

**BEEF AS A SOURCE OF VITAMIN B-12, IRON AND ZINC TO IMPROVE
DEVELOPMENT OF INFANTS FED LOW AMOUNTS OF ANIMAL PRODUCTS**

NARRATIVE SUMMARY

In a low-income area of Guatemala City we reported previously that almost two-thirds of infants aged 7 to 12 months had plasma vitamin B-12 concentrations that indicated depletion or deficiency of the vitamin. The only dietary source of this vitamin is animal source foods (ASF) and diets of mothers and young children in these communities, as in most poor regions of the world, are low in ASF and consequently vitamin B-12. Because it is known that severe vitamin B-12 deficiency has adverse, possibly permanent effects on infant and child development, this study was designed to test associations between B-12 status and indicators of child health and function, and the effect of food-based interventions on B-12 status and child growth, cognitive and motor development, activity and behavior. In addition, the effect of beef supplementation on iron and zinc status will be determined. Subjects were 304 Guatemalan children, aged 1 year at enrollment, and their mothers. 80% of the infants were still consuming some breast milk. The three equi-caloric dietary interventions, provided daily under supervision for 9 months, were: a beef meal, a control meal (commercial turkey-rice and fruit), and the control meal + added vitamin B-12 equivalent to the daily recommended intake. Indicators of child function and development were measured throughout the intervention and outcomes in the beef and B-12 supplemented groups will be compared to those in the control group. The field phase of the study ended in August 2005 and laboratory and statistical analyses are in progress. Analysis of baseline data on the 304 children confirms the high prevalence of vitamin B-12 depletion in the infants (30% deficient and 20% marginal status) and their mothers (36% deficient and 33% marginal status). Low plasma B-12 concentrations in infants were predicted by lower maternal plasma B-12, less vitamin B-12 intake

from complementary foods (primarily powdered cow's milk), more dependence on breast feeding, and smaller household size. Infants with plasma vitamin B-12 levels that indicated deficiency had poorer motor skills related to secure walking, and those consuming less than the average vitamin B-12 intake had a lower mental score on the Bayley assessment exam than those consuming above the average amount. Mothers with deficient plasma vitamin B-12 concentrations had a 60% greater risk of depressive symptoms, after controlling for socioeconomic and other variables. These preliminary analyses of baseline data confirm the high prevalence of vitamin B-12 deficiency in mothers and infants in this population, and suggest that maternal depletion is one cause of the infant depletion. Deficient plasma B-12 levels predicted poorer motor and mental development of the children, and greater risk of depressive symptoms in the mothers. Analysis of the effects of the beef and vitamin B-12 interventions on child development is in progress.

RESEARCH

Problem Statement and Approach. The high global prevalence of vitamin B-12 deficiency has been recognized relatively recently. For example the World Health Organization held a Consultation on Vitamin B-12 in October, 2005, the first for more than 40 years. A review by the Principal Investigator of this project concluded that about 40% of individuals studied in Latin America had deficient or marginal plasma vitamin B-12 concentrations. In our previous research in Guatemala City we observed that plasma vitamin B-12 was deficient or marginal in about one third of school children, and 30% of lactating women at three months postpartum and 12% of their breastfeeding infants, and two thirds

of infants aged 7 to 12 months. The main cause of the vitamin B-12 deficiency appears to be a low intake of animal products, as B-12 is only found in animal source foods unless the foods are fortified with the vitamin.

This research is designed to answer several important questions based on these observations. The most important is whether the low plasma vitamin B-12 levels in infants are associated with adverse effects on their mental and motor development, activity and behavior, and on maternal depressive symptoms. Severe deficiency has been shown to have these effects in other studies. Second, we propose to determine whether meat or vitamin B-12 supplementation of young children with a high prevalence of vitamin B-12 deficiency improves their nutritional status, growth and development. Third, predictors of infant vitamin B-12 deficiency at baseline will be investigated, including their diet, maternal B-12 status, and breast milk B-12 concentrations.

Specifically, the research will evaluate the effects of supplementation with beef, or vitamin B-12, on the cognitive and motor development, activity, temperament, growth and morbidity of these young children. The three randomized intervention groups (recruitment goal 80 children per group, completion goal 60 per group), providing a similar amount of calories, are: 1) a beef supplement; 2) a low meat control meal (commercial turkey and rice dinner and fruit infant foods); and 3) the same control meal fortified with vitamin B-12.

The relevance to global agriculture and development is that a low intake of animal products may cause vitamin B-12 deficiency and have permanent adverse effects on child development and human capital formation. Documentation of such adverse effects caused by vitamin B-12 deficiency could increase attention to the need for increased animal source food intake and production in low income populations of developing countries, and the importance of animal source foods for normal human development.

Progress. After an initial pilot testing phase, the intervention started in October 2003 with funding from the National Cattlemen's Beef Association. Funding from the GL-CRSP was added

starting in October 2004. From October 2003 to December 2004, children were enrolled into the three randomized groups fed the supplemental foods daily, under observation. At 0, 3, 6 and 9 months of intervention, data were collected on usual diet, anthropometry, cognition and development, behavior, activity, and morbidity. Maternal measures at baseline included plasma B-12, a breast milk sample for B-12 analysis, anthropometry, diet, socioeconomic and other household data, and reported depressive symptoms. Blood samples to assess nutritional status were collected at baseline and after the nine months of supplementation. The intended number of children completed the

Table 1. Reasons for withdrawal from the study.

Reason	N	%
Moved to other community	28	47
Parents did not want to continue	13	22
Lack of interest/time	10	17
Child did not like the supplement	6	10
Severe disease/death*	2	3
Lack of trust	1	1
TOTAL	60	100

study (241, compared to the desired final sample size of 180). All fieldwork was completed exactly on schedule, by September 2005. Some baseline analyses have been completed, and final sample and data analysis and publication are in progress. There were no substantial modifications from the original protocol or work plan, and no changes from the planned schedule of work.

Preliminary results. Some analyses have been conducted on the baseline interviews and samples taken from 304 infants and their mothers, when infants were 12 months of age. Eighty percent of the infants were partially breastfed, 29.8% had marginal plasma vitamin B-12 concentrations (200-300 pg/mL) and 19.5% had deficiency (<200 pg/mL). Of the mothers, 37.5% had marginal vitamin B-12 status and 32.5% were deficient. Anemia was present in 14.5% of infants and 9.8% of mothers, and 39.4% of infants were iron deficient. Lower infant plasma vitamin B-12 concentrations were predicted by lower

maternal plasma vitamin B-12, higher B-12 intake from complementary foods (almost all of which came from cow's milk), higher frequency of breastfeeding (because the breast milk contains much less vitamin B-12 than cow's milk), and smaller household size ($P < 0.0001$).

In the mothers, 43% had a high depressive symptoms score, which was predicted by lower plasma vitamin B-12 concentrations, a lower platelet count, a higher body mass index, and having more children (all $P < 0.05$). The relative risk for having more depressive symptoms was 1.6 for women with deficient vs. adequate plasma vitamin B-12 concentrations ($P < 0.005$).

The baseline analyses also suggest that vitamin B-12 deficiency is adversely affecting infant development. Infants with deficient plasma vitamin B-12 concentrations had poorer performance of motor skills related to secure walking, compared to adequate infants ($P < 0.005$), and those consuming $< 1.44 \mu\text{g}/\text{d}$ of vitamin B-12 from complementary foods had a lower mean Mental Development Index score than infants consuming $> 1.44 \mu\text{g}/\text{d}$ ($P < 0.05$).

In summary, as expected the infants and their mothers have a very high prevalence of vitamin B-12 deficiency. This is accompanied by a higher rate of maternal depressive symptoms, and poorer walking skills and mental development in the infants. The causal role of vitamin B-12 deficiency in these adverse functional outcomes will be evaluated during the coming year using the data from the longitudinal beef or vitamin B-12 supplementation study.

GENDER

A total of 158 boys and 146 girls were enrolled into the study, and gender differences in outcomes at baseline and post-intervention will be evaluated. Differences in initial B-12 status and other measures will be examined by gender at baseline, as will responses to intervention. No gender differences have been observed in B-12 status in our previous studies.

The mothers of all the 304 infants also participated in this research. This research has demonstrated that 70% of the mothers are vitamin

B-12 depleted or deficient and this is associated with a higher risk of deficiency in their infants at 12 months postpartum (and presumably much earlier during the first year of life), and with higher risk of maternal depressive symptoms. We also plan to analyze the breast milk vitamin B-12 concentration of these women and baseline to confirm that maternal deficiency vitamin leads to lower secretion of the vitamin in breast milk and subsequently greater risk of infant depletion. The potential implications are that these women need to learn the importance of increased animal source food intake during pregnancy and lactation, they may also need supplementation with the vitamin, and a national B-12 fortification program may need to be considered.

A group of 12 women from the community were trained as Community Motivators for this project. They were responsible for contacting mothers, setting up clinic visits, distributing and observing the consumption of the food supplements, collecting morbidity data, and alerting the project supervisory personnel about medical or other problems. This training has provided these women with qualifications that will improve their future employment opportunities and their knowledge of the importance of nutrition for child development.

Other training included two licensed Guatemalan psychologists who were instructed by the lead Guatemalan psychologist and Dr. Maureen Black on the application of child development tests. The Field Director, a female Guatemalan physician, has been trained in data entry and analysis.

POLICY

This research makes several important contributions to our knowledge of nutritional status problems of mothers and infants in areas of the world where the consumption of animal source foods is low. Inadequate intake of these foods causes maternal depletion of vitamin B-12, which during pregnancy results in low B-12 stores in the infant at birth, and inadequate amounts of the vitamin in breast milk.

Several changes in policy need to be made as a

result of the baseline analyses completed to date. The first is to improve the intake of animal source foods by mothers and young children. In these peri-urban communities, where a wide variety of cheaper meats is available, the most feasible strategy is probably education of the mother and the household about the importance of consumption of these foods (which are also the main source of other nutrients such as absorbable iron and zinc) for healthy pregnancy outcome and child development. This strategy was successful for improving animal source food consumption in Lima, Peru. Encouragement to include dried or whole milk intake of mothers, infants and children would also increase their B-12 intake substantially. Another potential strategy, which is under discussion, is the addition of vitamin B-12 as a fortificant to wheat or maize flour. Wheat flour is currently fortified with folic acid but not vitamin B-12 in Guatemala and many countries in the world.

Activities are already underway to ensure that policy-makers are involved in evaluating the results of the study and aware of potential strategies to improve the situation.

Preliminary results on the vitamin B-12 deficiency problem in Guatemala and other Latin American countries were shared with the Nutrition office at the Pan American Health Organization (PAHO) in Washington D.C. PAHO is the regional office of the World Health Organization responsible for Latin America. This led to a Consultation on Folate and Vitamin B-12 Deficiency in the Americas to recommend the addition of vitamin B-12 as well as folic acid as fortificants for flour in Central and South America (see Allen, L., Folate and B-12 status in the Americas, *Nutr. Rev.* June 2004, and Flour Fortification with Iron, Folic Acid and Vitamin B-12, PAHO, Washington D.C., 2004).

Results of the baseline analyses were reported briefly in an invited lecture by Dr. Lindsay Allen on dietary problems of preschoolers, at the International Congress of Nutrition in Durban, South Africa, September 2005.

Dr. Allen was one of the organizers of a WHO Consultation on Folate and Vitamin B-12, held in Geneva, October 2005. She worked with WHO in the development of a global data base of the

prevalence of vitamin B-12 deficiency which revealed that this is a widespread problem, and presented a paper on the evidence that a lack of animal source foods was the main cause of vitamin B-12 deficiency in developing countries.

Dr. Allen was invited to present a lecture on the Animal Source Foods as a Source of Micronutrients in Developing Countries, at the Board for International Food and Agricultural Development meeting, held in Des Moines, Iowa, October 2005, in conjunction with the World Food Prize ceremony.

Once the results of the food-based intervention study are completed in 2006, additional activities will include the following.

The results and their implications will be shared at a scientific meeting at INCAP, Guatemala. INCAP serves the seven countries of Central America and a meeting will be selected where their representatives are present.

Results will be reported at the Society for International Nutrition Research annual meeting, the congress of the Latin American Nutrition Society, and in multiple publications.

OUTREACH

The primary outreach target will be women and children in regions of the world where the intake of animal source foods, and subsequently of vitamin B-12, is low. The mothers and children in this study are broadly representative of the high proportion of people in low income countries who consume inadequate amounts of animal source foods. We have learned from this Guatemalan study that consumption of cow's milk, rich in vitamin B-12, improves the vitamin B-12 status of these young children so it should be encouraged as a complementary food after the recommended six-month initial period of exclusive breast feeding. It is also apparent that the nutritional status of these women is inadequate to support the nutrient requirements and normal development of their infants. In the GL-CRSP funded Child Nutrition Project in Kenya, we demonstrated that inclusion of small amounts of meat or milk in snacks provided at school virtually eliminated the high prevalence of severe vitamin B-12 deficiency. This is a feasible

strategy in many situations. Additional extension approaches include the need for nutrition education of women and households on the importance of animal source foods; and increased emphasis on household animal source food production and preservation in agricultural development and training programs.

Dr. Ramirez is responsible for research on food security at INCAP and will follow up appropriately with the agricultural community through INCAP on the need for increasing animal source food intake, once the results of the study are known.

DEVELOPMENTAL IMPACT

Environmental impact and relevance. There is no direct environmental impact of this project. In the longer-term, it must be recognized that sufficient production and consumption of animal source foods is required for optimal human nutritional status and capital formation, and that this need should not be ignored even where there are environmental constraints.

Agricultural sustainability. This research will make it more evident that the concept of agricultural sustainability must include production and consumption of animal source foods, because of their demonstrated importance in improving dietary quality (micronutrient content of the diet). In low income countries, unless staple crops can be fortified with micronutrients such as vitamin B-12, and fortified foods are consumed in reasonable amounts by subsistence households, animal source foods are the only dietary source of some micronutrients such as vitamin B-12, and the major source of iron, zinc and other micronutrients. It is becoming increasingly accepted that it is infeasible to provide vitamin-mineral supplements for all populations in developing countries who need them; at present these are given only to some pregnant women and a few young children. Thus the definition of agricultural sustainability must include a minimum level of animal source food production and consumption by the population.

Contributions to U.S. agriculture. The nutritional risks associated with avoidance of animal source foods, and especially meat, are not adequately

appreciated in the U.S. or the world in general. For example, the practice of restricting the meat intake of children in the United States has become more common because some parents believe that “red meat” has adverse effects on health. In a recent study at UC Davis, for example, wealthier parents fed far less red meat to their children than did those in lower socioeconomic groups; 91% of lower income children and 100% of higher income children failed to meet the minimum number of meat servings. In an analysis of data from the National Health and Nutrition Education Survey (NHANES III), we found that plasma vitamin B-12 levels were significantly lower in those with lower intakes of meat. Many studies are revealing that lacto-ovo vegetarians or those who consume low amounts of meat, in industrialized countries have poor vitamin B-12 status, so meat needs to be consumed to ensure vitamin B-12 intake is adequate. In general, the study will reveal the importance of meat consumption for the lactating mother, and the normal development and nutritional status of young children.

Contributions to host country. INCAP is one of the premier nutrition institutions in Latin America, and for more than 50 years has been dedicated to research and its application to prevent and treat malnutrition. INCAP has collaborated with UC Davis in several previous studies that confirm the high prevalence of vitamin B-12 deficiency in lactating women, their infants, preschoolers and schoolers in peri-urban Guatemala City. The proposed research will enable INCAP to understand the prevalence and etiology of vitamin B-12 deficiency during the critical first year of life, as well as the adverse consequences of this deficiency, and eventually the need and application of strategies to prevent this wide-spread problem. This project has also increased the expertise of investigators at INCAP and enabled an important exchange with senior researchers who are working on this topic. The collaborative research has provided training and research skills with new methods of assessment, laboratory techniques, community-based research and especially in measures of child development. This is a critical area of interest to INCAP. The project has contributed to INCAP’s technical capacity and involvement in the area of

micronutrients and will result in peer-reviewed and locally/regionally relevant publications. INCAP will transfer the information learned to its member states, increase awareness about the importance of vitamin B-12 deficiency, and guide related policy decisions such as the need to support animal source production and consumption, to fortify maize and wheat flour or other foods with vitamin B-12, and/or provide vitamin B-12 containing micronutrient supplements to infants, pregnant and lactating women. Moreover, the research is highly relevant to the current incentive supported by the GL-CRSP to increase awareness of the nutritional importance of consuming animal source foods, and will inform development specialists and nutritionists in the region (and world) about their special importance for child development.

Linkages and networking. Dr. Ramirez is closely connected with Central America's focus on sustainable agriculture and will share the results of this research with the member countries. Dr. Allen will continue to work closely with the World Health Organization in the publication of the Consultation on Folate and Vitamin B-12, where the baseline results of this study were presented. Dr. Black will share the child development results at meetings concerned with child health. The team has been partially supported by the National Cattlemen's Beef Association which will publicize the results of this research. Preliminary results on the importance of animal source foods were presented to the Board on International Food and Agricultural Development, and at the American Association for the Advancement of Science, by the PI in 2005. The PI meets with the GL-CRSP investigators who will be updated on the results of this research, including those in projects studying constraints to animal source food consumption.

Collaboration with international research centers (IARCS) and other CRSPs. Drs. Allen and Graham have worked previously with the GL-CRSP's Child Nutrition Program in Kenya, which included beef and milk supplementation of schoolers. Dr. Allen's group demonstrated the beneficial effects of the animal source food intervention on vitamin B-12 status of the Kenyan children. More recently Drs. Allen and Graham received GL-CRSP funding to analyze the contribution of animal

source foods to the dietary intake of the Kenyan children, in collaboration with Dr. Marie Ruel of the International Food Policy Research Institute (IFPRI, a member of the CGIAR). They compared dietary diversity and animal source food intake as indicators of dietary adequacy and determined that dietary diversity was the best indicator of dietary quality when animal source food intake was very low, and that above a minimum level of intake, animal source foods significantly improve dietary quality.

OTHER CONTRIBUTIONS

Support for free markets and broad-based economic growth. This research will demonstrate the importance of animal source foods for adequate human nutrition, normal child development and human capital formation, thereby enabling broad-based economic growth. It will provide impetus to activities such as: nutrition education concerning the need to consume animal source foods; promotion of initiatives to increase animal source food production and consumption at the household, regional and national level; and innovative ways to preserve animal source foods and to feed them to young children.

Contributions to and compliance with mission objectives. Achievement of food security and consumption of a nutritionally adequate diet is an important objective of the GL-CRSP, and has long been an objective of USAID. This research will encourage renewed emphasis on the importance of animal source foods in agricultural production, and the role that animal production must play to enable normal human development and capital formation.

Concern for individuals. This project made a substantial contribution to the women and children who participated in this research. Mothers and infants who were severely vitamin B-12 deficient at baseline were all treated with injections of the vitamin. Individuals with illness, and children diagnosed with severe developmental delays, were referred for medical attention and appropriate care. One meal per day was provided to each of the children during the 9 months they participated in the study. The mothers, their households, community motivators and project staff all gained an increased

awareness of the importance of nutrition for child development.

Support for democracy. This research can demonstrate the fact that population groups, including those with limited resources, can improve their nutritional status and human capital formation through adequate consumption of animal source foods. Increased attention to this issue will reduce risk of functional impairments such as poor child development and increased risk of maternal depression, creating individual opportunities for achievement and more stable and democratic societies.

Humanitarian assistance. If households cannot obtain access to a food supply that provides sufficient nutrients to support their needs, this engenders the need for short term humanitarian support such as feeding programs, and remedial treatment with vitamin mineral supplements. In contrast, making animal source food production and consumption a focus of development programs will increase sustainability of a nutritionally adequate food supply for the population.

LEVERAGED FUNDS AND LINKED PROJECTS

This project has been able to leverage personnel funds and with support in-kind of two co-investigators to ensure that the study is administered and progresses toward successful completion. There have been no linkages to other grants/projects used for the conduct of this study.

USDA-Agricultural Research Service (\$14,250 per year; salary). Dr. Lindsay Allen is the Principal Investigator and the primary person responsible for all aspects of the study during FY04-05. She provides 10% of her time to this project.

USDA-Agricultural Research Service (\$9,942 per year; salary). Dr. Joanne Graham is a Postdoc supervising Dr. Allen's laboratory and is the main day-to-day UC Davis communicator with the field and with INCAP. She provided 20% of her time to this project. Duties included obtaining lab and other supplies, ensuring timely shipment of supplies including food, developing protocols for lab assays at INCAP and UCD and sample collection at INCAP, and some data analysis.

Support In-kind - Dr. Maureen Black (consultant) is a trained child development expert/psychologist who has years of experience in assessing the impact of nutrient intervention studies on child development in developing countries, and in assessing mother-child interactions. She was responsible for training and development in all areas of child development in this study.

Support In-kind - Dr. Margaret Bentley (consultant) is a Medical/Nutritional Anthropologist who has international experience in the assessment of food intake and feeding practices of infants and children; assessment of child activity (including a zinc intervention trial with INCAP in Guatemala which showed positive effects on activity); and ethnographic techniques that will enable us to understand the mother's perception of their child's health and development relevant to the cultural norm, and issues such as how to motivate the parents to adhere to the intervention. She provided guidance in the training of staff for the assessments of child activity, feeding and health and designed the pilot trials of infant acceptance of the supplementary foods.

TRAINING

Degree

Katherine Jones, PhD, 2005, Nutrition, University of California, Davis. Completed.

Kathleen Deegan, PhD, 2007, Nutrition, University of California, Davis. In-progress.

Non-Degree

Dr. Manuel Ramirez-Zea attended a two week training session with the project statistician at the University of California, Davis in the Fall, 2004, to learn and coordinate data cleaning methods, and data analysis procedures with the Davis team. Partial support was provided by Fogarty.

COLLABORATING PERSONNEL

United States

Allen, Lindsay. Lead Principal Investigator, USDA - Western Hum Nutr Res Ctr and University of California, Davis.

Bentley, Margaret (Peggy). University of North Carolina.

Black, Maureen. University of Maryland.

Graham, Joanne. Postdoc, USDA - Western Hum Nutr Res Ctr.

Jones, Katherine. Graduate Student, UC Davis.

Guatemala

Ramirez, Manuel. Project Director, INCAP.

Torun, Benjamin. Consultant, INCAP.

Zuleta, Clara. Project Supervisor, INCAP.

Mejia, Rosa Mery. Psychologist, INCAP.

Navas, Lilian. Field Supervisor, INCAP.

Ramos, Luisa. Secretary, INCAP.

Salazar, Irma. Data Entry, INCAP.

Muz, Ana. Psychology Student, INCAP.

Hernandez, Lorena. Psychology Student, INCAP.

de Juarez, Thelma. Interviewer, INCAP.

de Maldonado, Tania. Interviewer, INCAP.

Castro, Alvaro. Driver, INCAP.

COLLABORATING INSTITUTIONS

USDA, ARS Western Human Nutrition Research Center and Dept. of Nutritional Biology
University of California, Davis

Davis, CA 95616

Phone: (530) 752 5269

Fax: (530) 752 5271

Instituto de Nutricion de Centro America y Panama (INCAP)

Calzada Roosevelt, Zona 11

Apartado Postal 1188, Guatemala City

Guatemala

Phone: (502) 2471 9913

Fax: (502) 2473 6529

PUBLICATIONS

Jones, K.M. (2005) Vitamin B-12 Deficiency in Guatemalan Mother and Infants: Prevalence, Predictors, and Associations with Maternal Depression and Infant Development. Ph.D. thesis, University of California, Davis.

ABSTRACTS AND PRESENTATIONS

Jones, K.M., Black, M.M., Mejia, R.M., Ramirez-Zea, M., Zuleta, C., Allen, L.H. Cognitive function, motor skills, and behavior of Guatemalan infants with highly prevalent deficient and marginal plasma vitamin B-12 concentrations. 2005. Submitted for presentation at Experimental Biology 2006.

LEAD PRINCIPAL INVESTIGATOR

Lindsay Allen, Emeritus Professor, Department of Nutrition, and Center Director, USDA - Western Human Nutrition Research Center. University of California, Davis. Davis, CA 95616. Tel: (530) 752-5920 or (530) 752-5268. Email: lhallen@ucdavis.edu.