Title
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High-nutrition biscuits to increase animal protein in diets of HIV-infected Kenyan women and their children: A study in progress

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Abstract

Background—Preliminary evidence suggests that improved nutrition early in HIV infection may delay progression to AIDS and delay the initiation or improve the effectiveness of antiretroviral drug therapy. There are few studies that evaluate food-based interventions in drug-naïve, HIV-infected women and their children. Meat provides several nutrients identified as important in maintaining immune function and lean body mass.

Objective—To design supplemental meat and soybean biscuits for use in a randomized trial examining the effect of meat in the diet of drug-naïve, HIV-infected rural Kenyan women on changes in weight, lean body mass, morbidity, nutritional status, and activities of daily living of the women and growth and development of their children.

Methods—We designed three supplemental biscuits: one with added dried beef, another with added soybean flour, and a wheat biscuit to serve as a control biscuit to be used in a randomized feeding intervention in drug-naïve, HIV-infected rural Kenyan women and their children. The nutritional contents of the different types of biscuit were examined and compared.

Results—The three biscuits were isocaloric. Meat biscuits provided more lysine, vitamin B_{12}, and bioavailable zinc. Soybean biscuits provided more total and absorbable iron; however, higher fiber and phytate contents may inhibit nutrient absorption. Data analysis for clinical outcomes of the trial is ongoing.

Conclusions—The “biscuit model” is useful for nutrition supplementation studies because it can be provided in a blinded and randomized fashion, safely and privately in a home under directly

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Authors’ contributions

Judith Ernst, Department of Nutrition and Dietetics, Indiana University School of Health and Rehabilitation Sciences, was the overall principal investigator of the study, designed the intervention and the anthropometric and body composition studies, and drafted the paper. Grace Ettyang, Department of Epidemiology and Nutrition, School of Public Health, College of Health Sciences, Moi University, was the Kenyan principal investigator and was heavily involved in the design and production of the biscuits and oversaw conduct of the overall study. Charlotte G. Neumann, Department of Community Health Sciences, School of Public Health and Department of Pediatrics, School of Medicine, University of California, Los Angeles, oversaw the basic design and the cognitive and morbidity aspects of the study.

observed consumption by a highly stigmatized population. It is well received by adults and children, and the biscuits can be produced locally with available, simple, affordable technology.

Keywords

Food supplement; HIV-positive women; protein; vitamin B_{12}

Introduction

Many of the 25 million people with human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS) estimated to be living in sub-Saharan Africa also suffer from malnutrition. Reproductive-aged women and their infants and young children are among the most vulnerable to malnutrition and progression of HIV to AIDS. Most HIV-infected individuals live in resource-poor areas of the world where they are at increased risk for immune suppression because of food scarcity, poor diet quality, heavy infection burden, malaria, and intestinal parasitic infection [1, 2]. The scientific community has evolved in its appreciation of the value of food as an integral component of comprehensive care for individuals with HIV and AIDS [3]. It is now well recognized that those who are food insecure and malnourished are more likely to fail drug treatment regimens [4].

Effects of HIV on the human system

With the stress of a chronic infection like HIV, the immune system becomes severely compromised and loses its ability to resist infection. Disease progression from HIV to AIDS is defined and monitored by the level of CD4 lymphocytes, HIV viral load, the loss of lean body mass and body fat, and the incidence of coinfections such as tuberculosis, pneumonia, and fungal infections. The micronutrient status of the body also declines with HIV, with decreases in serum levels of vitamins B_{12} and A, zinc, and selenium correlating with disease progression from HIV to AIDS [5, 6]. Evidence suggests that improved nutrition early in HIV infection may delay progression to AIDS and delay the initiation or improve the effectiveness of antiretroviral drug therapy [7–9]. However, few studies evaluate how to optimize food-based interventions in HIV-infected individuals.

Impact of animal-source foods in other populations

Meat provides several critical, bioavailable nutrients that are important in maintaining normal immune function, resistance to infection, and lean body mass. These include complete protein, lysine, vitamin B_{12}, and bioavailable iron and zinc [10]. Lysine has been shown to improve immune function and protein status, weight gain, and linear growth in children and hemoglobin in women [11, 12].

Rural Kenyan diets contain very little animal-source food and may be marginal in total protein quality and content, vitamin B_{12}, available iron and zinc, and lysine, and the high phytate and fiber content reduces the bioavailability of dietary iron and zinc [13–15]. Iron deficiency, vitamin B_{12} deficiency, and malaria all contribute to anemia among HIV-infected and -noninfected Kenyan women [16–18].

A number of studies in developing countries suggest that greater priority should also be given to the correction of mild to moderate zinc deficiency in children, pregnant women, and lactating mothers [19, 20]. Zinc supplementation has positive effects in malaria, diarrhea, and respiratory infections (including pneumonia), and improves immune function in susceptible children [21–23]. A modest zinc supplement reduced diarrhea in HIV-infected children without increasing HIV viral load [24].

This project builds upon the knowledge gained from the USAID Global Livestock Collaborative Research Support Program (GL-CRSP) Child Nutrition Project (CNP) that showed increased arm muscle accretion in school-aged children in Embu District, Kenya, who received animal-source foods, with a much greater impact from meat than from milk [25, 26]. Near reversal of vitamin B_{12} deficiency, improved weight gain, increased physical activity, improved cognitive outcomes, and decreases in certain morbidity outcomes were also observed in children who received meat [25–29]. The CNP provided evidence that animal-source foods, particularly meat, may be a critical component in the diets of schoolchildren. Similar studies have not been conducted in women and children who are HIV-infected.

Methods

Study design

We designed a three-arm, randomized study of 225 HIV-positive, drug-naïve women and their youngest children, between 6 months and 8 years of age, with and without HIV. The participants were among clients enrolled in the US Agency for International Development-supported Academic Model Providing Access to Healthcare (AMPATH) partnership in western Kenya [30], operated under the joint direction of the Moi Teaching and Referral Hospital and the Moi University and Indiana University Schools of Medicine.

The women and their children were randomly assigned to receive one of three isocaloric intervention biscuits that contained either dried beef, soybean flour, or just wheat flour. The biscuits were provided 5 days per week for 18 months in Turbo, a 3,218-km^2 division of Uasin Gishu District of Kenya. The feeding intervention biscuits were delivered to women's homes daily by a directly observed treatment fieldworker, who observed intake and returned the leftovers to a central location for quantification. HIV-infected children in participating families received the same intervention biscuit as the mother and sibling. At enrollment, these women were drug-naïve and, based on their CD4 counts, did not meet the cutoff point for treatment with antiretroviral drugs, and therefore it was hypothesized that a food intervention might have a positive impact on their health and delay the need for antiretroviral therapy. Feeding was carried out for 18 months, with follow-up of mothers and children at 6 months afterwards. The groups were matched for distance of subject households from the treatment clinic.

Intervention food

The research team developed three types of isocaloric intervention biscuits made with wheat flour. Given the current recommendation for nonpregnant HIV-infected and -noninfected women of 0.75 g/kg of protein daily [31, 32], an HIV-infected woman weighing approximately 60 kg was assumed to require approximately 45 g (0.75 g/kg) of protein per day. It was estimated that at least 50% of protein needs would be available from household food resources, and that the meat or soybean biscuits would provide the other approximately 50% of protein needs to the mothers, whereas the wheat biscuit would only provide minimal protein of poorer quality. Dried beef powder or soybean flour was added to the basic recipe to provide 4.0 g of total protein per 100 kcal in the beef and soybean biscuits. Dried beef strips were obtained from Farmer's Choice butcheries (Nairobi, Kenya) and were processed into a powder using a commercial blender (Vitamix Pro 3) for the meat biscuits. Soybean flour (packaged by Nakumatt under their brand name) was purchased from Nakumatt Supermarket in Eldoret, Kenya, and roasted after purchase by a consistent method. Refined, unfortified wheat flour manufactured in Kenya (EXE, Unga Ltd) was used in all biscuit recipes.

The biscuits were prepared, packaged in opaque wrappers, weighed, labeled, and stored in a research bakery specifically designed with standardized mixing, weighing, baking, and storage equipment that allowed for a reliable, safe, and reproducible product. The production bakery was operated by research project staff specifically trained in quantity food production, with oversight for quality control and safety by co-principal investigators Ettyang and Ernst and the field research project coordinator. The food preparation staff were required to wear clean uniforms, aprons, and hair nets and have initial and periodic medical examinations, with testing for tuberculosis and stool examinations for parasites. They were required to wash their hands and work with gloves. The kitchen was inspected by the local department of public health for sanitation and cleanliness. Nutrient and bacterial analyses of the developed foods were performed in a reliable food laboratory (Covance Laboratories, Madison, Wisconsin, USA). Analyses were repeated quarterly for quality control of macro- and micronutrient, phytate, and fiber contents. Biscuits, ready for distribution, were delivered to the field twice each week. Each biscuit was labeled during packaging with a gram weight and a participant identification number in accord with the randomization to type 1, 2, or 3. Biscuit weights were recorded daily onto individual participant worksheets when dispensed to the directly observed treatment fieldworker, who indicated at the feeding site on the same worksheet if the entire amount was eaten (yes or no). Any uneaten portion was returned in the wrapper with the label to a central location for quantification, and the remaining amount was also recorded on the worksheet. The reason for incomplete consumption was also recorded.

For young children or those with difficulty chewing, a known and consistent amount of water (boiled and filtered) was added to the biscuits when they were served in the home to make them into a porridge. The daily serving size of the intervention biscuit varied with age: one biscuit for infants less than 1 year of age, two biscuits for children 1 to 8 years of age, and three biscuits for women.

Ethical approval

The study was approved by the Institutional and Ethics Review Boards at Indiana and Moi Universities and the University of California, Los Angeles. Informed, written consent was obtained, by specifically trained staff, from women participants and from parents on behalf of their children. Children aged 7 years or older were given the opportunity to provide assent.

Outcome measures

Repeated measurements of women and children, collected over the course of 2 years by trained enumerators, included estimates of lean body mass; muscle strength (women and children older than 3 years); immune function; infection; skin tests for delayed cutaneous hypersensitivity using candida, tuberculin, and tetanus skin test antigens; overall health; nutrient, energy, and protein intakes; micronutrient status (iron, zinc, vitamins B_{12} and A, and folic acid); quality of life; usual activity of women; and growth, development, and activity of children.

Results

The intervention biscuits were accepted by both mothers and children. Out of 99,642 feeding observations, the prescribed amount was consumed 91.8% of the time by women and 91.3% by children; the minimum amount of biscuit consumed over the entire study was 94.7% by women and 88.9% by children. The biscuit samples were repeatedly tested for bacteria and found to be negative for *Escherichia coli, Salmonella, Shigella, Staphylococcus*, and *Clostridium perfringens*.

The adult biscuit intervention supplied approximately 530 kcal/day. The meat and soybean biscuits supplied approximately 21 g protein/day, and the wheat biscuit supplied approximately 7 g protein/day (table 1). Figure 1 gives the percentages of the recommended intakes of protein, lysine, and vitamin B_{12} provided by the intervention biscuits. Even though the amounts of protein consumed from the meat and soybean biscuits were similar, the daily intake of lysine was significantly greater for the meat biscuit (73 mg lysine/g protein or 88% of the recommendation) than for the soybean biscuit (45 mg lysine/g protein or 50% of the recommendation) (fig. 1) [33, 34]. The wheat biscuit provided only a trace of approximately 0.02 mg lysine/g of protein. Vitamin B_{12} intake was also greatest from the meat biscuit, which provided 1.32 µg/day or 55% of the recommendation [13, 35]. The soybean biscuit provided a minimal amount of vitamin B_{12}, and the wheat biscuit provided no vitamin B_{12} (table 1 and fig. 1). The meat biscuit had the highest estimated zinc absorption (30%), as compared with the soybean and wheat biscuits (15%) (table 1) [13]. Total iron content was highest in the soybean biscuit, even with only 5% absorption, compared with 15% and 10% absorption from the meat and wheat biscuits, respectively [13]. The soybean biscuit, however, contained phytate with high ratios to iron and zinc of 5.1 and 38.8, respectively (table 1).

The daily intakes of nutrients from meat, soybean, and wheat supplements given to infants and children study participants are indicated in table 2. Protein intake from the meat and soybean biscuits provided children with 64% to 108% of the recommended amount, whereas the wheat biscuit provided only 25% to 42% of the recommended amount, and this was from protein of poorer quality (fig. 2) [32, 35]. Infants and children in the meat and soybean groups received lysine in amounts that met 80% to 135% of recommended intakes; those in the wheat group did not receive lysine from the supplement (fig. 2) [33, 34, 36]. Vitamin B_{12} was provided mainly to those in the meat group (table 2). The meat biscuit provided higher amounts of absorbable zinc and lower amounts of fiber and phytate (table 2). Infants and children who received soybean biscuits received greater amounts of absorbable iron; however, the phytate-to-iron ratio was highest in the soybean biscuit (table 2).

Discussion

Data analysis regarding the impact of the intervention biscuits on functional outcomes of women and their children has not yet been completed. The data on the composition of the biscuits show that the meat and soybean biscuits provided significant amounts of protein in relation to energy. The amount of protein provided to children older than 1 year (14 g/day) was similar to the amount that benefited school-aged children in the CNP study conducted in Embu, Kenya [27].

Women, infants, and children in the meat group received the most lysine and vitamin B_{12} and greater amounts of total and absorbable zinc than those in the other groups. Given the nutrients provided, those who received the meat biscuit are expected to show a greater improvement in study outcomes. Of interest is the higher amount of absorbable iron provided by the soybean biscuit than the meat biscuit. This was due to the high amount of iron in the soybean flour. However, the soybean biscuit contains fiber as well as phytate, with ratios to iron and zinc that are known to inhibit mineral absorption. Therefore, an impact from the higher iron intake in those in the soybean group may not be observed.

This paper presents an important and novel food-based intervention based on locally available ingredients in rural Kenya. The findings of this study may have implications for the development of initiatives that are sustainable and/or subsidized by the local, regional, and/or global economies, which ensure that all individuals infected with HIV have access to foods providing nutrients in sufficient quantity and quality to optimize health and well-being. The knowledge gained may significantly impact other populations at high risk for decreased immune function and nutritional status.

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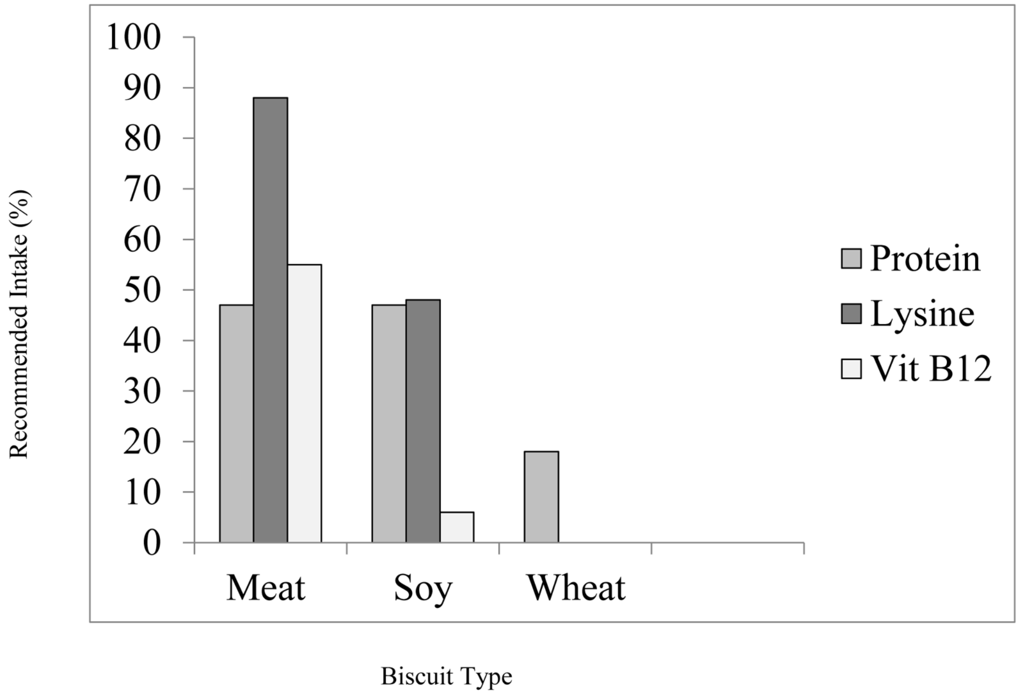

FIG. 1.

Estimates of daily intakes of protein, lysine, and vitamin $B_{12}$from meat, soybean, and wheat biscuit supplements for women study participants

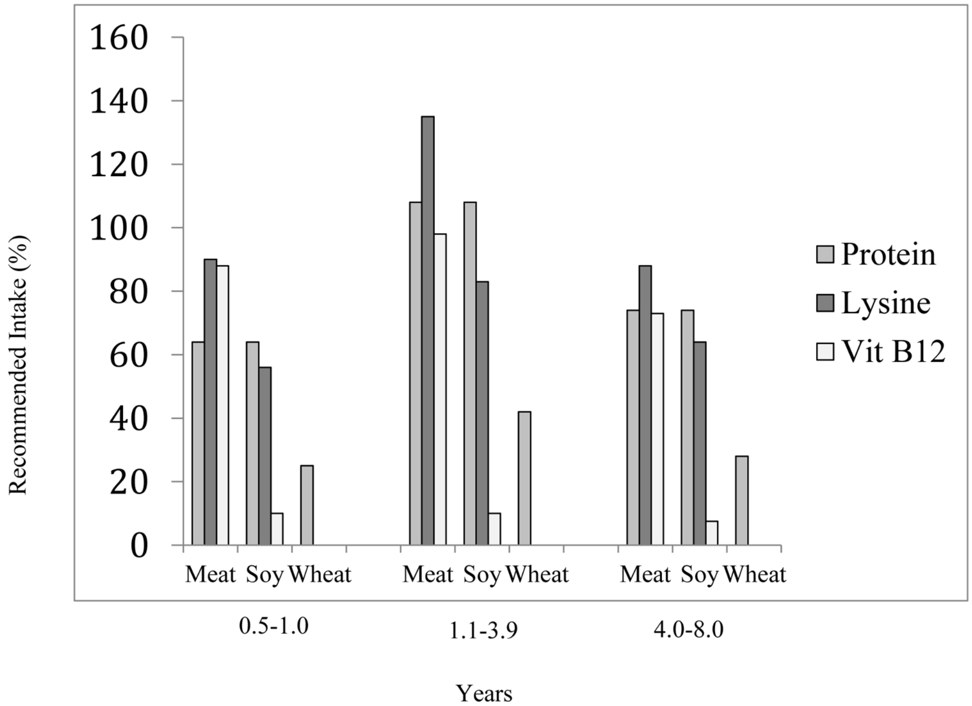

FIG. 2.

Estimates of daily intakes of protein, lysine, and vitamin B_{12} from meat, soybean, and wheat biscuit supplements for infant and child study participants

TABLE 1

Daily amounts of nutrients received by woman study participants from the biscuit supplement

Nutrient	Biscuit type		
	Meat	Soybean[a]	Wheat
Biscuit (g)	120	110	115
Energy (kcal)	530	530	530
Protein (g)	21	21	7
ASF protein (g)	16	0	0
Vitamin B_{12} (μg)	1.32	0.14	0
Total iron (mg)	1.78	8.40	0.64
Absorbable iron (mg)[b]	0.27	0.42	0.06
Total zinc (mg)	1.80	1.28	0.36
Absorbable zinc (mg)[c]	0.54	0.19	0.05
Fiber (g)	1.20	10.40	1.60

ASF, animal-source foods

[a]Soybean biscuit phytate:iron ratio, 5.1; phytate:zinc ratio, 38.8.

[b]Absorbable iron: meat, 15%; soybean, 5%; wheat, 10%.

[c]Absorbable zinc: meat, 30%; soybean and wheat, 15%.

TABLE 2

Daily amounts of nutrients received by child study participants from the biscuit supplement

Nutrient	Age (yr)	Biscuit type		
		Meat	Soybean[a]	Wheat
Biscuit (g)	0.5–1.0	40	37	38
	1.1–8.0	80	74	76
Energy (kcal)	0.5–1.0	175	175	175
	1.1–8.0	350	350	350
Protein (g)	0.5–1.0	7	7	2.7
	1.1–8.0	14	14	5.4
ASF protein (g)	0.5–1.0	5.3	0	0
	1.1–8.0	10.6	0	0
Vitamin B_{12} (μg)	0.5–1.0	0.44	0.05	0
	1.1–8.0	0.88	0.10	0
Total iron (mg)	0.5–1.0	0.59	2.80	0.21
	1.1–8.0	1.20	5.60	0.42
Absorbable iron (mg)[b]	0.5–1.0	0.09	0.14	0.02
	1.1–8.0	0.18	0.28	0.04
Total zinc (mg)	0.5–1.0	0.60	0.43	0.12
	1.1–8.0	1.20	0.86	0.24
Absorbable zinc (mg)[c]	0.5–1.0	0.18	0.06	0.02
	1.1–8.0	0.36	0.13	0.04
Fiber (g)	0.5–1.0	0.40	3.50	0.50
	1.1–8.0	0.80	6.93	1.07

ASF, animal-source foods

[a] Soybean biscuit phytate:iron ratio, 5.1; phytate:zinc ratio, 38.8.

[b] Absorbable iron: meat, 15%; soybean, 5%; wheat, 10%.

[c] Absorbable zinc: meat, 30%; soybean and wheat, 15%.