
Principle Investigators
Barbara Piperata, PhD
Associate Professor
Department of Anthropology
The Ohio State University
Columbus, Ohio, USA
Tel: (614) 292-2766
E-mail: piperata.1@osu.edu

Jeehea Sonya Haw, MD
Instructor of Medicine
Division of Endocrinology, Metabolism and Lipids
Emory Univ. School of Medicine
Atlanta, Georgia, USA
E-mail: jeehea.sonya.haw@emory.edu

Warren Wilson, PhD
Associate Professor
Department of Anthropology & Archaeology
University of Calgary
Calgary, Alberta, Canada
Tel.: 403-220-2665
E-mail: wwilson@ucalgary.ca

Summary
Each year millions of pregnant women, mothers, and children experience severe illness or death, largely from preventable or treatable causes. Ninety-nine percent of these deaths occur in the developing world. Attention to maternal and child health (MCH) has been growing, and improving MCH is seen as critical to fostering economic development. The urgency of this issue is reflected in the fact the MCH is the number one international development priority for the Canadian government and one of the three primary global health goals of the United States government. It is also reflected in two of the Millennium Development Goals (MDGs); MDG 4 (reduce child mortality) and MDG 5 (improve maternal health). Of the eight MDGs, these two are the most at risk of not being met. Achieving improvements in MCH Achieving this requires a clear understanding of both health status and cultural variables which influence health. Medical anthropology, by synthesizing data on both health outcomes and cultural context, is well suited to inform our understanding of both health outcomes and culturally-appropriate public-health interventions. Toward that end, in collaboration with epidemiologists, a sociologist, local NGOs and front-line health workers in the region, this project is designed to document health outcomes and causes of these outcomes among rural women and children who inhabit two of Nicaragua’s poorest regions. The health outcome variables explored in this study include nutritional status, mental health, blood pressure, and biomarkers of immune function, stress, and metabolism. The causal variables explored in this study include food insecurity, the network of friends and family upon which one can rely (social capital), digestive system function, and other cultural and demographic factors. The immediate outcome of the project will be the identification of barriers to maternal and child health in this region, which in the long term should lead to the development of new evidence-based and locally-relevant solutions.
Project Outline

Purpose:

1) *short-term*: the identification of
   a) MCH outcomes in one rural community in Nicaragua;
   b) the identification of barriers to maternal and child health MCH in this community.

2) *long-term*:
   a) explore the relationship between food insecurity and health outcomes;
   b) explore the relationship between food insecurity and two other measures of dietary adequacy;
   c) the development of new evidence-based and locally-relevant public health solutions.

Methods:

1) document the health and anticipated predictors of these health outcomes for 250 mothers and one of their children.

2) *Health outcome variables considered*:
   a) Self-Reported Health,
   b) Anthropometrics (weight, height, sitting height, waist circumference),
   c) Arterial Blood Pressure,
   d) Biomarkers (immune and metabolic function).

3) *Anticipated predictors of MCH considered*:
   a) Culture (via ethnography),
   b) Sociodemographics,
   c) Social capital,
   d) Maternal mental health,
   e) Food insecurity,
   f) Food frequency / dietary diversity,
   g) Gut function (environmental enteropathy).

4) analyze the relationship between anticipated predictors of health and the observed health outcomes with an emphasis on diet;

5) meet with all stakeholders subsequent to data analysis to discuss findings, possible public-health interventions, and further research needed.

Expectations:

1) Health outcomes in mothers and children will be predicted by diet (food insecurity & food frequencies), household wealth and the size of social networks.

2) Health outcomes in children will also be predicted by the function of their digestive system and mediated by their mother’s mental health.

Timeline:

May-July 2015 (Nicaragua)- collection of interview and health outcome all data.
August-November 2015 (Tuscaloosa, USA)- analysis of biosamples (blood & urine)
August 2015-May 2016 (Calgary, Canada and Columbus, Ohio)- data analysis and write up of key findings
June 2016 (Nicaragua)- meet with stakeholders to discuss findings and future directions.

Collaborators: Comunidad Connect (Nicaraguan NGO), Nicaragua Community Health Connection (Nicaraguan NGO), Project Wave of Optimism (Nicaraguan NGO), Jason DeCaro, PhD (University of Alabama), Kammi Schmeer, PhD (The Ohio State University).

Background

Thanks to renewed vigor in efforts to address global health issues, the past 15 years have witnessed notable declines of child and maternal mortality. There remain, however, persistent health inequities that require urgent scrutiny. Maternal mortality rates, for example, remain unacceptably high: every three seconds a child dies, almost all in poor countries and most often due to preventable causes. Of those causes, the most fundamental is undernutrition: about 805 million people, including 1 in 4 children, remain chronically undernourished and progress in addressing this issue has slowed in the past decade. In developing countries 56% of child deaths are attributable to undernutrition's potentiating effects and 83% of these are attributable to mild-to-moderate as opposed to severe malnutrition. In addition, childhood undernutrition chronically compromises immune function, cognitive development, physical work capacity, and adult economic productivity.

The unfinished agenda concerning maternal and child health is particularly concentrated in developing countries, with dire, long-term implications for social cohesion, stability, and development. In 2014, the WHO’s Director General emphasized that from a technical standpoint we know how to tackle the persistent causes of maternal and child mortality, “but we must find the right approach in each and every setting”. Indeed, the causes of death differ substantially from one country to another, highlighting the need to expand understanding of child and maternal health and predictors at a local level rather than in geopolitical regions. Moreover, decades of research have documented that health varies in relation to culture. The collection and analysis of data on cultural variables which mediate health outcomes in each setting is consequently required to save the lives of women and children. The discipline of medical anthropology, with its consideration of both biological and cultural variables associated with health outcomes, is well-suited to such research. Not surprisingly, medical anthropologists concerned with health inequities have provided a wealth of knowledge which has informed public health interventions.

Objective

The objective of this project is to investigate the health of women and children who inhabit a rural Nicaraguan community to provide innovative, locally-grounded research on health outcomes and predictors in this region. The health outcome variables considered, selected on the basis of known public-health issues in Central America, include nutritional status, mental health of the mother, blood pressure, patterns of illness, and immune and metabolic function. Of these, nutritional status, blood pressure, patterns of illness and immune and metabolic function will be combined to create an index of overall health outcomes (also known as the cumulative burden or allostatic load) in light of the environmental challenges faced (Figure 1). The variables considered in this study which may explain these health outcomes include diet (food insecurity and 24-hour dietary recalls), social support networks (social capital) and other cultural factors,
and digestive tract function. These variables have been selected as they have been found to have strong explanatory value elsewhere in Latin America.

![Conceptual framework of the study](image)

Figure 1. The conceptual framework of the study.

Although food insecurity, social capital, gut function and sociodemographic characteristics, have been linked to maternal and child health individually, no study has considered each of these variables in a single model. Further, no study has assessed the cumulative burden of these variables. In this we will build upon similar work we’ve completed in Brazil, Tanzania, Guyana, and elsewhere in Nicaragua.

**Why combine a number of markers of health to assess “cumulative burden”? Allostatic Load & Overload**

Here, this measure of overall health or cumulative burden provides a comprehensive way of thinking about the well-being of the participants. For the past 25 years, cumulative burden has also been conceptualized as a phenomenon known as “allostatic load”. We can only survive within a very narrow thermal, chemical, and physical range and have a suite of physiological systems which keeps us within this range. For example, in healthy individuals, the consumption of sugars results in the release of insulin by the pancreas which helps to maintain a safe and narrow range of blood-sugar levels. In another case, if a person travels to high altitude, the lower air pressure at high altitude makes it harder to obtain sufficient oxygen in each breath to fuel our metabolic processes. Consequently, at high altitude a suite of systems automatically turn on to ensure that tissues obtain sufficient oxygen. Another example can be seen when a person is in a cold environment; their body will elevate its metabolic rate to ensure that their body remains warm enough to function.

Our systems interact to maintain physiological homeostasis (such as safe blood-sugar levels, enough oxygen or sufficient heat) in the face of environmental challenges and are collectively called allostatic systems. While allostatic systems can be activated and quickly deactivated if an environmental challenge, or stressor, abates, they carry a cost over the long run. When allostatic systems are activated chronically over a course of years or decades, pathologies develop - such as diabetes, atherosclerosis, growth faltering, or Alzheimer’s. This cost - the cumulative burden of stressors- is allostatic load.

This burden can be illustrated by a see-saw (Figure 2). With either the 5 kg children or 500 kg Sumo wrestlers, the system is in balance. However, having Sumo wrestlers, illustrative of a greater environmental challenge, on the see-saw exerts more strain on the system. Over time, greater environmental challenges will greatly compromise the see-saw. Likewise the strain...
on a living organism due to repeated ups and downs of physiologic response, as well as by the elevated activity of physiologic systems under challenge, and the changes in metabolism all increase the wear and tear on a number of organs and tissues.

Figure 2: Allostatic load

Any operational definition of allostatic load must include some combination of mediators and/or markers that can gauge how far this process has gone - how much cumulative burden has developed. Often researchers mix mediators and markers. So, for instance, in US adults, it might be common to include mediators like stress hormones (e.g. cortisol and epinephrine), as well as markers of dietary adequacy and cholesterol, all rolled into one index. This allows a researcher to capture ongoing allostatic processes, the mediators, as well as the cumulative effects of prior allostatic processes, the markers.

Why does the number of systems matter? The concept of allostatic load or “cumulative burden” is useful as a construct because it tries to cast a broad net. Over time, stressors are likely to affect many physiological systems through many proximal pathways. The more systems we capture, the better we’re doing at gauging the overall cumulative burden of stressors or, the health, of the population. Toward this end, some researchers combine data across 6, 7, even as many as 10 systems to achieve a comprehensive allostatic load index. Here we seek to create a comprehensive measure of cumulative burden / allostatic load using X markers to better understand the well-being of Nicaraguan mothers and children.

What is known?
1) Food insecurity: Food insecurity is a term that describes the lack of physical and economic access to sufficient and nutritious food for the entire household. Food insecurity has been linked to child stunting, underweight and under-five mortality. It is a significant public health and humanitarian problem within Nicaragua. This problem was exacerbated in 2014-2015 by both Nicaragua’s worst drought in 32 years and the largest epidemic of coffee leaf rust, a virus which decimates the coffee crop, since 1976.
2) Demographic characteristics: Socioeconomic status, marital status, maternal education and a number of other demographic characteristics have been linked with MCH in developing countries.
3) Social support networks (social capital): This is a measure of the extent to which people are embedded within their family relationships, social networks and communities, and have a sense of civic identity. Essentially, we seek to know how many friends an individual can turn to when faced with a problem. In our work in Tanzania and that of others elsewhere, a larger network of
friends has been found to explain lower stress levels in mothers and better overall health outcomes in their children, regardless of food insecurity levels.

4) Maternal mental health: While the relationship is inconsistent, maternal mental health has been linked to child health in developing countries. Most recently, in the work of one of our team members in Leon, Nicaragua and our work in Tanzania maternal anxiety and depression levels were strongly predictive of child health outcomes.

5) Digestive tract function: Nutrition supplementation programs often fail to improve the health of targeted populations. Recent research suggests that this may be due to compromised function of the digestive tract. Diseases of the small intestine, characterized by reduced capacity to absorb nutrients from food, commonly affect people in developing countries, generally due to chronic infection. In turn, reduced absorptive capacity has been found to explain up to 64% of compromised nutritional status in children in West Africa and East Asia.

**Procedures**

**Sample**

The unit of analysis for this study is households with the mother present and one child between the ages of 0.0-1.0 or 3.0 – 11.0 years. Our age criteria are based on our interest in understanding the impact of the household food environment on young children aged 3.0-11.0 years who are still young enough to be heavily dependent on adults for securing food and 0.0-1.0 years who are breastfeeding which enables us to analyze gut function as described below. Sample size calculations are based on the expected relationship between food insecurity and child cumulative burden, because this is likely to be the weakest effect tested: we have hypothesized it is mediated by maternal mental health and in previous studies the effect of food insecurity on child growth outcomes has been small.

Data were collected in 250 households in Los Robles, a community of approximately 2,200 individuals in a rural region in Nicaragua’s Department of Jinotega. The sample was selected for four reasons: 1) women and children are the cohorts most at risk cohorts for poor health outcomes in developing countries; 2) populations in rural regions are more at risk for poor health outcomes than those in urban areas; 3) the Department of Jinotega manifests the worst MCH in Nicaragua; 4) in December 2014 a new health clinic was opened in Los Robles, providing the citizens with an alternative to the older clinic which is two-hour walk away and these data provide a baseline to evaluate the impact of this change; 5) this study provides an opportunity to compare the same health outcomes and predictors collected in 2013-14 by Piperata and colleagues in Leon, an urban setting in Nicaragua.
Variables considered

Health outcome variables considered (dependent variables):

*Self-Reported Health & Maternal-reported health of child:* Mothers were interviewed about their health and that of their child. These data provide the maternal perspective on their health and that of their child.

*Anthropometrics:* Standard procedures were implemented to collect anthropometric measures for each mother and child, including standing and sitting height, and weight. Anthropometry provides the single most portable, precise, replicable, universally applicable, inexpensive and non-invasive technique for assessing the health and nutritional status of populations. As variation in growth is generally best explained by diet, anthropometrics, along with food insecurity, dietary intake data, and transferrin levels, will serve as a fourth indicator of dietary adequacy. In addition, waist circumference was collected for mothers. Waist circumference is the simplest way to measure abdominal obesity—the extra fat found around the middle that is an important factor in health, independent of body-mass index (BMI).

*Blood Pressure:* Three arterial blood pressure measurements were taken at five minute intervals, and the results employ the mean of the three readings. Three readings were averaged because this minimizes intra-individual variability. All readings were made with the respondent seated and the right arm supported at heart level.

*Immune & metabolic function & Iron levels via Dried Blood Spots:* Although many important markers of health are present in serum or plasma, the collection is an invasive procedure. In addition, once collected, these samples must be immediately centrifuged, separated, and quick-frozen or assayed, requiring ready access to lab facilities. Recently, researchers have developed protocols which surmount these obstacles. The Dried Blood Spot (DBS) protocol requires only a finger prick and the resulting drops of blood placed on filter paper. The filter paper can be easily transported and subsequently assayed for biomarkers of health.

While field-based research on morbidity often relies on caregiver or self-reports of overt symptoms, typically recalled over a 7- or 14-day period, this approach is subject to underreporting, particularly for longer periods of time. Additionally, linguistic and cultural factors help define idealized states of health, and may contribute to variation in the experience and reporting of symptoms. Physical examinations by healthcare professionals can provide a more objective assessment of child morbidity, but are time-intensive and may not be feasible in this field settings. The recent developments in DBS assessment inflammation and immune markers address these shortcomings, providing a powerful new tool to assess the immunocompetence, metabolic function, and aspects of dietary adequacy of peoples in isolated locations.

The collection of DBS samples is straightforward and non-invasive in comparison with venipuncture. The process entails first cleaning the participant’s finger with isopropyl alcohol, and then pricking the finger with a disposable lancet and the drops of blood are then spotted onto standardized filter paper. Samples are allowed to dry overnight at ambient temperatures, and
then stored in plastic containers at -20°C prior to transport to a laboratory for analysis. For this project 10 drops of blood were collected for 250 mother-child dyads and will be assayed for nutritional adequacy (transferrin), immune function indicators of both acute stress (C-reactive protein) and chronic stress (Epstein-Barr virus antibodies), and glucose challenge metabolic function (hemoglobin A1C).

Iron deficiency (ID) is the most common micronutrient deficiency worldwide, and transferrin receptor (TfR) level has been identified as an important measure of iron status that is not confounded by inflammation. Concentrations of TfR remain stable for at least 4 weeks when blood spots are stored at room temperature. The DBS protocol for TfR is precise and reliable in the detection of ID with both the presence and absence of infection, agrees well with plasma TfR and is feasible in remote settings such as Los Robles.

C-reactive protein (CRP) is an inflammatory protein produced by liver hepatocytes in response to messenger cytokines, primarily IL-6. CRP is an important component of innate immunity and has been used clinically for decades as an indicator of active infection. Trace amounts of CRP are normally present in circulation, but concentrations increase more than 100-fold during the 24-72 hours following infection. Since CRP has been shown to increase in response to a wide array of pathogenic agents as well as psychological stressors, it is a potentially useful marker of stress. Concentrations of CRP above 5—10 mg/L have been associated with detrimental child growth outcomes, poor iron status, sub-clinical infectious morbidity, and infection and inflammatory processes in many field-based studies with infants, children, and adolescents. Serum/plasma CRP concentrations greater than 10 mg/L are assumed to represent an acute inflammatory response, likely to an infectious agent. Within most populations, high-sensitivity CRP (hs-CRP) assays show >95% of subjects with values of <10 mg/L. Although there seems to be population-to-population consistency in this, data for ethnic populations are limited and the current “normal” values are derived almost exclusively from European or European-American populations.

Epstein-Barr virus (EBV) is a ubiquitous human γ herpes virus which infects and establishes a mostly asymptomatic life-long infection in B lymphocytes. In LMICs, nearly 100% of individuals are sero-positive for EBV by the age of five years. Once infected, individuals harbor the virus for life in infected cells, and adequate cell-mediated immune function is critical for maintaining the virus in a latent state. During latency, an intact EBV genome resides in the cell as an extrachromosomal plasmid and evades surveillance and elimination by cytotoxic T-lymphocytes (CTLs) through down-regulation of many latent-cycle antigens that are the target of CTL attack. Stress–induced immunosuppression which may be triggered by episodes of nutritional, infectious, or psychological stress, allow the virus to reactivate and release viral antigens into circulation, to which a secondary humoral antibody response may emerge. As a result, EBV-specific antibodies provide an indirect, functional measure of cell-mediated immune activity, such that increased EBV antibody titers indicate lower cell-mediated immunity.

The utility of the EBV antibody model has been demonstrated in several studies of stress-induced immunosuppression. Elevated EBV antibody titers have been associated with a number of stressors. Alternatively, stress management interventions have been associated with decreases in EBV antibody titers.
Finally, in comparison with other measures of immunocompetence, meta-analysis has identified EBV antibodies as among the strongest and most consistent correlates of chronic stress. EBV-specific antibody titers can be expected to rise 2-7 days after viral antigen re-exposure with a consequent antibody response on the order of days or weeks. EBV antibody levels are therefore not subject to short-term fluctuation, acute context effects, or diurnal variation, and a single sample can be used as an immunological measure of chronic stress.

Metabolic function was measured with both non-fasting blood glucose tests and the collection of DBS to determine glycated hemoglobin A1C (HbA1C) levels. A random capillary glucose test (aka non-fasting or casual blood glucose test) was conducted on all mothers, to screen for metabolic function with a focus on those at risk for diabetes. A random capillary glucose test assumes a recent meal and therefore has higher reference values than the non-random (aka fasting) glucose test. If a healthy adult without diabetes is given random glucose tests throughout the day, their glucose levels would remain relatively stable regardless of dietary intake or stress. Alternatively, in people with diabetes and prediabetes, glucose levels can vary widely or be consistently high over the course of the day. The reference values for a "normal" random glucose test in an average adult are 79 - 140 mg/dl (4.4 - 7.8 mmol/l), between 140 - 200 mg/dl is considered pre-diabetes, and > 200 mg/dl is considered diabetes according to American Diabetes Association guidelines.

In this study, this test was given twice to 250 mothers who had eaten within the last few hours: at the start of the interview and one hour after consuming a glucose load of 50g anhydrous glucose dissolved in water > 200 mg/dL. Prior to the first test, the mother was asked if she had eaten anything in the past few hours. Those who had not eaten were excluded from the study. In this test, the subject’s finger was pricked using a disposable lancet and the blood sugar measured with a Walgreens Trueresult Blood Glucose Monitoring System.

The HbA1C provides an estimate of a person’s average blood sugar level for the past few months. This test does so by measuring the percentage of blood sugar attached to hemoglobin; the higher the HbA1C is, the higher your recent blood sugar levels have been. An A1C level of 6.5 percent or higher is indicative of diabetes. A result between 5.7 and 6.4 percent indicates prediabetes. Normal levels are below 5.7 percent. An advantage of HbA1C over blood glucose measurement include greater specimen stability; relative to blood glucose, HbA1C levels fluctuate less day-to-day due to stress and illness. A disadvantage of utilization of HbA1C over blood glucose measurement is that conditions that shorten red blood cell (RBC) survival e.g., hemolytic anemia, homozygous sickle cell trait, pregnancy, or recent significant blood loss, will reduce exposure of RBCs to glucose, thereby lowering the HbA1C test value and resulting in a false negative. Consequently, HbA1C values will not be calculated for pregnant women. As well, specimens with >10% fetal hemoglobin may have a falsely decreased HbA1C test result. Finally, if the onset of diabetes is rapid, blood glucose levels will more correctly reflect glycemia than HbA1C levels.

To measure HbA1C, blood was collected for 250 women following the DBS protocol described above. The blood was collected after a one-hour oral glucose tolerance test (OGTT), following a glucose load of 50g anhydrous glucose dissolved in water > 200 mg/dL.

The DBSs will be assayed for CRP, EBV and transferrin at Dr. Jason DeCaro’s Developmental Ecology and Human Biology Lab at the University of Alabama, Tuscaloosa. Dr.
DeCaro is an expert in this field, has conducted similar research with WW in Tanzania, and spent one week with the team in Nicaragua to observe data collection. The DBS will be assayed for HbA1C in Dr. Alan Potter’s Department of Laboratory Medicine at the University of Washington. All samples spent less than 96 hours at ambient temperatures; 24 hours to dry immediately post-collection and less than 72 hours for transportation from Nicaragua to the University of Alabama. The HbA1C samples may require 24 more hours at ambient temperatures for transport to the University of Washington.

**Anticipated predictors of MCH considered (independent variables):**

Independent variables were selected based on their power to explain health outcomes in similar settings. We prioritized diet as it has been found to have the strongest predictive power of health outcomes in low-to-middle income countries (LMICs).

**Ethnography:** Wilson conducted participant observation by living in the region from January through June 2015. Participant observation connects the researcher to the most basic of human experiences, discovering through immersion and participation the hows and whys of human behavior in a particular context. During this time, he participated in community activities, such as the harvesting and transport of coffee, road repair, the preparation of farms for maize and bean cultivation, and varied daily activities commonly undertaken by the citizens of Los Robles. Qualitative observations were be recorded daily in his field journal. As well, he frequently asked questions designed to uncover the meaning behind observed behaviors.

**Sociodemographics:** Age, sex, date of birth, relationship to male and female head of household, occupation for past five years, years of education, marital status, family size data, numbers of new births, deaths, patterns of immigration and emigration etc. were collected for each household. Objective socioeconomic status (OSS) will be determined on the basis of household production, composition, wage labor, and assets. Material goods, duration of wage labor, and receipt and use of remittances will be summed to create an OSS score. To measure household wealth, we recorded the condition of the home, access to basic resources, and type and number of assets that a household owns.

**Social capital and Subjective social status:** Social capital is the extent to which people are embedded within their family relationships, social networks and communities, and have a sense of belonging and civic identity. Generally, in times of need, those with more social capital find that they have a larger network of friends upon whom they can rely. Previous research finds that social capital is a strong predictor of ill-health. Social capital was measured using a series of questions concerning friends one can turn to in times of need. Subjective social status was measured using the MacArthur Subjective Social Status Scale (MSSSS). The MSSSS is an instrument which tries to capture individuals’ sense of their place in the social ladder, taking into account standing on multiple dimensions of socioeconomic status and social position. It has been validated for use in developing countries with minor adaptations to local contexts.
Maternal mental health: A robust body of research persuasively argues for more attention to be paid to mental health in developing countries, noting that social factors known to be linked to poor mental health are on the rise throughout the developing world. We are particularly interested in understanding the relationship between maternal mental and physical health and household-level food security as previous work by members of this research team and others indicate food insecurity can undermine both mental and physical well-being. As well, two of our team members found that in Tanzania poor maternal mental health undermines a mother’s ability to buffer her children from low food availability. To assess mental health, we will used the Self Reporting Questionnaire (SRQ-20), a 20-question symptoms checklist. The SRQ-20 shows high validity and has been used extensively in vulnerable populations across a range of cultural contexts including Nicaragua. The SRQ-20 elicits information about common psychoneurotic complaints and can be completed within five minutes, enabling researchers to screen large groups of patients in a busy medical setting where it would be impractical to perform more extensive evaluations of emotional symptomatology.

Food insecurity: Food insecurity was measured using the Latin American and Caribbean Food Security Scale (ELCSA). It assesses levels of perceived food security and has been validated throughout Latin America, including in Nicaragua. The use of the ELCSA enables us to compare our findings with those collected elsewhere by two of our team members in Nicaragua and others in Latin America.

Dietary intake: Dietary intake will be assessed via a food frequency questionnaire. The resulting data will be analyzed for dietary diversity.

Digestive function: Despite a multiple intervention programs, undernutrition remains the number one risk to health worldwide, killing more people than AIDS, malaria and tuberculosis combined. The reason behind failed nutritional therapy is unclear, but recent research suggests that it may be explained by chronic infection which damages the intestinal tract, a condition known as environmental enteropathy (EE), a condition marked by compromised nutrient absorption and intestinal permeability. As well, EE hinders oral vaccine effectiveness leading to undernutrition and further infection.

EE will be evaluated using the lactose:creatinine urinary (L:C) test. Urine was collected on a sterile pad placed inside a locally purchased diaper for 50 children <12 months of age in Los Robles. Once the child urinated, the pad was removed and two, two ml samples of urine were extracted using a syringe and placed in an airtight vial with two drops of chlorhexidine digluconate. The urine samples will be analyzed at Dr. Jason DeCaro’s Developmental Ecology and Human Biology Lab at the University of Alabama, Tuscaloosa.

Data collection team

The data collection team consisted of WW, four local, female assistants (aka Brigidistas) with years of experience in public health, a local physician, Dr. Alfredo Alaniz, employed in the the Los Robles clinic and four students, a graduate and an undergraduate student from the University of Calgary and two MPH students from Emory University. WW worked closely with
the *Brigidistas* and Dr. Alaniz from January to June 2015 as described below, trained the N. American students in data collection procedures over a one-week period and subsequently supervised data collection.

**Instrument refinement and team training**

From January through April 2015, WW and the 13 *Brigidistas* who work in Los Robles adapted the sociodemographics, social capital, subjective social status (MacArthur Subjective Social Status Scale), maternal mental health (SRQ-20), food frequency and food insecurity (ELSCA) instruments to the local context in four steps. First, in January, February, and March 2015, WW and the *Brigidistas* spent a total of six days practicing with the instruments. The *Brigidistas* were from Los Robles and used local knowledge to refine each instrument. After several months in Los Robles, WW had gained a bit of an understanding of local culture enabling him to also contribute to refinements of the instruments. Second, under WW’s supervision, in April 2015, the team spent two days at the Los Robles health post conducting interviews first with each other and subsequently with two focus groups each with four randomly selected mothers from Los Robles to ensure that the instruments were culturally relevant. These eight mothers were not compensated for their time and were subsequently excluded from the research sample. WW altered the instruments accordingly after the focus groups. Third, one week later, the *Brigidistas* used the refined instruments in individual interviews with six Los Robles mothers. These six mothers were not compensated for their time and were subsequently excluded from the research sample. WW then used pooled respondent feedback to rephrase portions of the interview that were unclear. Fourth, the following week WW spent two days training four *Brigidistas* to conduct interviews using the refined questionnaires. Training elements included explanation and discussion of each item, learning how to interview by role playing and with real respondents.

**Data collection**

250 mother-child dyads were recruited opportunistically, either in a visit to their homes or when they visited the Los Robles Health Post. The recruitment of participants in their homes required the team to visit every house in Los Robles with a mother and child within the age ranges described above. Exceptions to this were those households which had already been surveyed while they were visiting the health post.

All structured interviews were carried out by one of four *Brigidistas* on the team. The collection of anthropometric data was carried out by the four students and WW. The collection of the biomarker data was carried out by Dr. Alaniz, the four students, and WW. Data were collected in the Los Robles Health Post or participants’ homes. Data collection generally required no more than 1.5 hours and proceeded in the following six steps:

1. Ask the potential participant if they know whether or not they are diabetic. If not, the interview continued. Ask the participant if they have eaten in the past few hours. If yes, the interview continued. (*Brigidista* administered, 2 minutes)
2. Describe the study to the participant, read the letter of consent to the informant and obtain their signature or oral consent if non-literate. (*Brigidista* administered, 10-15 minutes)
3. Collect the first measure of blood glucose, collect 5 DBSs, and have the participant drink the solution for the oral glucose test. (student, Dr. Alaniz, or WW administered, 10 minutes)

4. Conduct the interview (Brigidista administered, 30-40 minutes)

5. Collect anthropometric data (student or WW administered, 10 minutes)*

6. One hour post consumption of the glucose solution, collect the second measure of blood glucose and 5 more DBSs (10 minutes).

*In those cases when step 4, the interview, took longer than 40 minutes, step 5, the collection of anthropometric data, was conducted after step 6, the collection of the second round of blood glucose and DBSs.

Data analyses

Self-reported health, blood pressure, and dried blood spot assay data will be combined with standardized measures of growth and nutritional status to a construct multi-measure, cumulative-burden, index of child and maternal well-being as the main dependent variables. This approach is more attractive than a single measure because it quantifies the cumulative burden of adversity reflected in the body’s responses to multiple stressors across developmental time. Ordinary least squares regression will be used to test the model, relying on procedures developed to identify mediation.

Institutional Ethical Clearance

The research was approved by the Nicaraguan Comité Institucional de Revisión Ética (CIRE), del Centro Nacional de Diagnóstico y Referencia (CNDR) del Ministerio de Salud de Nicaragua (MINSA) (Ethics ID: NIC-MINSA/CNDR CIRE-25/05/15-060) and the Conjoint Health Research Ethics Board in the Faculty of Medicine at the University of Calgary (Ethics ID: REB15-0232).