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ORIGINAL RESEARCH

# Effects of biscuit-type feeding supplementation on the neurocognitive outcomes of HIV-affected school-age children: a randomized, double-blind, controlled intervention trial in Kenya

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## Abstract

**Objective:** To determine if meat or soy protein dietary supplementation will enhance the neurocognitive performance of HIV-affected children at-risk of malnutrition and food insecurity.

**Methods:** A randomized, double-blind, controlled intervention trial evaluated the effect of nutritional supplementation on the neurocognitive outcomes of 49 HIV-affected school-age children in western Kenya. The intervention consisted in providing the mother, target child, and siblings with one of three isocaloric biscuit-type supplements – soy, wheat, or beef – on 5 days per week for 18 months. Neurocognitive outcomes of the target children were assessed by a battery of eight measures and followed up longitudinally for up to 24 months.

**Results:** Mixed effects modeling demonstrated significant differences in the rates of increase over time among all three groups (*F* test degrees of freedom of 2, $P<0.05$) for Raven's progressive matrices performance, but not for verbal meaning, arithmetic, digit span backward, forward, and total, embedded figure test, and Beery visual–motor integration scores.

**Conclusion:** HIV-affected school-age children provided with soy protein supplementation showed greater improvement in nonverbal cognitive (fluid intelligence) performance compared with peers who received isocaloric beef or wheat biscuits. Soy nutrients may have an enhancing effect on neurocognitive skills in HIV-affected school-age children.

**Keywords:** Neurocognition; neurodevelopment; malnutrition; HIV; nutrition; Africa; animal source foods; meat; soy; proteins

## Introduction

Mothers with human immunodeficiency virus (HIV) infection and their offspring, HIV positive and HIV negative, are among those most susceptible to the effects of malnutrition and disease [1, 2]. Most HIV-affected families live in impoverished areas of the world, where an adverse spiral of food insecurity, poor dietary quality, opportunistic infections, lethargy, and encephalopathy can impact both the caregivers' and the child's health, as well as deplete caregivers' abilities to cope with and attend to the child's developmental care, ultimately affecting the neurocognitive outcomes of the child [3–5].

While it is known that pediatric HIV infection is associated with a range of cognitive problems, and brain alterations are detectable even in the early postnatal period [6–8], children and youths who are not infected by vertical transmission (but whose families are affected by HIV) are nonetheless shown to have cognitive and school achievement deficits compared with same-age peers without HIV exposure [9, 10]. Children living in less economically developed areas are particularly at risk. A literature review indicated that in comparison with resource-rich settings, in resource-poor settings infants and young children born to HIV-positive mothers demonstrated greater neurodevelopmental delay compared with HIV-unexposed counterparts, but data on older children remain lacking [11].

In sub-Saharan Africa, where maternal HIV infection remains pervasive, malnutrition further undermines the neurodevelopmental status of children in HIV-affected families [12]. In this geographic terrain, the diet is mainly plant based, low in animal-source foods (ASFs), low in energy density, and deficient in vitamin $B_{12}$, and contains high amounts of phytate and fiber that reduce the bioavailability of micronutrients such as iron, calcium, and zinc [13, 14]. Iron deficiency is the most prevalent micronutrient deficiency worldwide, and leads to microcytic anemia, lethargy, and impaired cognitive, immune, and endocrine function [15]. Intestinal parasites and high-phytate diets also contribute to iron deficiency. Zinc deficiency is associated with immune dysfunction, diarrhea, and acute respiratory infections [16, 17]. Vitamin $B_{12}$ deficiency is classically associated with pernicious anemia, but on a global scale, deficiency due to food-bound cobalamin malabsorption is more common [18]. Severe vitamin $B_{12}$ deficiency results in neurologic deficits, impairs brain development [19], and adversely impacts school performance [20, 21].

ASFs can provide a variety of micronutrients that are deficient and poorly absorbed from plant-source diets, especially vitamin $B_{12}$ [22]. There is evidence that relatively small amounts of ASFs, added to a vegetarian diet, can substantially increase nutrient adequacy, particularly of bioavailable iron and zinc [22]. School breakfast programs have been associated with improved academic performance [23, 24]. The Child Nutrition Project (CNP) feeding intervention study provided evidence on the impact of an ASF nutrition intervention on learning and cognitive development outcomes in Kenyan school-age children. This randomized study provided three types of isocaloric school snacks 5 days a week to schoolchildren in rural Kenya (plain *githeri* (a vegetable porridge) plus added oil, *githeri* plus milk, or *githeri* plus meat) and had a control (no feeding) group. Children in the CNP study in the meat group significantly outperformed their counterparts in the milk, energy, and control groups on Raven's progressive matrices (RPM) [25]. The CNP feeding intervention also affected end-of-term school test scores over time. The meat group children showed greater improvements compared with the control group children in English, Kiembu, Kiswahili, geography, and arithmetic test scores [26]. Intake of riboflavin, available iron, iron, energy per body weight, zinc, and vitamin $B_{12}$ were positively associated with the changes in the school test scores [27]. Greater gains in RPM scores were also associated with intake of available iron. However, a soy porridge was not included in this intervention trial.

Soy is a rich source of protein, and includes all essential amino acids. It is also replete with isoflavones, which constitute one class of phytoestrogens found largely in soybeans and soy-derived products, with the aglycone genistein and daidzein being the most potent of the isoflavones present in soy foods [28]. Isoflavones from soy foods are more bioavailable in children than in adults [29], and at concentrations present in soy infant formula inhibit rotavirus infection *in vitro* [30]. However, soy-protein products contain appreciable amounts of phytate and fiber, which inhibit iron absorption. Soy protein itself is still relatively inhibitory to iron absorption, even with phytate removal [31].

The wheat biscuit was developed to be equal in energy to the soy and meat biscuits, to serve as the comparison product in this randomized controlled trial. The wheat biscuit was lower in protein content and quality, and the source of protein was solely derived from the wheat flour ingredient, whereas the meat and soy biscuits had dried beef powder or soy flour respectively added to the basic recipe made of wheat flour. Caloric supplementation without the addition of protein did not improve children's cognitive performance in the aforementioned CNP study [25].

Previous studies have demonstrated positive effects of soy-protein supplementation in alleviating malnutrition. Fortified blended flours, such as corn–soy blend (CSB), prepared as porridge are the most widely used foods in supplementary feeding programs [32]. In children with moderate acute malnutrition, however, recovery rates remain below 75%, lower than the rate achieved with peanut-paste-based ready-to-use supplementary foods (RUSFs) [33]. Hence, concerns about the nutritional adequacy of these blended foods and problems in home preparation of CSB (e.g., overdilution of the porridge or inadequate boiling of water) have led to the development of fortified CSBs. In a prospective, randomized, investigator-blinded, controlled noninferiority trial involving rural Malawian children aged 6–59 months with moderate acute malnutrition, a novel, locally produced, fortified blended flour (CSB++) that included dry skim milk and that was more energy dense proved to be not inferior to a locally produced soy RUSF and an imported soy/whey RUSF in facilitating recovery from moderate acute malnutrition [34]. A randomized study of 81 children with moderate acute malnutrition in Cameroon showed comparable recovery rates in children who were treated with an improved CSB (CSB+) versus RUSF [35]. A school feeding program involving 383 schoolchildren in Ghana demonstrated that the use of a multiple-micronutrient-fortified CSB was a key contributor to micronutrient adequacy [36].

Soybean-enhanced supplemental snacks may also provide an appropriate, affordable, and sustainable solution to the problem of malnutrition, since they are is energy dense and can be made with locally available foods in low-income countries in sub-Saharan Africa [37, 38]. Biscuit-based soy supplementation has been found to be accepted by pregnant women [39], but its efficacy in this form has not been assessed in children. In HIV-affected families, the 'biscuit model' confers further advantages in that it can be provided in a blinded and randomized fashion, safely and privately in a home under directly observed consumption by a highly stigmatized population [40].

The current study was a three-arm randomized, double-blind nutrition intervention trial of the effects of ASF versus soy supplementation on the neurocognitive performance of HIV-affected, nutritionally at risk school-age children in Kenya. The intervention consisted in providing one of three isocaloric biscuit-type supplements – soy, wheat, or beef – to the mother, target child, and siblings. It was hypothesized that children in the meat group would have better neurocognitive outcomes than those randomized to the soy and wheat groups.

## Materials and methods

### Study design

This was a three-arm randomized, double-blind nutrition intervention trial with a sample of 49 school-age children born to HIV-positive drug-naïve women in three communities in western Kenya (Turbo, Mautuma, and Soi). The uniform nutrition intervention consisted in feeding the mother and children biscuits 5 days per week for 18 months. Women, with HIV and also free from opportunistic or other serious infections, and the target children between 4 and 8 years old were randomly assigned to receive one of three isocaloric intervention biscuits that contained either beef, roasted soy flour, or wheat flour (used as the control biscuit). The study was a three-armed intervention trial to ascertain if the addition of soy and meat protein to a basic recipe of wheat will improve the cognitive performance of HIV-affected children. At enrollment, these women were not yet ill enough to warrant treatment with antiretroviral therapy (ART), and therefore it was hypothesized that a food intervention may have a positive impact on their health and delay the need for ART. Groups were matched for the distance of participant households from the treatment clinic and a government health center. The location of the households was determined by Global Positioning System mapping. The intervention spanned 18 months to ensure inclusion of all participants during all of the seasons, including the dry season from November to March, when there was the greatest vulnerability to food scarcity. Subsequently, the mother and child/children were followed up at 6 months after intervention. Participants were enrolled over a 1.5-year period (December 2008 to June 2010). Data collection began in December 2008 and ended in June 2012.

### Population

The study population of women received medical care either at a local government health center or at the Academic Model Providing Access to Health care partnership clinics in western Kenya, supported in part by United States Agency for International Development [41, 42]. This partnership operates under the joint direction of Moi Teaching and Referral Hospital and the Moi University and Indiana University schools of medicine and cares for more than 160,000 HIV-infected adults and children at more than 500 clinical sites throughout western Kenya. Women were included if they were drug naïve and classified as having WHO stage 1 or 2 HIV infection. At the beginning of the study, women were excluded if the initial CD4 cell count was less than 250/µL, or if the participant had one or more opportunistic infections, was pregnant, was allergic to meat, soy, or wheat, and/or did not have permission from her spouse/family to participate in the study. During the time of the study, however, the recommendations for ART initiation were liberalized in Kenya from a CD4 cell count of 200/µL or less to 350/µL or less. Therefore, the inclusion criteria regarding baseline CD4 cell count was changed from more than 250/µL to more than 400/µL. During the course of the study and after the study, it was discovered that some women received ART and/or were pregnant at the baseline, and even though they received the intervention, their data and the data from their child were excluded from the intervention effect analyses. Some target children were also found to be HIV positive and receiving ART at the baseline. The data from these children were excluded from the intervention effect analyses. HIV-positive target children who were drug naïve were not excluded.

### Intervention food

Details on the 'biscuit model' and nutritional composition of the intervention biscuits for this study have been published [40]. The research team developed the intervention foods to be isocaloric biscuits made with wheat flour. Dried beef powder or soy flour was added to the basic recipe in amounts to provide similar amounts of total protein in the beef and soy biscuits; 4.0 g protein per 100 kcal. Dried beef strips produced by Farmer's Choice butcheries in Nairobi were processed into a powder in a Vitamix commercial blender. Soy flour was purchased solely through Nakumat supermarket in Eldoret, Kenya, and then roasted after purchase with a consistent method. Refined unfortified wheat flour manufactured in Kenya (EXE) was used as an ingredient in all of the biscuit recipes. The biscuits were prepared, packaged in opaque wrappers, weighed, labeled, and stored in a research bakery specifically designed with standardized mixing, weighing, and baking and storage equipment that allowed the production of a reliable, safe, and reproducible product. The production bakery was set up and operated by research project staff specifically trained in quantity food production, with oversight for quality control and safety by co-principal investigators and the field research project coordinator. Food preparation staff members were 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 their hands and work with disposable gloves. The kitchen was inspected by the local department of public health for sanitation and cleanliness. Nutrient and bacterial analyses of the foods developed were performed in a reliable food laboratory (Covance Laboratories, Madison, Wisconsin, United States), and repeated quarterly for quality control of macronutrient, micronutrient, phytate, and fiber contents.

### Delivery of biscuits and direct observation of treatment

Biscuits, ready for distribution, were delivered to the field twice per week. Directly observed treatment (DOT) was used to ensure adherence and to quantify intake. A DOT field worker delivered the supplement daily and returned any leftovers to a central location for quantification. The reasons for any leftovers were recorded daily for each of the participants. For young children or those children with difficulty chewing, a fixed amount of water (boiled and filtered) was added to biscuits when they were served in the home to make them into a porridge consistency. Adherence was assessed daily as part of the DOT method, whereby the individual dispensing the intervention watches the participant consume the biscuit. This modality has been reliable in the dispensing of medication for treatment of tuberculosis [43].

### Socioeconomic status measure

The socioeconomic status (SES) score adapted for the local population was previously validated [44], and was further updated for the study population in 2013. The SES score comprises 33 binary items and three scaled items: land score, animal score, and goods score.

### Outcome measures

There were eight measures: digit span forward, digit span backward, digit span total, RPM, verbal meaning, arithmetic, embedded figure test, and Beery test of visual–motor integration. Children were assessed every 6 months starting from the time that they were enrolled in the study.

This battery of tests had been used in previous research in rural Kenya involving school-age children [25, 26], had been shown to be appropriate for the local cultural context, and the individual measures were demonstrated to capture multiple cognitive constructs associated with micronutrient status. Construct validity and reliability have been measured in the Kenyan population [25, 27, 45]. The cognitive testing team consisted of a supervisor and three enumerators, who were trained by a Kenyan psychologist who served as the lead field psychologist in the CNP study and is well versed in administering neurocognitive testing for both research and clinical purposes. Each enumerator was assigned a panel of children to follow up, so serial assessments were administered by the same enumerator familiar with the child and the family, in the home setting. Oversight for reliability training and quality control were further provided by a developmental pediatrician and a neuropsychologist. The cognitive team members, including training personnel, were blinded to the randomization group, as well as the history of the child and the family.

RPM [46] is a nonverbal test of cognitive performance, abstract reasoning, and problem-solving (fluid intelligence). Each child was presented with a matrix-like arrangement of symbols and asked to select the correct symbol to complete the pattern in multiple-choice format. No time limit was set to solve each problem. Thirty-six items were administered to each child. The verbal meaning test measures expressive language abilities and verbal skills. The test was designed in East Africa and had previously been used in Kenya, Uganda, and Tanzania [47]. The child was presented with four pictures and asked to point to the one named by the tester. Thirty-six items were administered to the children, with simpler items requiring recognition of simple nouns, while the most advanced items tested more abstract concepts. No time limit was set to identify each picture. The arithmetic test was adapted from the revised edition of the Weschler Intelligence Scale for Children [48] and assesses basic knowledge of arithmetic. The child was asked to add and subtract simple numbers and then proceed to more difficult items involving division, multiplication, and decimals. The test consisted of 19 orally presented arithmetic word problems and a time limit of 30 s to reply verbally with the answer to each question. The digit span tests are subsections of the revised edition of the Weschler Intelligence Scale for Children, and measure the child's ability to remember and orally repeat a sequence of numbers. The test consisted of 14 items for forward and backward recall. The tester read increasingly longer strings of numbers, which the child was asked to recall and repeat in forward order for the digit span forward test, and in reverse order for the digit span backward test. Although both forward and backward digit recall are reliant on short-term concentration, each has been noted to also require different mental processes: forward recall requires basic short-term auditory memory, while backward recall invokes more complex processes involving mental transformations. Digit span scores were thus analyzed in two different ways: separately and together as a digit span total score. The Beery–Buktenica Developmental Test of Visual–Motor Integration, sixth edition [49] was used to assess the extent to which individuals can integrate their visual and motor abilities. The participant is instructed to copy 30 geometric designs in increasing order of difficulty without a time limit. The designs are scored according to the visual–motor integration manual on the basis of accuracy and quality standards. Discontinuation item is reached when the participant fails to copy three consecutive designs that can be scored.

### Ethics

This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human participants were approved by the institutional and ethics review boards at the University of California, Los Angeles, Indiana University, and Moi University. Informed and written consent was obtained, by specifically trained staff, from female participants and from parents on behalf of their children. Children aged 7 years or older were also given the opportunity to give consent.

This study was part of a main study. The main study was registered at ClinicalTrials.gov (NCT00562874). CONSORT guidelines were followed in the design and reporting of this randomized controlled trial.

### *Statistical analyses*

Descriptive statistics were used to summarize baseline characteristics of the study population. Frequencies were reported for categorical variables, and the mean and standard deviation were reported for continuous variables. Chi-square tests were used to test for significant differences at the baseline across the three groups, and Fisher's exact tests were used in cases with small cell counts. Linear mixed effects models with random intercepts were used to model the covariance structures of the outcomes. Longitudinal analyses included only observations for school-age children older than 48 months. We plotted data in profile plots and histograms and tabulated summaries of covariates by treatment group and location. The data were longitudinal, with repeated measures nested within participants. Analysis generally followed methods described by Weiss [50]. Other covariates included indicators for study location (Mautuma, Soi, and Turbo), sex, baseline age, and the treatment group by time interaction. An age-squared covariate was found to be not significant, so it was not included in the final models. Participants were randomized to the three treatment groups after the baseline so no main effect for the treatment group is included to increase power. Data analysis was performed with the statistical software package Stata/SE 14. Validity of the models was confirmed by standard statistical methods. Differences in the rates of change of neurocognitive measurements by biscuit types were tested with an *F* test. We considered $P<0.05$ to be statistically significant.

## Results

The flow of participants is depicted in Fig. 1. Forty-nine children were enrolled as school-age children, age 4–8 years, with 18 (37%), 20 (41%), and 11 (22%) in the soy, beef, and wheat groups respectively. Forty-four percent were male. There were 196 visits. The attrition at each stage of the study is also shown in Fig. 1. For example, at 6 months, three children in the soy group had dropped out of the biscuit intervention: one child who did not remain in the intervention after the baseline, and two children who stopped their biscuit intake after 5.5 and 3 months respectively, leaving 15 children from the baseline count of 18.

Table 1 shows the baseline characteristics of age, sex, height, weight, BMI, head circumference, hemoglobin level and CD4 cell count, which did not differ significantly among children in the three randomized groups. Maternal characteristics are presented in Table 2. One mother had two children in the study. Maternal age, education, SES score, CD4 cell count, and hemoglobin level did not differ significantly across the three groups.

Baseline cognitive scores are presented in Table 3. There were no significant differences among soy, beef, and wheat groups in all but one outcome. The wheat group had an average verbal meaning score lower than the other two groups at the baseline.

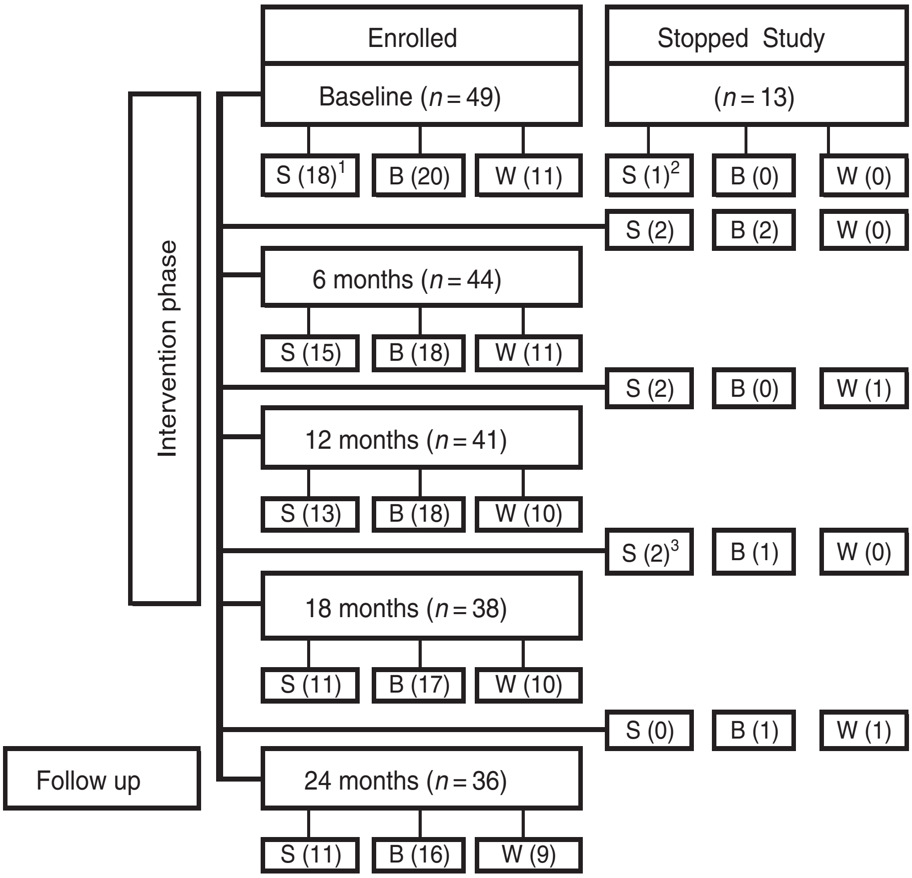
### *Mixed effects model results*

Table 4 shows the results from the mixed effects models for the eight outcomes. Outcome variables did not differ by study location or sex. There were significant increases in the outcomes' scores over time for all three biscuit groups, as would be expected through the developmental maturation of the children. The *F* tests reveal that these increases over time were not significantly different across biscuit groups for most outcomes. However, there were significant differences between biscuit groups in the RPM scores ($P=0.012$), with the scores of children in the soy group being almost two times higher than those in the beef and wheat groups. In pairwise tests, there was no significant difference between the beef and wheat groups ($P=0.849$). A complete list of pairwise test results for all outcomes is given in Table 5.

## Discussion

This study was a randomized, double-blind, controlled intervention trial to assess the effects of beef, soy, or wheat biscuit-type supplements on the neurocognitive outcomes of HIV-affected school-age children in Kenya. Mixed modeling analysis revealed that neurocognitive performance improved over time in all three groups. The rate of increase in nonverbal cognitive scores (fluid intelligence) was greater for children who received soy protein compared with children provided with beef or wheat supplementation.

Findings from our previous studies (see the introductory section) demonstrated improved academic and cognitive functioning among school-age children in Kenya receiving ASF supplementation [25–27]. The main differences in



[1] n=1 child HIV+ but drug naïve at baseline

[2] Received ≤1 day of intervention

[3] $n=1$ HIV+ child began antiretroviral drugs at 15 months

Fig. 1. Numbers of children randomized to receive a biscuit with soy (S), beef (B), or wheat (W) at enrollment who remained in the study or stopped participating in the study during or after the 18-month intervention phase.

the interventions between previous studies and the current research were the lack of soy supplementation in the previous school feeding studies, extension of feeding supplementation to mothers and siblings, and use of the biscuit model for supplementation rather than *githeri*-based mid-morning snacks. Hence, the present study offers valuable information on the effects of ASF versus soy supplementation in HIV-affected families and established the feasibility of biscuit-type supplementation using local resources.

The child biscuit intervention provided 14 g of protein per day [51]. Protein intake from the meat and soy biscuits provided children with 64–108% of their recommended levels, in contrast to the wheat biscuit supplement, which provided a lesser amount (25–42% of the recommendation) of incomplete protein, which is also of inferior quality [52, 53]. Children in the meat and soy groups both received lysine in amounts that met 80–135% of the recommended intake, while those in the wheat group did not receive lysine from the supplement [54, 55]. Vitamin $B_{12}$ was provided primarily to those in the meat group in an amount of 0.88 mg/day (1–8 years), representing 73–98% of the recommended intake. Higher amounts of absorbable zinc were available to those receiving meat, and the

Table 1. Children's characteristics by intervention group (*n*=49)

| | Soy (*n*=18) | Beef (*n*=20) | Wheat (*n*=11) | All (*n*=49) | *P* |
|---|---|---|---|---|---|
| Location | | | | | |
| Turbo | 11 (61.1%) | 12 (60.0%) | 2 (18.2%) | 26 (51.0%) | 0.117 |
| Soi | 3 (16.7%) | 5 (25.0%) | 6 (54.6%) | 14 (29.0%) | |
| Mautuma | 4 (22.2%) | 3 (15.0%) | 3 (27.3%) | 10 (20.0%) | |
| Baseline characteristics | | | | | |
| Males | 8 (47.0%) | 10 (50.0%) | 3 (27.3%) | 21 (43.8%) | 0.448 |
| Baseline age (months)[a] | 73.2 (17.3) | 68.4 (17.8) | 62.7 (15.6) | 68.9 (17.3) | 0.289 |
| CD4 cell count (/μL)[a] | 1046.3 (540.1) | 950.9 (406.0) | 1199.2 (467.0) | 1041.5 (469.2) | 0.378 |
| Hemoglobin (g/dL)[a] | 12.86 (0.9) | 12.02 (1.4) | 12.35 (1.3) | 12.39 (1.2) | 0.129 |
| Weight (kg)[a] | 18.1 (3.0) | 17.1 (3.6) | 15.5 (2.4) | 17.0 (3.2) | 0.124 |
| Height (cm)[a] | 110.7 (8.5) | 106.0 (10.9) | 105.2 (6.4) | 107.4 (9.3) | 0.219 |
| BMI (kg/m$^2$)[a] | 14.7 (1.3) | 15.0 (1.2) | 14.0 (1.1) | 14.6 (1.3) | 0.100 |
| Head circumference (cm)[a] | 49.9 (1.7) | 51.0 (2.2) | 49.1 (1.2) | 50.1 (1.9) | 0.117 |

Data were missing in 2% of individuals for sex, in 4% of individuals for CD4 cell count and hemoglobin level, in 6% of individuals for weight, height, and BMI, and in 53% of individuals for head circumference.

[a] The mean is given, with the standard deviation in parentheses.

Table 2. Characteristics of mothers by intervention group (*n*=48)

| | Soy (*n*=18) | Beef (*n*=19) | Wheat (*n*=11) | All (*n*=48) | *P* |
|---|---|---|---|---|---|
| Location | | | | | |
| Turbo | 11 (61.1%) | 11 (57.9%) | 2 (18.2%) | 24 (50.0%) | 0.134 |
| Soi | 3 (16.7%) | 5 (26.3%) | 6 (54.6%) | 14 (29.2%) | |
| Mautuma | 4 (22.2%) | 3 (15.8%) | 3 (27.3%) | 10 (20.8%) | |
| Education[a] | | | | | |
| Primary education | 12 (66.7%) | 10 (55.6%) | 9 (81.8%) | 32 (66.0%) | 0.464 |
| Secondary education | 6 (33.3%) | 6 (33.3%) | 2 (18.2%) | 14 (29.8%) | |
| Middle college | 0 (0%) | 2 (11.1%) | 0 (0%) | 2 (4.3%) | |
| Baseline age (years)[a] | 37.8 (6.8) | 35.3 (5.8) | 33.0 (7.8) | 35.7 (6.8) | 0.168 |
| CD4 cell count (/μL)[a] | 430.4 (134.1) | 466.0 (177.4) | 501.1 (198.1) | 461.3 (166.6) | 0.552 |
| Hemoglobin (g/dL)[a] | 12.4 (2.0) | 12.3 (2.1) | 11.9 (1.2) | 12.3 (1.9) | 0.729 |
| SES score[a] | 0.31 (0.15) | 0.30 (0.19) | 0.27 (0.19) | 0.30 (0.17) | 0.881 |

Data were missing in 4% of individuals for hemoglobin level and CD4 cell count, and in 2% of individuals for education.

SES, socioeconomic status.

[a] The mean is given, with the standard deviation in parentheses.

meat biscuit supplements were also lower in fiber and phytate, thus increasing bioavailability of the micronutrient. Children in the soy biscuit intervention group received greater amounts of absorbable iron because of the high amount of iron in soy flour, but the soy biscuit also contained fiber as well as phytate, with ratios with respect to iron that are known to inhibit mineral absorption. It is possible that higher iron content in the soy biscuit had a noticeable effect on children's cognitive performance, despite the inhibitory effects of high fiber and phytate in the soy biscuits.

Table 3. Baseline cognitive scores by intervention group (*n*=49)

| Outcomes | Soy (*n*=18) | Beef (*n*=20) | Wheat (*n*=11) | All (*n*=49) | *P* |
|---|---|---|---|---|---|
| Digital span forward | 3.44 (1.2) | 3.05 (1.8) | 2.63 (1.3) | 3.10 (1.5) | 0.366 |
| Digital span backward | 1.33 (1.4) | 0.85 (1.4) | 0.45 (0.8) | 0.94 (1.3) | 0.212 |
| Digital span total | 4.78 (2.3) | 3.90 (2.9) | 3.09 (1.6) | 4.04 (2.5) | 0.196 |
| Raven's progressive matrices total | 13.6 (2.9) | 13.6 (3.4) | 12.9 (5.4) | 13.4 (3.7) | 0.867 |
| Verbal meaning total | 26.2 (6.2) | 25.1 (6.1) | 19.9 (8.1) | 23.3 (6.9) | 0.046 |
| Arithmetic total | 4.67 (2.9) | 4.20 (3.0) | 2.63 (2.4) | 4.02 (2.9) | 0.174 |
| Embedded figure test total | 9.50 (1.9) | 9.15 (2.4) | 8.00 (3.3) | 9.02 (2.5) | 0.269 |
| Beery VMI total | 7.44 (3.5) | 6.70 (2.6) | 5.81 (2.7) | 6.78 (3.0) | 0.368 |

VMI, visual–motor integration. The mean is given, with the standard deviation in parentheses.

Table 4. Mixed effects models for cognitive outcomes (*n*=49)

| | Digit span forward | | | Digit span backward | | | Digit span total | | | Raven's matrix total | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Est | SE | *P* | Est | SE | *P* | Est | SE | *P* | Est | SE | *P* |
| Study location | | | | | | | | | | | | |
| Turbo | – | – | – | – | – | – | – | – | – | – | – | – |
| Soi | 0.55 | 0.35 | 0.117 | 0.64 | 0.32 | 0.042 | 1.14 | 0.61 | 0.063 | 1.46 | 0.92 | 0.112 |
| Mautuma | 0.28 | 0.37 | 0.446 | –0.11 | 0.34 | 0.747 | 0.09 | 0.66 | 0.893 | 0.75 | 0.97 | 0.441 |
| Female | 0.41 | 0.29 | 0.153 | 0.47 | 0.27 | 0.079 | 0.90 | 0.52 | 0.080 | –0.39 | 0.77 | 0.615 |
| Baseline age | 0.46 | 0.10 | <0.001 | 0.59 | 0.09 | <0.001 | 1.06 | 1.18 | <0.001 | 0.62 | 0.27 | 0.024 |
| Time×soy | 0.79 | 0.16 | <0.001 | 1.01 | 0.15 | <0.001 | 1.73 | 0.24 | <0.001 | 4.06 | 0.56 | <0.001 |
| Time×beef | 0.66 | 0.14 | <0.001 | 0.73 | 0.13 | <0.001 | 1.40 | 0.21 | <0.001 | 2.19 | 0.50 | <0.001 |
| Time×wheat | 0.57 | 0.17 | 0.001 | 0.59 | 0.16 | <0.001 | 1.21 | 0.26 | <0.001 | 2.33 | 0.61 | <0.001 |
| Test of interaction | | *F* | *P* | | *F* | *P* | | *F* | *P* | | *F* | *P* |
| Time×biscuit group | | 0.48 | 0.620 | | 2.07 | 0.126 | | 1.18 | 0.309 | | 4.43 | 0.012 |

| | Verbal meaning total | | | Arithmetic total | | | Embedded figure test total | | | Beery VMI total | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Est | SE | *P* | Est | SE | *P* | Est | SE | *P* | Est | SE | *P* |
| Study location | | | | | | | | | | | | |
| Turbo | – | – | – | – | – | – | – | – | – | – | – | – |
| Soi | 1.98 | 1.48 | 0.181 | 1.57 | 0.59 | 0.008 | 0.67 | 0.49 | 0.172 | 1.61 | 0.65 | 0.014 |
| Mautuma | –1.73 | 1.58 | 0.274 | 0.63 | 0.63 | 0.318 | –0.59 | 0.52 | 0.252 | –0.16 | 0.70 | 0.821 |
| Female | –0.03 | 1.25 | 0.976 | 0.72 | 0.50 | 0.149 | –0.05 | 0.41 | 0.897 | –0.02 | 0.55 | 0.996 |
| Baseline age | 1.63 | 0.44 | <0.001 | 1.12 | 0.17 | <0.001 | 0.39 | 0.14 | 0.007 | 0.74 | 0.19 | <0.001 |
| Time×soy | 4.99 | 0.64 | <0.001 | 2.30 | 0.23 | <0.001 | 1.13 | 0.23 | <0.001 | 1.55 | 0.32 | <0.001 |
| Time×beef | 3.90 | 0.57 | <0.001 | 1.75 | 0.20 | <0.001 | 1.07 | 0.21 | <0.001 | 1.40 | 0.28 | <0.001 |
| Time×wheat | 4.40 | 0.69 | <0.001 | 1.73 | 0.25 | <0.001 | 1.28 | 0.26 | <0.001 | 1.93 | 0.35 | <0.001 |
| Test of interaction | | *F* | *P* | | *F* | *P* | | *F* | *P* | | *F* | *P* |
| Time×biscuit group | | 0.93 | 0.396 | | 2.26 | 0.105 | | 0.23 | 0.794 | | 0.77 | 0.464 |

Est, estimate; SE, standard error; VMI, visual–motor integration.

Table 5. Pairwise differences of test scores for cognitive outcomes by biscuit group in mixed models ($n$=49)

| Outcomes | Soy–beef | | Soy–wheat | | Beef–wheat | |
|---|---|---|---|---|---|---|
| | Est[a] | 95% CI | Est[a] | 95% CI | Est[a] | 95% CI |
| Digit span forward | 0.13 (0.20) | −0.27 to 0.52 | 0.22 (0.23) | −0.23 to 0.67 | 0.09 (0.22) | −0.33 to 0.51 |
| Digit span backward | 0.28 (0.19) | −0.09 to 0.64 | 0.42 (0.21) | 0.00–0.84 | 0.14 (0.20) | −0.25 to 0.54 |
| Digit span total | 0.33 (0.31) | −0.27 to 0.94 | 0.52 (0.35) | −0.17 to 1.20 | 0.18 (0.33) | −0.46 to 0.83 |
| Raven’s progressive matrices total | 1.87 (0.67)[b] | 0.56–3.18 | 1.73 (0.77)[b] | 0.22–3.23 | −0.14 (0.72) | −1.56 to 1.28 |
| Verbal meaning total | 1.10 (0.81) | −0.48 to 2.68 | 0.60 (0.91) | −1.19 to 2.38 | −0.50 (0.86) | −2.19 to 1.19 |
| Arithmetic total | 0.56 (0.29) | −0.01 to 1.13 | 0.58 (0.33) | −0.07 to 1.23 | 0.02 (0.31) | −0.59 to 0.63 |
| Embedded figure test total | 0.07 (0.29) | −0.51 to 0.64 | −0.15 (0.33) | −0.80 to 0.51 | −0.21 (0.32) | −0.83 to 0.40 |
| Beery VMI total | 0.15 (0.40) | −0.63 to 0.93 | −0.38 (0.45) | −1.36 to 0.31 | −0.52 (0.43) | −0.36 to 4.48 |

CI, confidence interval; Est, estimate; VMI, visual–motor integration.

[a] The standard error is given in parentheses.

[b] $P<0.05$.

There is evidence to suggest that flavonoids, abundant in soy, have the potential to enhance human memory and neurocognitive performance through their ability to protect vulnerable neurons, enhance existing neuronal function, and stimulate neurogenesis [56]. Flavonoids may exert effects on long-term potentiation, and consequently strengthen memory and cognitive performance, through their interactions with these neuronal signaling pathways, which include the phosphatidylinositol-3 kinase–protein kinase B, protein kinase C, protein kinase A, Ca–calmodulin kinase, and mitogen-activated protein kinase mechanisms [57–59]. Isoflavones can also increase choline acetyltransferase and messenger RNA levels of neurotrophins in the hippocampus and frontal cortex [60]. Animal studies have shown that flavonoids improve visuospatial memory (or visuospatial working memory) [61, 62]. Human clinical studies on neurocognitive-enhancing effects of flavonoids, however, have been mostly focused on older adults, including individuals with HIV dementia, and postmenopausal women, with little research focused on children [63–65]. After 6 weeks of soy supplementation, postmenopausal women demonstrated improved frontal lobe function, specifically in mental flexibility and planning ability, compared with women in the placebo group [28]. Neurocognitive improvements from a high-soy diet were found to extend to young adults and spatial memory of men as well [57]. A study on student volunteers who received a high-soy diet showed significant improvements in short-term memory (immediate recall of prose and 4-s delayed matching to sample of patterns) and long-term memory (picture recall after 20 min) and in mental flexibility (rule shifting and reversal) [66].

Soy isoflavones are also implicated in immune functioning [58]. Specifically, genistein is structurally similar to 17β-estradiol, and it suppresses antigen-specific immune response *in vivo* and lymphocyte proliferation response in vitro [59]. Soy isoflavones may also adjunctively preserve neuronal functioning and sustain neurocognitive abilities of HIV-1-infected persons, by diminishing apoptotic signaling induced by the HIV-1 viral protein Tat [67]. It is thus possible that soy nutrients, including soy isoflavones, may have a role in improving the mother’s immune functioning and mental capacities, and these effects may be carried over to the developmental stimulation of the child. Siblings may also have benefitted from the feeding intervention, and older siblings may have the capacity to provide caretaker support and academic fostering as well.

Fluid intelligence is a major component of intellect, central to executive functioning, logical reasoning, and other frontal functions [68–70]. There is some evidence from optical topography to suggest that visuospatial working memory shares a common neural system with general fluid intelligence within the lateral prefrontal cortex during the preschool years [71]. Although there is a paucity of data on the effects of soy isoflavones on the cognitive functioning of children, it is possible that the improvements in spatial memory, mental flexibility, and working memory noted in adults receiving high-soy diets may similarly apply to improvement of fluid intelligence in HIV-affected school-age children provided with soy supplementation, mediated by neural pathways in the frontal and prefrontal regions.

The strengths of this study include the randomized, double-blind controlled design; the provision of nutritional supplementation to the mother and siblings as well as the target child to study the effects in HIV-affected families; the use of detailed, longitudinal assessments based on the age of enrollment; the use of DOT to ensure 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 the Academic Model Providing Access to Health care partnership in western Kenya. Limitations included the relatively small sample size of 49 school-age children, which may limit the power to detect differences in the test scores across biscuits. The dropout rate was high in working with families in this resource-poor area. Families' willingness to be followed up over time could not be ensured, and some fathers might not have wanted the mother and child to continue in the study. Some families, particularly those who dropped out within the first 6 months, were wary because of the stigma associated with HIV infection [72]. Inclement weather conditions affected contact with families, as road travel to meet in person was the only means of contact without telephone or digital access. Families might not have been present at the appointed day/time (e.g., went to town for medical appointment or to run an errand), necessitating another road trip to meet the family for follow-up. If a family missed an appointment, other logistical issues had to be solved (e.g., the assessment team might not be able to meet other families as scheduled). Another limitation is that it was not possible to elucidate mechanisms by which the food supplement modulated neurocognitive and academic skills in this study. Pathways mediated by the effects of supplemental feeding for mothers and siblings as well as mother–child and child–siblings interactions could not be clarified.

In conclusion, the results from this three-arm randomized, double-blind nutrition intervention trial of the neurocognitive-enhancing effects of ASF supplementation on HIV-affected, nutritionally at risk school-age children in Kenya indicated that nonverbal cognitive (fluid intelligence) performance improvements in school-age children over time were greater for children who received soy protein than those who received ASFs. It is possible that soy nutrients, including soy isoflavones, may augment nonverbal cognitive skills in HIV-affected school-age children, and these effects may be mediated by the mother's health improvement and her ability to provide developmental and educational stimulation. Further studies are needed to elucidate the neurocognitive-enhancing effects of soy biscuit intervention on HIV-affected children. Soy protein may be a good candidate for future nutrition interventions as it can be grown locally and may be less expensive than meat in poor rural areas.

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## Conflicts of interest

The authors declare no conflict of interest.

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## Significance statement

Research on neurocognitive functioning of children prenatally exposed to HIV, and at risk of malnutrition, has been lacking. This study employs locally-manufactured isocaloric biscuits made with wheat, soy and beef, to deliver nutritional supplementation in a standardized, quantifiable and sustainable way in poor rural areas. The main finding from this 3-arm randomized, double-blind controlled intervention trial, was that school-aged HIV-affected children provided with soy protein supplementation attained non-verbal cognitive scores that were almost two times higher than those in the beef and wheat groups. The finding that soy was advantageous to beef was unanticipated, but there is evidence from the literature that soy nutrients, including soy isoflavones, may play a role in augmenting fluid intelligence.

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