurodevelopmental outcomes through ASF supplementation.

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AUTHORS’ CONTRIBUTIONS

K.K.L. drafted the manuscript, participated in field work, assisted in quality control of data collection, and had primary responsibility for the contents of this manuscript. S.R. performed the statistical analysis and participated in the writing of the manuscript. S.D.S. adapted the assessment measures for the local cultural context supervised field work. Q.C. performed statistical analysis. R.E.W. designed the statistical analysis and edited the manuscript. C.E. assisted with supervision of the statistical analysis and edited the manuscript. G.E. guided field research and data collection on-site, and provided consultancy for study design. J.E. was Principal Investigator of the main NIH grant, designed the larger, umbrella study, guided overall field research and data collection, and edited the manuscript. C.G.N. was the Principal Investigator, was responsible for the study design, and oversaw overall research activities. All authors read and approved the final manuscript.

REFERENCES


39. Siekmann JH, Allen LH, Bwibo NO, Demment MW, Murphy SP, Neumann CG. Kenyan schoolchildren have multiple micronutrient deficiencies, but increased plasma vitamin B-12 is the only detectable micronutrient response to meat or milk supplementation. J Nutr. 2003; 133: 3972S-3980S.


63. Verdun D, Siika A, Sawe C, Ernst J. Experience and Challenges in the Recruitment and Retention of HIV-infected Rural Kenyan Women and Their Children into a Randomized Nutrition Intervention Study. Global Livestock CRSP, UC Davis. 2010; Research Brief 10-02-HNP.