A pilot randomized controlled trial of a new supplementary food designed to enhance cognitive performance during prevention and treatment of malnutrition in childhood

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Running title: New food formulation for prevention and treatment of MAM

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Abstract

Background. Cognitive impairment associated with childhood malnutrition and stunting is generally considered irreversible.

Objective. To test a new nutritional supplement for prevention and treatment of moderate-acute malnutrition (MAM) focused on enhancing cognitive performance.

Methods. An 11-week village-randomized controlled pilot trial was conducted in 78 children aged 1-3 or 5-7 years living in villages in Guinea-Bissau. The supplement formulation contained 291 kcal for young children and 350 kcal/day for older children, and included 5 nutrients and 2 flavan-3-ol rich ingredients not present in current food-based recommendations for MAM. Local bakers prepared the supplement from a combination of locally-sourced items and an imported mix of ingredients, and it was administered by community health workers 5 days per week. The primary outcome was executive function abilities at 11 weeks. Secondary outcomes included additional cognitive measures, and changes in z-scores for weight (WAZ) and height (HAZ), and hemoglobin levels at 11 weeks. An index of cerebral blood flow was also measured at 11 weeks to explore the use of this measurement as a biological index of cognitive impairment.

Results. There were no significant differences in any outcome between groups at baseline. There was a beneficial effect of randomization to the supplement on working memory at 11 weeks in 1-3 year children (P<0.05). This difference contrasted with no effect in older children and was not associated with faster growth rate. In addition, cerebral blood flow correlated with task switching performance (P<0.05).

Conclusions. These preliminary data suggest that cognitive impairment can be monitored with measurement of cerebral blood flow. In addition, the data provide preliminary data suggesting it may be possible to improve poor cognitive performance associated with in young children through changes in the nutritional formulation of supplementary foods used to prevent and treat MAM. Powered studies of the new supplement formulation are needed.
Key Words: Stunting, moderate-acute malnutrition (MAM), Guinea-Bissau, ready-to-use supplementary food, blended fortified foods, cognition, weight-for-age z-score, height-for-age z-score, cerebral blood flow, near-infrared spectroscopy.
Introduction

Malnutrition and stunting occurring in early childhood are associated with a range of cognitive impairments including deficits in executive functioning (e.g. attention span, working memory), leading to poor school performance in later childhood (1-3). These functional problems can be traced to a range of structural and neurochemical deficiencies including glial and dendritic development in areas of the brain including the prefrontal cortex and hippocampal structures (4, 5). Moreover, interventions designed to prevent or treat malnutrition generally find little or no improvement in cognitive measures when administered solely as a nutrition supplement without other intervention components such as medical treatment or social enrichment (4, 6-9), which has led to the perception that the effects of childhood malnutrition on cognition are largely irreversible (10).

An alternative possible explanation for the long-term impairment in cognition associated with stunting and malnutrition is that recovery with food-based treatments is possible but typical foods used for this purpose are not yet nutritionally optimal. Current recommendations for ready-to-use supplementary foods (RUSFs) and fortified blended foods (FBFs) do not include several nutrients that research in other populations has indicated are important for cognitive health. For example, choline is recognized as an essential nutrient required for synthesis of the neurotransmitter acetylcholine and maintenance of cell membranes (11), and low levels of dietary choline reduce brain volume in animal models such as young pigs (12). In addition, stunting in early childhood is recognized to be associated with impaired cognition (13) and is also associated with low circulating choline (14). Nevertheless, this type of evidence does not rise to the level of being high quality, and choline is not currently included in World Health Organization (WHO) recommendations for prevention and treatment of malnutrition. The trace
elements chromium and molybdenum are also not included in current WHO requirements and, while there is little evidence for any effect on cognition in children, deficiency states in elderly and young adults have been suggested to be linked to impaired cognition (15-17). Omega-3 polyunsaturated fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and their relationship to omega-6 fatty acids, are also not included in current recommendations for RUSFs and FBFs. However, omega-3 fatty acids are important components of neuronal membranes, and while their effects appear limited in healthy infants born at term (18) some studies in children and the elderly indicate they may have broad cognitive benefits in nutritionally-challenged populations including to promote neuronal growth and influence signal processing and neural transmission (19-21).

There are also foods containing classes of bioactive chemicals that are not currently categorized as essential nutrients but which cross the blood-brain barrier and have neuropsychological effects that may be beneficial for cognitive repair in the prevention and treatment of malnutrition (22, 23, 24, 25). In particular, cocoa and green tea contain a class of polyphenols with an array of bioactions including antioxidation, anti-inflammation, and glucoregulation that have been documented to promote neurogenesis, reduce neuronal injury, and increase vasodilation and cerebral blood flow in non-malnourished individuals (26). There have been no studies to date examining whether the consumption of cocoa or green tea could promote recovery of cognitive damage resulting from childhood malnutrition, but the accumulating evidence from other populations justifies studies exploring their effectiveness. Cocoa and green tea also contain caffeine, which we hypothesized might have synergistic benefits for cognitive protection during prevention and treatment of malnutrition, due to its recognized neurocognitive stimulatory effects (27).
We designed a nutritional formulation to improve cognitive function during nutrition interventions to prevent and treat stunting and moderate-acute malnutrition (MAM), based on the considerations described above, and conducted a pilot study to obtain data for power calculations for a future powered trial on its impact on cognitive performance and growth. The work was conducted in village children in Guinea-Bissau, West Africa.

**Methods**

**Study location and participants**

Guinea-Bissau is a small low-income country in West Africa with a population of 1.7 million. Families living in rural villages cultivate most of the food they eat, including rice (the staple food), millet, corn, sorghum, groundnuts, cassava, sweet potatoes, mangoes, and domestic animals. In addition they catch wild foods including fish and small mammals, and grow cashews to sell for additional rice and other popular foods including sugar, oil and bread.

The study was conducted in two Mandinka tribe villages in the Oio district of Guinea-Bissau, which is one of the poorest regions of the country and has very high rates of stunting and moderate-acute malnutrition (28). The villages were a convenience sample that were comparable in terms of number of inhabitants, ethnicity, religion and interest in study participation, and were also sufficiently large to make study recruitment feasible. Inclusion criteria were: parents willing to have their child participate (no child refused), and the child was reported to be 2-3 years of age (20 per village) or 6-7 years of age (20 per village). Moderate-acute malnutrition (MAM) was not an inclusion criteria out of concern that the supplement could benefit children with non-obvious nutritional deficiencies (e.g. iron) and because in a previous study we found that enrolling only malnourished children resulted in non-adherence to an assessment-only control regimen (29). An additional inclusion criterion for the older age group was that the child was enrolled in first grade in the village elementary school. Exclusion criteria
were: the child had one or more food allergies, he or she would not be in the village for the duration of the study, or had severe acute malnutrition (SAM) as indicated by a mid-upper arm circumference (MUAC) in the red zone of a tape measure (in which case the parents were advised to take their child to the nearest tertiary clinic). Children were enrolled in the study based on parental report of age. However, when parents presented participants’ birth certificates at the end of the study, some subjects were found to be outside the original age range criteria. These data were retained in analyses with Institutional Review Board (IRB) permission, and children’s ages were 1-3 years and 5-7 years.

IRB permission to conduct the study and all of the measurements was provided by the The National Committee of Ethics in Health, which is the relevant sub-body of the government of Guinea-Bissau, and informed consent was obtained in the local language. In addition, Tufts University provided permission for all measurements except near infrared spectroscopy (NIRS), and NIRS was approved as a sub-study by the Massachusetts General Hospital IRB. A post-hoc agreement was implemented to share de-identified data between the two U.S. institutions. Following an explanation of the study protocol, all mothers or legal guardians of children agreed to participation, and provided their informed consent with a signature or thumbprint. The consent process took place in the presence of a member of the research team and a community health worker. Participating families received an allotment of rice as an expression of appreciation for their participation.

Study Protocol

This 11-week pilot study was a village-randomized controlled study comparing supplementation to an assessment-only control in 2 villages. Two age groups, 1-3 and 5-7 years, were studied (participant demographic information reported in Table 1) and we hypothesized significant effects of the supplement on cognitive function in both age groups. The supplement was
provided 5 days per week for 11 weeks to intervention children in the village Community Health Center. The mothers or primary caretakers brought children to the supplement center and children were watched by the community health workers while they ate their food. If any supplement was not consumed, the consumed amount was recorded and the child was sent home with the remainder for use later in the day. Children who did not come were marked absent and received no supplement that day. The primary outcome was children’s executive function abilities (working memory, reverse categorization), and secondary outcomes included growth, hemoglobin, and skin carotenoids. In addition, an index of cerebral blood flow ($\text{CBF}_i$) and an index of tissue absorption ($\mu_{\text{ai}}$) was measured at 12 weeks via a NIRS method called diffuse correlation spectroscopy (DCS) (30) to evaluate NIRS as a method potentially suitable for assessment of cognitive function in large population studies.

Baseline assessments included demographic information, cognitive variables, anthropometry (weight, height, head circumference and MUAC), and skin carotenoids in all children. After baseline, villages were assigned a number and their treatment (intervention or control) was based on a randomization schema generated in SAS. The villagers randomized to the intervention group received the new supplement at the community health center 5 mornings per week. Community health center workers distributed the supplement and tracked supplement attendance and consumption after receiving training by the research staff from the International Partnership for Human Development and the U.S. team. When a child’s caregiver was routinely someone other than the mother, that caregiver was allowed to bring the child for supplement distribution. Outcome assessments obtained at baseline were repeated at the end of the study period before the supplement distribution was completed. In addition, measurements of $\text{CBF}_i$ and tissue absorption with NIRS-DCS were only performed at the end of the study.

Concerning our brain blood flow assessments, we hypothesized that the measure of $\text{CBF}_i$ and
μai by NIRS-DCS would provide an assessment of the level of neuronal maturation. Oxygen, delivered to the brain through the blood, is consumed by oxidative glucose metabolism to produce adenosine triphosphate (ATP), which provide energy to the neurons. Increases in neuronal activity cause increases in cerebral metabolism, which are associated with increases in blood volume and blood flow (the so called neurovascular-coupling). The coupling between neuronal activity, cerebral oxygen metabolism and cerebral blood flow is present during functional activity, resting state and brain developmental maturation. In particular, cerebral metabolic rate of oxygen (CMRO₂) and cerebral metabolic rate of glucose (CMRGl₇) are known to increase with age in children and these increases matches behavioral, neurophysiological, and anatomical maturation occurring during development (31-33). While these changes have been demonstrated with PET, we have recently replicated these findings in infants non-invasively with NIRS-DCS. For example, in premature infants we have demonstrated CBF increases with postmenstrual age (34) and is higher in brain regions that are known to mature earlier (35). We also found that cerebral blood volume increases three-fold during the first year of life across frontal, temporal and parietal regions (36)—results consistent with increases in glucose metabolism observed with PET (37).

The supplementary food

The supplement formulation used in this study was designed as a substitute for typical ready to use supplementary foods (RUSFs) and fortified blended foods (FBFs), which are used in a variety of ways in low-income countries including in supplementary feeding programs for mothers and children to prevent growth faltering, for community treatment of MAM, and for school feeding programs (38, 39). Due to practical considerations, the supplementary nutrients were delivered in a combination of a locally prepared biscuit and chewable micronutrient gummies (Children’s MV-Alive, Nature’s Way Products LLC, WI; Vitamin Friend’s Iron, Vitamin Friends LLC, CA) but future use of the formulation could include combinations of the ingredients.
and nutrients into a single mix or ready-to-use product. The general goals of the new supplement were to a) increase anticipated nutrient intakes from home food to levels recommended by the WHO (18); and b) to levels recommended by the Institute of Medicine for those nutrients that are not recommended by WHO (see reference 19); and c) include cocoa and green tea as ingredients for their flavan-3-ol (and caffeine) contents. Specifically, the new nutrients included choline provided at 50% adequacy due to the difficulty of incorporating the total recommended intake into a single food serving (11); fish oil containing the essential omega-3 polyunsaturated fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (19); two additional micronutrients (molybdenum, chromium) that have been associated with reduced cognition in both elderly and younger adults (15-17) and are defined as essential nutrients in U.S. national requirements for healthy children (40); high flavan-3-ol sources of cocoa and green tea (26); 50% greater levels of Vitamin K than WHO recommendations (41); and calcium levels naturally existing in ingredients without additional fortification to facilitate iron absorption, taking into consideration both the negative effect of high levels of calcium on non-heme iron absorption and the potential for added polyphenols to reduce absorption (42).

The biscuit was designed to provide 300 kcal/day for the older children and 250 kcal/day for the younger children, and was prepared locally from a combination of the mix of new ingredients and the locally-available ingredients peanuts, blended vegetable oil, honey, sugar, eggs and dried powdered moringa (moringa oleifera). The calculated macronutrient composition of the biscuit as formulated is given in Table 2. Village bakers were trained by the research staff to prepare the biscuits using hygienic techniques, keep records of production and deliver the baked goods to community health centers for distribution, and quality control worked well except for occasional batches that were overcooked. Community health workers distributed the supplement in the morning, made records of attendance, observed consumption and recorded amounts consumed. Both community health workers and bakers received a small stipend for
their work. Oversight of quality control for supplement preparation and study record-keeping was conducted in twice-weekly visits by a local study coordinator.

Cognitive assessments

The cognitive assessments included two measures of executive functioning in early childhood: working memory and task-switching abilities. Specific tasks were chosen based on their potential to be culturally adapted for the local villages, to be reliably conducted by the local research team, to require minimal instructions to the children, and to fit within a 15-minute testing timeframe (given the expected time needed for the other study measures and uncertainty of how long individual children in this specific setting would be interested in participating in the assessments).

The assessments were administered in a quiet room in the local village by a member of the local research team who had been trained by the senior researcher who designed the tests (PM) and followed a script designed by author PM for all test implementation. Tasks were administered in the local language, either solely by the local research team or with an additional translator who repeated the local researchers’ instructions and was instructed not to interfere or provide additional task directions to the children. Children could participate alone or could sit on the knee of their mother or a caregiver, as they preferred. Any other adults present were seated behind the child, not told of the specific behaviors or measures of interest for each task, and were instructed to not help or provide answers to the child. If a child became fussy or upset, the individual task or entire assessment was stopped; and caregivers were also instructed that they could stop the assessments at any time. All sessions were video-recorded for later blinded coding by coders unaware of participant randomization. Sessions were also reviewed by experienced test administrators for protocol adherence.
Working memory was assessed in both young and older children with the *Spin the Pots* task (43); age modifications guided by (44). Children were presented with an array of small opaque cups, each covered by a lid with a distinct color and/or pattern, all placed upon a circular base platform. At the start of the task, children were shown that the lids could be removed and that stickers could be hidden inside each cup. The test administrator hid stickers in a subset of the total number of cups (young children: 4 of 6 total cups; older children: 8 of 10 total cups) and then covered the entire array with an opaque cover. The test administrator then lifted the cover and allowed the child to search for a sticker. If the child found a sticker, the child could keep the sticker; if they did not find a sticker, the test administrator let the child see the bottom of the empty cup and told them there was not a sticker there. The circular base was then covered and rotated 180°. The cover was then removed and the child was allowed to search again. This procedure continued until the child found all the hidden stickers or until they reached a predetermined set of trials (young children: 12 trials; older children: 18 trials). Children’s performance was assessed by the total number of stickers found as well as a categorical assessment of whether children found all of the hidden stickers.

Task-switching was assessed through a *Reverse Categorization task* modeled after Carlson et al. (45). The 1-3 year old children were presented with a basket full of animals and told that the basket included both ‘mommy’ animals and ‘baby’ animals. They were then shown two boxes: one box had an image of an adult animal on the front of the box and the other box had an image of a baby animal on the front of the box. The test administrator directed the child’s attention to the front of the boxes and labeled them the “Mommy” and “Baby” boxes. The test administrator then told the child they were going to play a game in which they put ‘baby’ animals in the ‘baby’ box and ‘mommy’ animals in the ‘mommy’ box. The test administrator then demonstrated accurate sorting with 6 animals (3 mommy, 3 baby) during a pre-switch phase. He instructed the child, “This is a [baby/mommy] animal, so it goes in the [baby/mommy] box.” The child was then
allowed to sort the next 6 animals (3 mommy, 3 baby). For each animal, the test administrator labeled the animal (‘baby’ vs. ‘mommy’) and asked the child where the animal should go. Children were praised for accurate sorting or corrected with a restatement of the rule, for each of the 6 trials. Children were next told that they were now going to play a “silly game” in which they put all the ‘mommy’ animals in the ‘baby’ box and all of the ‘baby’ animals in the ‘mommy’ box. During this post-switch phase, children were then allowed to sort 12 additional animals (6 mommy, 6 baby). The experimenter labeled the size and restated the rule for each animal; however, they did not correct inaccurate sorting. The procedure was similar for the 6-year-old children, with the following exceptions. During the pre-switch phase, the test administrator labeled the size of only the first two animals (e.g., mommy cow), subsequently only labeling the kind of animal (e.g., cow) for the remaining 4 animals. During the post-switch phase, the test administrator labeled only the kind, but not the size of the animal. He also only reminded the children of the rule on the 1st and 7th trial. Similar to the 2-year-old children, 6-year-old children were corrected for errors during the pre-switch, but not the post-switch phases. We coded the total number of accurate sorts during the post-study phase.

Two additional cognitive assessments were also conducted but are not reported or discussed here. An object-directed manual exploration task, which was conducted only in 2-year-old children, was designed to assess children’s rate of habituation and novelty preferences and involved free play with a series of small animal toys. Review of the videotaped procedures revealed test administrator difficulty in adhering to study protocol; therefore, the assessment was deemed ineligible for data analysis. A delay of gratification test, which was conducted only in the 6-year-old children, involved measuring how long children could wait to play with a novel set of toy cars and rollercoaster. Since almost none of the children were willing to wait any length of time, there was too little variability to assess differences between or changes within
conditions. As both of these tasks occurred as the final cognitive assessments for the respective age groups, and thus could have no impact on the earlier cognitive assessments.

**Anthropometric and biochemical assessments**

Outcome assessments were performed by a group of trained medical/nursing staff seconded from the Guinea-Bissau Ministry of Health who had no role in study design or supplement distribution.

Non-fasting weight was measured at baseline and 11 weeks using a digital calibrated scale weighing to ±0.1 kg (floor scale model 813, Seca, Chino, CA). Height was assessed using an upright stadiometer measuring to 0.1 cm (model 213, Seca). Mid-upper arm circumference (MUAC) was taken at the midpoint between the acromion process of scapula and olecranon process with a paper tape using standardized methods (46).

Hemoglobin was measured in duplicate by pulse co-oximetry, a non-invasive technique validated for anemia screening (27) which uses a multiple wavelength spectrophotometric sensor situated in a comfortable finger clip (Pronto-7, Masimo Corp, Irving CA). Due to technical challenges in the field, the hemoglobin measurements could not be collected at baseline, so only 11-week data are available.

Skin carotenoid content was measured in duplicate in the palm of each hand by Resonance Raman Spectroscopy (47, 48), to provide an index that could potentially serve as an independent marker of supplement adherence (since the supplement contained carotenoids). Measurements were made in the palm of the hand because the carotenoid concentration is high and differences in pigmentation among various skin types are minimal at this location, and the stratum corneum thickness of the palm (~400 μm) is high compared with other skin
sites. Measurements were made with a NuSkin scanner (Pharmanex Global Research, UT),
which uses a laser power of <10 mW and an exposure time of 30 seconds per measurement
with an elliptical spot size of 2 mm by 3 mm.

Cerebral blood flow by near infrared spectroscopy

To assess cerebral hemodynamics we used NIRS-DCS. DCS uses near-infrared light and, in addition to
quantifying an index of tissue absorbance ($\mu_{a,i}$) by measuring light attenuation at 785 nm, it also quantifies
cerebral blood flow (CBF$_i$) by measuring the temporal fluctuations of the light speckle pattern generated
by the dynamic scattering of red blood cells (49, 50). Numerous studies in humans and in animals have
shown that CBF$_i$ agree very well with CBF values measured with gold standard methods (30, 51, 52). By
simultaneously measuring both cerebral hemoglobin oxygenation (SO$_2$) and CBF$_i$ in previous studies in
infants we have shown that CBF$_i$ is more tightly correlated with cerebral oxygen metabolism than SO$_2$
(53). To measure both parameters, a combination of two NIRS devices is needed. For this pilot study we
only had room for one device and chose the DCS, since CBF is a superior indicator of brain development
(34, 35, 54) and brain health (55) compared to SO$_2$.

For this study we used a custom DCS device built at the Martinos Center at the Massachusetts
General Hospital. The system includes 4 photon counting avalanche photodiode detectors, one
long coherence length laser at 785 nm, and a custom FPGA based software correlator.

Acquisition rate was set at 2 Hz per data point. Custom fiber optics monitors were used to
measure the children enrolled in the study. The light power at the probe was around 20 mW and
diffused over a 5 mm diameter spot. This was well under ANSI standards limits for laser light
exposure. Light was detected at 0.5, 1.5, 2 and 2.5 cm distances of separation from the source.
Because of the low light level detected at the largest distance, only two separations (1.5 and 2.0
cm) where used to calculate $\mu_{a,i}$ (see (56) equation 2) and one separation (2 cm) was used to
calculate CBF$_i$ (57). In particular, to obtain the blood flow index, we fit Eq. (7) of (57) to the
measured temporal auto-correlation functions at 2 cm. The short separation (0.5 cm) DCS data
was also analyzed and used to discriminate extracerebral blood flow (scalp BF$_i$) from CBF$_i$. A scattering coefficient of 5 cm$^{-1}$ was assumed in all groups to calculate CBF$_i$ and $a_{mi}$ (58). An average of 20 data points over the 10 seconds of acquisition was sufficient to eliminate noise from the intensity and autocorrelation curves. For these measurements children were asked to seat still in a chair in front of a study staff, in a room with low light, and to remain still for the whole examination period, 1-2 minutes. Measurements consisted of positioning the optical monitor on the upper forehead of the subject and keeping it in place by hand for 20 seconds during data recording. Measurements were acquired in the left and right forehead corresponding to FP1 and FP2 in the EEG 10-20 system, and if the child moved or the detected light signal was low, measurements were repeated one more time. No pressure was applied to the monitor, which was sanitized between subjects (59).

**Data analyses**

This was a pilot study designed to generate data for power calculations for a future powered trial, and the primary hypothesis was that randomization to receive the new supplement would result in beneficial effects on cognition in both age groups. Subjects with missing or implausible values were excluded from analyses. Z-scores for weight-for-age (WAZ), height-for-age (HAZ) and weight-for-height (WHZ) were calculated with macros based on WHO child growth standards without any correction for height versus length in children under 2 years (60). All analyses were stratified by age group (young and older categories). Differences in baseline variables between the two supplement groups and control group were compared using chi-squared ($\chi^2$) for categorical variables, Student’s t-test for normally distributed continuous variables and the Wilcoxon Sign Rank Test for non-normal continuous variables. Primary analyses were intention-to-treat (ITT) based on the initial study group randomization. ANCOVA models adjusting for age and baseline levels were used to compare change from baseline
between the groups. Analyses were performed using SAS version 9.3 (SAS Institute, Inc., North Carolina), and statistical significance for all variables was set at a 2-sided P of 0.05.

An independent coder, blind to experimental condition, coded children’s performance on the cognitive tests at the baseline and outcome testing points as well as any instances of experimental error or interference. A second coder, also blind to experimental condition, coded 1/3 of the dataset; interrater reliability was high (r > .9). Most of the participant data for baseline data in 2-year-old children was deemed ineligible and is therefore not provided here. Some of these tests were excluded during coding because the test administrator could not speak the local language and the use of village interpreters resulted in some occasions where the interpreter may have prompted the correct result (n = 10). In other cases the 2-year-old children refused to participate or complete testing for the cognitive tasks (n = 27). In contrast, there were minimal data collection concerns with the 6-year-old children. We omitted 5 children from baseline analyses due to experimenter error and 1 child from baseline analyses due to potential translator interference. When tests were repeated at 11 weeks the test administrator was more experienced working with the 2-year-old children and in addition worked with a single trained interpreter. This allowed for data collection of acceptable quality at outcomes for most children. Therefore, in the analyses below, we report only comparisons between the intervention and control groups in the younger and older age groups at 11 weeks follow up. An age adjusted Poisson regression model was used to compare the number of stickers found between groups in separate models for younger and older children and for number of correctly sorted animals in older children. An age adjusted logic regression model was used to compare proportion of children who found all the stickers.

**Results**

Baseline and change data for child anthropometry and skin carotenoid content, and hemoglobin
at 11 weeks, are shown in Table 1. No children dropped out of the study but some did not come to the final outcome assessment and supplement attendance was 84% of possible days, with most children consuming most of the supplement under study observation. There were no significant differences between groups for any of the baseline variables, and both groups of children were undernourished as seen by negative mean z-scores for all anthropometric variables. Mean values for z-scores tended to be somewhat worse in the intervention group. Mean changes not different in the intervention group versus the control except for a small (0.1 cm) greater arm circumference effect in the control group in the older age group. It should be emphasized that the study was a pilot, and was underpowered to detect significant differences between groups.

Table 3 summarizes the cognitive data from both age groups. Results show that, for the younger age group, randomization to the supplemented group was associated with a large positive effect on children’s working memory. In the Spin the Pots task, younger children in the intervention group found more stickers (mean = 3.44 vs. 2.88) and tended to be more likely to find all the stickers than children in the control group (50% vs. 12.5%). The task-switching test is not reported for younger children due to lower numbers completing the test. For the 6-year-old children, randomization to the intervention group was not significantly associated with children’s working memory or task-switching abilities; however, raw scores were high in both conditions for the Spin the Pots task, suggesting potential ceiling effects as discussed below. In the Reverse Categorization task, children performed equally well across conditions on the pre-switch trials (mean correct across 6 total pre-switch trials: intervention group = 5.05 trials, control group = 5.15 trials; p=ns), confirming that any differences that might have emerged on the post-switch trials as a result of the supplement would have reflected changes in task-switching abilities, rather than rule learning or general attention.
To test the utility of CBFi measurements to assess brain cognitive abilities in children at risk of malnutrition we performed data analysis combining the intervention and control children. Mean values for CBFi at 2 cm in in the younger and older groups were $10.4 \pm 0.5$ and $11.0 \pm 0.6 \times 10^{-8}$ cm$^2$/s, respectively. Scalp BFi was significantly lower than CBFi, $1.6 \pm 0.1$ and $1.5 \pm 0.1 \times 10^{-8}$ cm$^2$/s, for young and older groups, respectively. Mean values for tissue ($\mu_{ai}$ @785 nm) in the younger and older groups were $0.18 \pm 0.01$ and $0.20 \pm 0.01$ cm$^{-1}$, respectively. There was a statistically significant correlation between the Reverse Categorization task and CBFi in the right frontal region in the older children ($R=0.44$, $P=0.038$). This correlation is illustrated in Figure 1 along with depiction of village measurements. Some anthropometric measures correlated with CBFi in the younger and older groups. In particular, CBFi in the right frontal region of older children it was negatively correlated with arterial oxygenation (-0.45, $P=0.034$) and head circumference ($R=-0.51$, $P=0.016$). In addition, CBFi in the younger children was negatively correlated with HAZ (right CBFi $R=-0.43$, $P=0.001$; left CBFi $R=-0.49$, $P=0.004$). Tissue absorption did not correlate with any anthropometric measure.

Discussion

This pilot randomized study tested a new nutritional formulation for use in undernourished and stunted children that, on theoretical grounds, could potentially contribute to structural and functional repair of brain structure and function. The results indicate improved executive function associated with short-term use of the solely food-based intervention, with significantly greater scores on a test of executive function found after just 11 weeks of supplementation in the younger group of children compared to assessment-only controls. The results are based on an underpowered sample, but justify further testing of the new formulation.
Like existing RUSFs and FBFS, the new formulation generally met WHO nutrient targets for
renourishing children, including specified targets for protein, vitamins and minerals. In addition
the new formulation contained 5 additional nutrients (DHA, EPA, choline, chromium and
molybdenum), which are not included in current WHO recommendations for malnutrition-related
food products, and two ingredients (cocoa and green tea powder) that are high in the subclass
of polyphenols that cross the blood-brain barrier and have potentially beneficial neurological
effects in other population types such as elderly adults. In addition, calcium levels were reduced
to facilitate iron absorption, given the known positive effect of iron on cognition and the
widespread anemia in communities of the type studied. To our knowledge none of the new
nutrients, specific ingredients and bioactive factors have previously been tested for use - either
separately or in combination - in nutrient recommendations to prevent or treat malnutrition. They
were included here based on an evaluation of research studies mostly performed in non-
malnourished adults and children that identified theoretical benefits including promotion of
neurogenesis and neuronal membrane growth and repair, enhanced neurotransmitter synthesis,
reduced apoptosis, increased signal processing and transmission, decreased inflammation, and
enhanced intestinal absorption and blood brain transport of the neuroactive factors (11, 26, 61-
64). Furthermore, the broad-reaching nature of these potential benefits suggested the potential
for synergistic benefits.

The preliminary finding of an association of supplement consumption with improved working
memory in younger children is noteworthy given evidence linking early working memory abilities
to longer-term cognitive development and academic achievement both in healthy, typically-
developing children (65), as well as in children born prematurely for whom there is greater
concern for cognitive impairments and delay (66). Although the apparently improved working
memory was limited to the younger, 2-year-old children in the current study, the intervention
was only for 11 weeks. In addition the older children’s high working memory abilities across both
suggest that this may represent ceiling performance under the current task parameters rather than a lack of effect at older ages, and increasing the working memory demands of the task (e.g., more hidden stickers, more empty cups, and/or longer delays before searching) may yield a measure more sensitive to detect intervention-related change. Relatedly, that we failed to find an impact of the supplement in the other cognitive assessments may also be due to the parameters and sensitivity of the tasks or the relatively short duration of supplementation, rather than representative of the supplement’s failure to impact these cognitive abilities. The need for cultural adaptation of the cognitive assessments in this pilot study, along with the lack of norms for this kind of population, may have resulted in tasks that lacked the sensitivity to detect significant change between conditions. Given these limitations future research would benefit from including additional time in their protocols prior to supplementation to pilot cognitive measures. However, the positive association of supplement consumption and improved working memory in younger children, and the positive relationship of cognition with brain blood flow, warrants a more targeted investigation of working memory in a fully powered sample in both the younger and older children.

Current neuroimaging tools to assess brain maturation are either ethically non-applicable (positive emission tomography, PET), too expensive (magnetic resonance imaging, MRI or functional MRI), or require professionals or well-trained operators to perform functional studies which consist of presenting repeated stimuli to the children and measuring neurovascular evoked responses (electroencephalogram-event related potential, EEG-ERP, functional NIRS). Several groups are currently testing the ability of fNIRS to assess cognitive function delays in malnourished children (67), though not yet to our knowledge in supplement interventions. These fNIRS studies rely on the evoked hemodynamic responses measured while children are performing cognitive tasks, and require devices with multiple channels, placement of optical monitors on the head similar to
EEG caps, and collection of data for several minutes. Our approach makes it possible to quantify resting state cerebral perfusion non-invasively, in few seconds with a portable device suitable for large studies. Thus, our approach can be done by non-experts and fully scales to the large number of subjects required for nutritional studies (for example, in this study, we measured 78 children in 4 days). In addition, this pilot study demonstrated the feasibility of measuring children in remote and low resources area with the NIRS-DCS device powered with a portable generator and temperatures averaging 36-40 degree Celsius. The test was well accepted by the children and their family and produced reasonable quality data. The NIRS-DCS study was only done at the end of the nutritional intervention and this limited our ability to assess changes in hemodynamic as a consequence of the intervention, and future studies should perform measurements before and after supplementation.

Using the NIRS-DCS methodology the correlation between the right frontal cerebral blood flow and the animal sorting test in 5-7 years old children demonstrated that children who perform poorly on cognitive testing have lower than average CBF_i. This is an important finding which need further validation in a larger population. The recovered parameters (CBF_i and \( \mu_{ai} \)) represent bulk hemodynamic values in a relative large volume (2x2x1.5 cm^3) and, as in NIRS, scalp and skull contribute to these values. As shown in this study, the large differences in blood flow indices between scalp and brain give us confidence that we can use the device in children ranging in age from 18 months to 7 years without excessive extracerebral contamination. Nevertheless, the negative correlation between head circumference and CBF_i in the older children, suggest some scalp contamination. This problem can be addressed by using larger source-detector separations. In this study, we were limited by the relatively low power of the laser source. For future studies we plan to increase the light power to 35 mW to increase the signal-to-noise ratio at larger separations. Another limitation of our study was that with only DCS we could not estimate scattering coefficient but had to assume it constant. We are aware of the
strong impact of the scattering coefficient in the quantification of CBF with DCS, and our calculated blood flow index reflect the product of blood flow and scattering. In future studies we plan to use a combined frequency-domain NIRS and DCS system (68) to quantify the scattering rather than assume it constant across children. The delivery of a non-invasive tool able to quantitatively assess brain function independent of neurodevelopmental tests (which are very challenging in field studies) would have a critical impact to directly assess the impact of a broad range of intervention to increase cognitive abilities and brain function in general.

If the cognitive function results found here are confirmed in a larger study they would have major implications for the prevention and treatment of malnutrition, since cognitive impairment resulting from stunting and malnutrition is currently assumed to be mostly irreversible (1, 2) and nutrition-only trials have demonstrated limited success (e.g. (9)). It should be noted that the method of implementing the formulation described here, namely a food product prepared locally (from provided and local ingredients) and a vitamin supplement, is feasible for scaling. It would also be possible to incorporate all the vitamins and minerals and unique nutrients into a single mix, which could be produced centrally and distributed, or to incorporate into ready-to-use foods by village bakers or other qualified individuals. Alternatively, the mix could be used to create shelf-stable food products, which could then be distributed to sites of consumption. All of these methods have the advantage that a portion of the ingredients are purchased locally so they reduce importation relative to typical lipid-based pastes while also facilitating local businesses.

Concerning study weaknesses, the protocol was designed as a small pilot designed to obtain data for power calculations and to examine the feasibility of preparing the supplement locally. Care was taken to use broadly comparable villages, but as a 2-village cluster randomized study it could not distinguish a supplement effect from a village by supplement interaction. As such the data should be considered preliminary and in need of verification in a powered trial. The lack of
power is likely the reason for the finding of no significant effects of the supplement on growth and hemoglobin, which would need a much larger study of longer duration to yield detectable changes (e.g. (69)). This pilot also used an assessment-only control which cannot distinguish between an effect of feeding any any specific influence of the supplement composition, whereas future studies should compare the new supplement to conventional products designed for the same purpose, namely supplemental nutrition for mothers and children for prevention and treatment of stunting and MAM, and for supplemental nutrition in school feeding programs.

There was also translation issues for cognitive testing at baseline which resulted in a loss of some data.

In summary, this study focused on developing methods for a future powered trial, because stunting and MAM remain widespread worldwide, and are associated with lasting cognitive impairment. The data from the new approach to formulating supplementary foods suggested improved executive function in undernourished young children. The results are preliminary, but given their potential importance they justify replication in a powered trial of longer duration.

Acknowledgements

This study was conducted with local implementation by the International Partnership for Human Development under the leadership of WP and ABS. SBR designed the supplement formulation with collaboration from ES and AK, and drafted the paper for review by the other authors. PM designed the cognitive testing methods and coded the videotaped sessions blinded and interpreted those results, ES designed and led the data safety and monitoring plan, MAF and KCW designed and built the DCS device and analyzed the DCS-NIRS data. MAF, PYL, and KH conducted the DCS-NIRS measurements and interpreted the DCS-NIRS results. CYOC provided input on flavonoids and measured levels in the supplement ingredients, EJ provided expertise on measuring skin carotenoids, ABS provided oversight of the field team and was
responsible for obtaining local IRB permission and recruiting staff to conduct the study and villages to enroll in the study, assisted by RC who also supervised supplement production and delivery. SBR led development of a plan for staff training and quality control, which was implemented by ST, who also entered data for analyses, which were conducted by CB. CB provided comments and assisted in interpreting the results. All authors have read and approved the final manuscript.

We thank Masimo Corp for donating a Pronto-7 pulse co-oximeter for the study, Pharmanex Global Research for providing a Resonance Raman Spectrometer, and Vitamin Friends LLC for donating Iron Gummies. We thank the subjects and their parents for participating, and Madeleine Gamache for help in preparing the manuscript.
References


57. Roche-Labarbe N, Carp SA, Surova A, Patel M, Boas DA, Grant PE, Franceschini MA. Noninvasive optical measures of CBV, StO(2), CBF index, and rCMRO(2) in human premature


TABLE 1 Summary statistics for anthropometry, skin carotenoids, grip strength and hemoglobin at baseline and 11-week values in all children enrolled in intervention and control sites

<table>
<thead>
<tr>
<th></th>
<th>Intervention</th>
<th>Control</th>
<th>Δ</th>
<th>Δ</th>
<th>P-value *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline 11 weeks Δ</td>
<td>Baseline 11 weeks Δ</td>
<td>P-value</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-3 Years</td>
<td>20</td>
<td>18</td>
<td>18</td>
<td>18</td>
<td>17</td>
</tr>
<tr>
<td>Age, years</td>
<td>2.4± 0.4</td>
<td>2.2 ± 0.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WAZ</td>
<td>-1.43 ± 0.94</td>
<td>-1.30 ± 0.99</td>
<td>0.01 ± 0.35</td>
<td>-1.20 ± 0.87</td>
<td>0.07 ± 0.30</td>
</tr>
<tr>
<td>HAZ</td>
<td>-2.08 ± 1.37</td>
<td>-2.03 ± 1.04</td>
<td>0.04 ± 0.70</td>
<td>-1.80 ± 0.87</td>
<td>0.07 ± 0.30</td>
</tr>
<tr>
<td>WHZ</td>
<td>-0.36 ± 0.81</td>
<td>-1.30 ± 0.99</td>
<td>-0.02 ± 0.61</td>
<td>-0.29 ± 0.69</td>
<td>-0.03 ± 0.55</td>
</tr>
<tr>
<td>Arm circumference, cm</td>
<td>14.93 ± 1.12</td>
<td>15.05 ± 1.11</td>
<td>0.13 ± 0.67</td>
<td>14.81 ± 1.14</td>
<td>0.20 ± 0.48</td>
</tr>
<tr>
<td>Head circumference, cm</td>
<td>47.04 ± 1.73</td>
<td>47.59 ± 1.53</td>
<td>0.35 ± 1.25</td>
<td>47.52 ± 1.94</td>
<td>-0.03 ± 0.79</td>
</tr>
<tr>
<td>Hemoglobin, g/dL</td>
<td>12.29 ± 0.92</td>
<td></td>
<td></td>
<td>11.96 ± 0.72</td>
<td></td>
</tr>
<tr>
<td>Skin carotenoid content, Raman counts x 10^3</td>
<td>24.6 ± 7.2 (n=15)</td>
<td>30.7 ± 8.9 (n=15)</td>
<td>6.1 ± 8.4 (n=15)</td>
<td>34.8 ± 6.7 (n=15)</td>
<td>38.1 ±10.6 (n=15)</td>
</tr>
<tr>
<td>5-7 Years</td>
<td>20</td>
<td>19</td>
<td>19</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Age, years</td>
<td>5.9 ± 0.3</td>
<td></td>
<td></td>
<td>6.1 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>WAZ</td>
<td>-1.78 ± 0.92</td>
<td>-1.77 ± 0.94</td>
<td>0.05 ± 0.24</td>
<td>-1.28 ± 1.35</td>
<td>-1.23 ± 1.05</td>
</tr>
<tr>
<td>HAZ</td>
<td>-1.74 ± 0.99</td>
<td>-1.72 ± 0.95</td>
<td>0.05 ± 0.13</td>
<td>-1.13 ± 0.99</td>
<td>-1.11 ± 1.31</td>
</tr>
<tr>
<td>BMI z-score</td>
<td>-0.96 ± 0.84 (n = 19)</td>
<td>-0.94 ± 0.80</td>
<td>0.02 ± 0.46</td>
<td>-0.79 ± 0.76</td>
<td>-0.74 ± 0.65</td>
</tr>
<tr>
<td>Arm circumference, cm</td>
<td>15.44 ± 1.30</td>
<td>15.30 ± 1.11</td>
<td>-0.15 ± 0.57</td>
<td>15.78 ± 0.84</td>
<td>0.24 ± 0.57</td>
</tr>
<tr>
<td>Head circumference, cm</td>
<td>50.0 ± 1.38</td>
<td>49.58 ± 1.42</td>
<td>-0.35 ± 0.74</td>
<td>50.13 ± 1.47</td>
<td>-0.20 ± 0.77</td>
</tr>
<tr>
<td></td>
<td>Hemoglobin, g/dL</td>
<td>Skin carotenoid content, Raman counts x 10^3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------------------------</td>
<td>------------------</td>
<td>---------------------------------------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12.45± 0.87</td>
<td>32.6 ± 12.1, 35.8 ± 9.1, 3.2 ±10.2, 37.4 ± 8.0, 39.8 ± 8.4, 2.4 ± 9.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12.49 ± 0.63</td>
<td>0.882</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* P value is adjusted for age in years and baseline values (change over time where available, cross sectional at 11 weeks otherwise). Values are means ± SD.
TABLE 2 Daily macronutrient and essential fatty acids provided in the locally-prepared biscuit supplement*

<table>
<thead>
<tr>
<th>Nutrients, units</th>
<th>2-year olds</th>
<th>6-year olds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy, kcal</td>
<td>291</td>
<td>350</td>
</tr>
<tr>
<td>Protein, % kcal</td>
<td>16.8</td>
<td>16.7</td>
</tr>
<tr>
<td>Carbohydrate, % kcal</td>
<td>29.4</td>
<td>28.6</td>
</tr>
<tr>
<td>Fat, % kcal</td>
<td>48.4</td>
<td>48.7</td>
</tr>
<tr>
<td>Eicosapentaenoic acid (EPA), mg</td>
<td>309</td>
<td>309</td>
</tr>
<tr>
<td>Docosahexaenoic acid (DHA), mg</td>
<td>209</td>
<td>209</td>
</tr>
<tr>
<td>Fiber, g</td>
<td>2.0</td>
<td>2.3</td>
</tr>
</tbody>
</table>

* The supplement was combined with a multivitamin gummie as described in Methods to meet the anticipated nutrient shortfalls in a typical home diet relative to WHO (18) and Institute of Medicine (19) nutrient targets, except for choline which was provided at 50% adequacy and calcium which was not fortified to promote absorption of other divalent cations such as iron.
### TABLE 3 Summary statistics for subset of children with cognitive test data at 11 weeks

<table>
<thead>
<tr>
<th></th>
<th>Intervention</th>
<th>Control</th>
<th>P-value *</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1-3 Years</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>16</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Baseline age, years</td>
<td>2.49 ± 0.40</td>
<td>2.23 ± 0.38</td>
<td></td>
</tr>
<tr>
<td>WAZ</td>
<td>-1.26 ± 1.03</td>
<td>-1.25 ± 0.84 (n = 15)</td>
<td>0.1526</td>
</tr>
<tr>
<td>HAZ</td>
<td>-2.01 ± 1.11</td>
<td>-1.85 ± 0.85 (n = 15)</td>
<td>0.6707</td>
</tr>
<tr>
<td>WHZ</td>
<td>-0.19 ± 0.92</td>
<td>-0.31 ± 0.75 (n = 15)</td>
<td>0.1272</td>
</tr>
<tr>
<td><strong>Cognition</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td># Stickers Found</td>
<td>3.44 ± 0.63</td>
<td>2.88 ± 0.62</td>
<td><strong>0.0435</strong>*</td>
</tr>
<tr>
<td>% Found All Stickers</td>
<td>50.0%</td>
<td>12.5%</td>
<td><strong>0.076</strong></td>
</tr>
<tr>
<td><strong>5-7 Years</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>20</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Baseline age, years</td>
<td>5.87 ± 0.28</td>
<td>6.11 ± 0.55</td>
<td></td>
</tr>
<tr>
<td>WAZ</td>
<td>-1.78 ± 0.92</td>
<td>-1.28 ± 0.99</td>
<td>0.7390</td>
</tr>
<tr>
<td>HAZ</td>
<td>-1.74 ± 0.99</td>
<td>-1.13 ± 1.35</td>
<td>0.4380</td>
</tr>
<tr>
<td>BMI z-score</td>
<td>-0.96 ± 0.84</td>
<td>-0.79 ± 0.76</td>
<td>0.6027</td>
</tr>
<tr>
<td></td>
<td>(n = 19)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cognition</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td># Stickers Found (max = 8)</td>
<td>6.60 ± 0.99</td>
<td>7.05 ± 0.83</td>
<td><strong>0.0755</strong>*</td>
</tr>
<tr>
<td>% Found All Stickers</td>
<td>25.0%</td>
<td>30%</td>
<td><strong>0.5384</strong></td>
</tr>
<tr>
<td>% Correctly Sorted Animals (max = 12)</td>
<td>8.90 ± 2.99</td>
<td>9.40 ± 2.89</td>
<td><strong>0.7809</strong>*</td>
</tr>
</tbody>
</table>

* Adjusted for age, ANCOVA model, **age adjusted logistic regression model, *** age adjusted Poison Regression Model
FIGURE LEGENDS

Figure 1 Relationship between brain blood flow and number of sorting tasks performed correctly, and illustration of measurement being conducted in rural village in Guinea-Bissau.