Improving Nutritional Status and CD4 Counts in HIV-Infected Children Through Nutritional Support

Principle Investigator
Maurice R. Bennink, Michigan State University, USA

Collaborating Scientists
Theobald Mosha, Sokoine University of Agriculture, Tanzania
Henry Laswai, Sokoine University of Agriculture, Tanzania
Elizabeth Ryan, Colorado State University, USA
Reuben Kadigi, Sokoine University of Agriculture, Tanzania.

Abstract of Research Achievements and Impacts
A randomized, prospective, community-based trial will be conducted to determine if HIV infected, HAARV naïve, 2 to 15 year old children and adolescents eating a bean-maize or cowpea-maize supplement will maintain a higher CD4 % than HIV infected, HAARV naïve, 2 to 15 year old children and adolescents eating a fish-maize supplement. The food supplements will be similar in nutrient and energy content with only the major source of protein (bean, cowpea, or fish) differing among the three study groups. The study will occur at two rural sites in Tanzania – the Turani and the Rombo areas – and 270 children and adolescents will be enrolled at each site. A local supervising and monitoring group was formed and trained for each research site. A laboratory at SUA was equipped to analyze biohazardous samples (HIV infected blood) and a two months supply of individual supplement packages (approximately 30,000 packages) were prepared. Dried blood spots will be prepared for each blood sample and these blood spots will be shipped to the United States. Protocols for eluting and analyzing viral RNA, ribosomal 16s RNA, and various proteins from dried blood spots were standardized. Viral RNA will be monitored to determine if dietary treatment affects innate ability to suppress the human immuno virus. Ribosomal 16s RNA is an indicator of bacterial products in blood and will reflect bacterial translocation from the gut. The proteins being analyzed (C-reactive protein, soluble tumor necrosis factor receptor p55 and interferon-γ) are markers of inflammation. These assessments will be performed to monitor HIV status and to evaluate hypotheses regarding treatment effectiveness. A cost comparison of the precooked supplements versus home prepared foods with the same ingredients versus anti-retroviral drug treatment was initiated. The baseline blood data and commencement of supplement feeding will occur in the beginning of FY11 and conclude at the end of FY12.

Project Problem Statement and Justification
The overall goal of the research is to determine if eating beans or cowpeas will improve the immune status of HIV positive children that are not being treated with antiretroviral drugs. The global theme addressed by this research is B – “To increase the utilization of bean and cowpea grain, food products and ingredients so as to expand market opportunities and improve community health and nutrition” and the topical area that will be addressed is #2 – “Achieving Nutritional Security for Improved Health of Target Populations”. HIV has caused an estimated 25 million deaths worldwide in just 27 years and there are approximately 33 million people in the world infected with HIV [1]. Around 2 million children less than 15 yr of age have HIV and 90% of the children living with – and dying from – HIV live in sub-Saharan Africa. Furthermore,
about 140,000 of these children live in Tanzania [2]. Most children living with HIV are innocent victims as they are infected during pregnancy, at birth or via breastfeeding. It is well known that insufficient intake of macronutrients and some micronutrients leads to a decrease in immune function and an increase in infectious diseases. Infections in turn cause nutrient loss that quickly leads to greater malnutrition and a vicious cycle is set in motion [3]. Since the human immunodeficiency virus destroys CD4 cells (immune cells), opportunistic infections are common place among those living with HIV [4,5]. In addition, most young children (not infected with HIV) in resource poor countries are under nourished or have marginal nutrition status. Since the insults of malnutrition and HIV on the immune system are synergistic, it is not surprising that young children with HIV are 2.5 – 4 times more likely to die than their counterparts that are not infected [1,6].

We previously showed that providing HIV+ children with a bean-maize supplement containing minerals and vitamins could reverse malnutrition if present and improve the immune system (increased CD4 counts) even though the children were not receiving highly active antiretroviral (HAARV) drugs. This is an extremely important finding since 50% of HIV+ people do not have access to HAARV drugs and consuming the bean based supplement could be an important stop gap until more people are able to obtain HAARV drugs. Children receiving HAARV treatment also benefited from the bean-based supplement in a second study we have done and so, the bean-based supplement would also be useful to children that have access to HAARV medicine. Cowpea is a widely consumed pulse in Africa, but there is no published data regarding the effect of feeding cowpeas to HIV+ children. If cowpea is also effective in improving nutritional and immunological status, consuming a bean-based or cowpea-based supplement could improve the lives of millions of HIV infected people which would at the same time benefit the entire bean/cowpea value-added chain from farmers to consumers.

**Planned Project Activities for April 1, 2009 - September 30, 2010**

**Objective 1:** Determine if HIV infected, HAARV naïve, 2 to 15 year old children and adolescents eating a bean-maize or cowpea-maize supplement will maintain higher CD4 % than HIV infected, HAARV naïve, 2 to 15 year old children and adolescents eating a fish-maize supplement.

**Approaches and Methods:**
1. Assemble research team members in Tanzania (field supervisors, nurses, technicians).
2. Install blood cell counter (cytometer) and train technicians to use and maintain the instrument.
3. Enroll 270 subjects into the HIV study.
4. Train one Ph.D. and three M.S. students to assist in research.
5. Provide field practical training in community nutrition and health for 10 undergraduates.
6. Analyze 540 blood samples for CD4 and related lymphocytes.

**Results and Outputs:** The study sites are remote from SUA – Turani is approximately 100 km from SUA and not easily accessible due to a 45 km stretch of very poorly maintained dirt road and Rombo is a rural area 550 km from SUA. Therefore, on-site, local research teams were assembled for each study site. The team members were trained to be responsible for the day to day administration and monitoring of the study. Community meetings were held to inform
families about the upcoming study and to gauge willingness to participate in the study. The meetings suggest that each site can provide the required 270 subjects.

Approximately 30,000 individual packages of food supplement (daily servings for about 2 months) have been prepared. This requires purchasing and transporting raw materials to SUA where the supplement is processed and weighed into packages that form individual daily servings.

A laboratory capable of receiving, processing and storing biohazardous materials was assembled at SUA. Except for the flow cell counter, all other pieces of equipment necessary for the study have been installed. Likewise, the laboratory has been stocked with the necessary supplies and pieces of small equipment.

Three MS students completed their coursework and received training to assist in the study and to gather data for their research dissertation. This project allowed 11 undergraduate students to receive practical field training in community nutrition and health. The project provides a “focal point” for the 11 undergraduates which is a great benefit not only for the students, but also for the Food Science and Nutrition Department at SUA. There is no cost to the project associated with the undergraduate training.

There was a delay in receiving and installing the flow cytometer. Therefore, no blood samples were collected since the CD4 counts have to be performed within hours of blood collection.

Protocols for eluting and analyzing viral RNA, ribosomal 16s RNA, and various proteins from dried blood spots were standardized. Viral RNA will be monitored to determine if dietary treatment affects innate ability to suppress the human immuno virus. Ribosomal 16s RNA is an indicator of bacterial products in blood and will reflect bacterial translocation from the gut. The proteins being analyzed (C-reactive protein, soluble tumor necrosis factor receptor p55 and interferon-γ) are inflammatory markers. These assessments will be performed to monitor HIV status and to provide hypotheses regarding treatment effectiveness. No actual blood spots from subjects were analyzed due to the delay in flow cytometer installation.

**Objective 2:**
Determine the relative costs of three dietary treatments compared to HAARV drug treatment.
Approaches and Methods:
1. Determine costs associated with cooking beans in a pot and preparing Ugali (corn based local food).
2. Determine costs associated with preparation of the bean-maize supplement and thin porridge from the supplement.
3. Determine costs associated with preparation of the fish-maize supplement and thin porridge from the supplement.

Results and Outputs:
Wholesale and retail prices for the various anti-retroviral treatments (ART) utilized in Tanzania were obtained from CTC/VCT areas, pharmacies, ministry of health, medical stores and pharmaceutical factories. Costs for raw ingredient for supplement preparation and distribution have been compiled. Determining the energy usage and cost for preparation of the various supplements and preparation of similar foods at the household level is technically more difficult and has not been completed yet.

Objective 3: Capacity building at SUA.

Results and Outputs:
One Ph.D. and 3 MS students received support and training for their respective research projects which are part of this research activity. The ability and capacity to conduct research related to HIV monitoring at SUA is a significant advancement for SUA. It is expected that other researchers at SUA will now be willing to engage in similar research activities which will increase the overall research capacity at SUA.

Institutional Capacity Building:

Degree Training:

Trainee # 1
First and Other Given Names: Pudensiana
Last Name: Kiwale
Citizenship: Tanzania
Gender: Female
Degree: Doctorate
Discipline: Agricultural Marketing
Host Country Institution to Benefit from Training: Tanzania
Training Location: SUA
Supervising CRSP PI: Reuben Kadigi
Start Date of Degree Program: August, 2009
Program Completion Date: August, 2012
Training Status during Fiscal Year 2010: Active
Type of CRSP Support (full, partial or indirect): Indirect
Trainee # 2
First and Other Given Names: Amos
Last Name: Nyangi
Citizenship: Tanzania
Gender: Male
Degree: MS
Discipline: Food Science
Host Country Institution to Benefit from Training: Tanzania
Training Location: SUA
Supervising CRSP PI: Henry Laswai
Start Date of Degree Program: August, 2009
Program Completion Date: August, 2011
Training Status during Fiscal Year 2010: Active
Type of CRSP Support (full, partial or indirect): Full

Trainee # 3
First and Other Given Names: Sarah
Last Name: Johnson
Citizenship: Tanzania
Gender: Female
Degree: MS
Discipline: Food Science
Host Country Institution to Benefit from Training: Tanzania
Training Location: SUA
Supervising CRSP PI: Henry Laswai
Start Date of Degree Program: August, 2009
Program Completion Date: August, 2011
Training Status during Fiscal Year 2010: Active
Type of CRSP Support (full, partial or indirect): Full

Trainee # 4
First and Other Given Names: Rosemary
Last Name: Marealle
Citizenship: Tanzania
Gender: Female
Degree: MS
Discipline: Nutrition
Host Country Institution to Benefit from Training: Tanzania
Training Location: SUA
Supervising CRSP PI: Theobald Mosha
Start Date of Degree Program: August, 2009
Program Completion Date: August, 2011
Training Status during Fiscal Year 2010: Active
Type of CRSP Support (full, partial or indirect): Full
Short Term Training:
Type of Training:
Description of Training Activity:
Status of this Activity as of September 30, 2010: Did not occur
When did the Short Term Training Activity occur?
Location of Short Term Training:
If Training was not completed as planned, provide a rationale: The HACCP/Food Safety Course was not offered by MSU in August or September as in previous years.
Who benefitted from this Short Term Training Activity?
Number of Beneficiaries by Gender: Male- Female- Total-

Equipment purchased (costing >$5000): None. A $49,000 flow cytometer has been “loaned” to SUA for the project duration by the manufacturer (BD Dicton) with the provision that there research reagents be purchased for determination of CD4 and other lymphocytes. The loan was possible in part due to prior arrangements between the Clinton Foundation and the manufacturer.

Explanation for Changes
There were two reasons why the baseline data was not collected and the supplementary feeding was not initiated. The first reason is that SUA had a two month delay in releasing their annual financial allocation from their government. SUA in turn delayed classes for two months and thus the academic obligations were not completed by the host country PIs until the end of Aug. Normally there would have been two months available to get the clinical trial underway. Equally important is the second reason – the “loaner” flow cytometer did not become available until late August. It took a month for SUA to approve the financial arrangements to purchase the reagents for the flow cytometer from a non-Tanzanian source. Apparently, once Tanzania has some money, they want it spent within the country and they do not want to allow funds to go to another country (Belgium in this case). (Note: Since this report is being prepared on 11-1-2010, I can add that the flow cytometer has been installed and training by the manufacturer has occurred. Now that their presidential election has occurred, the baseline data will be obtained immediately). The reason for not completing the short-term training for Drs. Mosha and Laswai is the HAACP course was not offered by MSU due to lack of sufficient enrollment. The course may be taught at some future date if the demand is sufficient.

Networking and Linkages with Stakeholders
In June 2010, Drs. Mosha and Bennink visited the Tanzanian USAID Mission and the World Bank to inform them of the research activities being conducted and planned by this activity. The host country PIs provided advice and assistance to two agricultural firms/enterprises: a) Tanzania Foods Limited – composite food producers for special groups; and b) Tanzania Power Foods – producer of foods for children, hospitals, and rehabilitation centers and for retail outlets. The host country PIs provided advice and assistance to three women’s; groups: a) WAVUMO – women’s group fighting against HIV/AIDS; b) TUNAJALI “We care” is an NGO women’s group that provides Home Based Care and counseling for HIV+ families; and c) FARAJA TRUST FUND – A women’s group dealing with women’s and children’s needs due to HIV infection.
Leveraged Funds
No direct leveraged funds were received. The Heinz Company Foundation supports two research projects with the PIs of this activity. The Heinz supported projects are synergistic to this activity, but their support does not directly increase the capacity of this activity. The loan of the flow cytometer avoids the need to spend to purchase one and is a huge savings for the project. Lastly, support for the PIs’ time and efforts comes from their respective institutions, not from USAID and should be considered “in kind” leveraged resources.

Scholarly Activities and Accomplishments:
None; the project was officially approved January 2010 and revised in June to include cowpeas and an additional study site. There has not been sufficient time to achieve scholarly activities.

Literature Cited

Contribution to Gender Equity Goal
Three women graduate students are receiving support for advanced degrees and Tanzania is in great need of women with advanced degrees. Tanzania Power Foods is a woman owned enterprise and host country PI interaction with her will assist her in maintaining her successful business. WAVUMO, TUNAJALI, and the FARAJA TRUST FUND are all women’s groups that benefitted from this USAID supported research activity.

Progress Report on Activities Funded Through Supplemental Funds No supplemental funds were used for this activity.
Dry Grain Pulses CRSP

Report on the Achievement of "Semi-Annual Indicators of Progress"
(For the Period: Jan 1, 2010 – March 31, 2010)

This form should be completed by the U.S. Lead PI and submitted to the MO by April 1, 2010.

Project Title:
Improving Nutritional Status and CD4 Counts in HIV-infected Children Through Nutritional Support

<table>
<thead>
<tr>
<th>Abbreviated name of institutions</th>
<th>SUA</th>
<th>MSU</th>
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<tbody>
<tr>
<td>Ticks mark the Yes or No column for identified benchmarks by institution</td>
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### Objective 1
- **Apply for IRB approvals**
  - Pattern: 1
  - Target: 0
  - Achieved: 1
  - 2/1/10

### Objective 2
- **Investigate costs of supplement production**
  - Target: 0
  - Achieved: 0

### Objective 3
- **Name of the PI reporting on benchmarks by institution**
  - T. Mosha
  - M. Bennink

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Name of the U.S. Lead PI submitting this Report to the MO:
Bennink

Signature: [Signature]
Date: May 27, 2-10

* Please provide an explanation for not achieving the benchmark indicators on a separate sheet.
Explanation for not achieving benchmark indicators

For PIII-MSU-3: Improving Nutritional Status and CD4 Counts in HIV-Infected Children Through Nutritional Support

Two benchmarks and one training output indicator were not achieved for this project for FY10 for the following reasons:

1. *Benchmarks* The flow cytometer that is “loaned” to our project did not become available from the manufacturer until late August. In addition, purchasing the reagents for the cytometer by SUA was a major problem and it took several weeks to finalize how this could be accomplished. Since the cytometer did not arrive at SUA until mid September and the reagents necessary to operate the machine won’t be available until about 10-12-2010, installation and training will take place the week of 10/18/2010. Obviously, no blood samples can be collected and analyzed until training has occurred.

2. *Training output indicator* The short term training for Drs Laswai and Mosha (HACCP/Food Safety Course) was not offered by MSU in September as is the usual custom. We hope that the course will be offered sometime during FY11 and that the short term training can then occur.
Dry Grain Pulses CRSP  
Research, Training and Outreach Workplans  
(October 1, 2009 – September 30, 2010)

PERFORMANCE INDICATORS  
for Foreign Assistance Framework and the Initiative to End Hunger in Africa (IEHA)

Project Title: Improving Nutritional Status and CD4 Counts in HIV-Infected Children  
Through Nutritional Support  
Lead U.S. PI and University: Maurice R. Bennink, Michigan State University  
Host Country(s): Tanzania

<table>
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<tr>
<th>Output Indicators</th>
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<th>2010 Actual (Sept 30, 2010)</th>
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<td>Number of HC partner organizations/institutions benefiting</td>
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