

JHSPH Institutional Review Board**RESEARCH PLAN**

PI: Benjamin Caballero, Manuel Ramirez-Zea, Paola Letona, D. Roche

Study Title: Cardiovascular disease risk factors in school-age children living in poor urban areas of Guatemala

IRB No.: 2642

PI Version Number/Date: Version 3 (April 13, 2010)

1. Aims/objectives/research question/hypotheses:

- To explore the common knowledge and attitudes about childhood environmental determinants of cardiovascular health among children and parents.

2. Background and rationale:

Cardiovascular health is deteriorating since early years of life in the developing world, due to rapid changes in lifestyle patterns. These alterations in the way of life throughout childhood are being reflected by a rapid increase in the prevalence of obesity and being driven by environmental (obesogenic) determinants, such as increase in use of motorized transport, fall in opportunities for recreational physical activity, increased sedentary recreation, greater quantities, variety, and marketing of energy-dense micronutrient-poor foods and beverages available, among others.¹ Obesity and sedentarism are the most prevalent risk factors that are inducing the CVD epidemic. Obesity has been increasing at a remarkable rate in the last decade in the developing world, even in countries like Guatemala, where the double burden of nutritional problems is one of the greatest in the world.² The data on physical activity patterns change is scarce. Unfortunately, most school- and home-based interventions have failed to prevent childhood obesity and few comparable data are available about cardiovascular alterations during childhood.³ To intervene in early ages becomes a critical subject, due to the fact that the direct and indirect costs related to the treatment of CVD are high, and prevention seems to be the only cost-effective alternative. Furthermore, children shape their food and activity preferences and develop essential motor skills before 7 years of age; thus, working before or around this age becomes extremely decisive. Therefore, it is crucial to start actions as early in life as possible, to revert or at least stop the CVD epidemic.⁴ The most important actions that have to be performed are the prevention of childhood obesity and sedentarism. A well-designed intervention model that takes into account the culture and specific necessities of its population will contribute to good quality of each individual life and wellbeing, and it will also save millions of dollars currently used in the treatment of CVD and its consequences. With this project we hope to promote the advance of health promoting schools in urban areas, particularly in the poorest communities where nutritional problems by deficiencies and excesses co-exist.

The aim of this first study is to collect baseline and formative research data for the subsequent development of a multilevel intervention. These studies are part of the research activities of the recently created Center for Excellence, funded by the NHLBI.

¹ Caballero, B. The global epidemic of obesity: an overview. *Epidemiol Rev.* 2007; 29:1-5.

² Duran, P.; Caballero, B., and de Onis, M. The association between stunting and overweight in Latin American and Caribbean preschool children. *Food Nutr Bull.* 2006 Dec; 27(4):300-5.

³ Summerbell CD, Waters E, Edmunds LD, Kelly S, Brown T, Campbell KJ. Interventions for preventing obesity in children (Review). *The Cochrane Collaboration.* Published by John Wiley & Sons, Ltd. 2006.

⁴ Caballero, B. Obesity prevention in children: opportunities and challenges. *Int J Obes Relat Metab Disord.* 2004 Nov; 28 Suppl 3:S90-5.

3. Participants:

The main target population consists of children 9-12 years of age (4th- 6th grade) enrolled in low-income urban elementary public (free) and private (monthly fee <US\$16) schools from three urban municipalities around Guatemala City (Villa Nueva, Santa Catarina Pinula, and Mixco). Schools will be selected in each phase by convenience, according to the receptivity and support of directors and teachers. Parents of those children will be also targeted.

We will recruit randomly up to 50 children (9-12 years of age, 4-6 grade only) and parents of four public and two private schools from the 3 municipalities for focus group discussions (FGD, at least 3 from each group, 6-8 participants). This formative research has already been done in a previous study in children from 1st to 3rd grade. Home visits will also be done up to 30 families (5 per school) to assess predisposing and reinforcing factors of health behavior. Exclusion criteria: individuals who are not in the school/community selected at the moment of the activity. Homogeneity/heterogeneity analysis will be used to decide how many groups/interviews are needed, after doing the first two with each population.

Identifiers will be collected, including: Participant's name in all tests, and home address and telephone number in the families participating in the home visits. These identifiers are needed to link students with parents and households. Once links are made, the data collected will be related with a non-personal ID number and the original contact information will be destroyed.

4. Study procedures:

Formative research will be performed in children and parents. Direct observations in and around schools, home visits, focus groups with children and parents will be conducted.

Direct observations will be made of the school environment, classroom and physical education classes, recess time, extracurricular activities, school surroundings, and the community in general. Focus groups will last up to 2 hours each and subjects will be asked about knowledge, attitude and practice related to health, obesity, diet, physical activity, and tobacco use; identification of individual and environmental factors that promote or discourage a healthy diet and physical activity; existing infrastructure at schools, homes, and neighborhoods that can help or hinder the implementation of the intervention; perception on changes in the school, home and neighborhood environments that can promote a healthy diet and physical activity, and; gender and ethnic aspects related to diet and physical activity. To facilitate the participation of the children and parents, the FGDs will be held at the school they attend (see Table 1).

Table 1. Information of the study groups, participants and activities.

Study Group	Number of participants	Activity	Duration
Public	Undefined	Direct observation	Undefined
Families	30	Home visit (Observation and interview)	1 hour
Children	50	FGDs	2 hours
Parents	50	FGDs	2 hours

Home visits will gather information on the household physical environment, socio-economic information will be obtained and family lifestyle and practices will be explored to assess predisposing and reinforcing factors of health behavior. Permission to take pictures of the kitchen space, food storage room, dining and family room will be verbally requested. All data will be integrated and analyzed using qualitative data software.

FGDs will be audio recorded. The study will last approximately six months (see Table 3).

5. Data Security and Protection of Subject Confidentiality

Hard copies of data collection forms have subjects' identifiers, and will be stored in a secure room, in locked cabinets. Data will be entered in an electronic study database without identifiers. A linkable identifier list will be kept by the PI in a locked cabinet, and will be destroyed once the electronic dataset is considered clean and final. All subsequent analyses will be performed in this database and will not include identifiable information. Audiotapes and digital voice files will be deleted after transcription is done.

6. Recruitment process:

We will recruit the study populations at public and private schools. We will randomly select children (grades 4-6) and parents of each school and invite them to participate in a FGD at their school. If someone doesn't want to participate, he/she will be substituted by another randomly selected subject, until the required sample is completed. We will use the list of children in each classroom to select participants. From each list of children, two pools of names will be created (children and parents) to randomly select potential candidates. Those lists of children will also be used to randomly select families for home visits. We will invite separately each pool of potential participants to a school meeting to explain the purpose of the study and how the FDG/home visits will be performed.

7. Consent process and documentation:

Written informed consent will be sought from all adults recruited and assent from all children. The consent form will be read and explained to every participant. Time for answering question will be given publicly at the school meetings and privately. The consent form will be administered in Spanish and a signed copy will be given to each participant. The junior investigator (Project Coordinator) will be in charge of explaining the study, procedures, and consent information to potential subjects before the study starts, both at a school meeting and privately with each subject. She will clarify that the participation in the research by them and/or their children is voluntary, that refusal to participate will bring no negative consequences, and that they can stop participating at any point after consent is granted without any penalty. Parents will be allowed to take home the consent form and decide later if they wish to participate, in which case we will offer to pick up the consent from their home. In the case of the FGD with children, after the parents have given their consent for their children's participation, the project coordinator will individually do the oral assent with each child.

8. Risks:

Children and adults enrolled in this study will be subject to minimal risks. Focus groups and home visits pose no physical risk, but some of the questions may create apprehension in some people. We will reassure children and parents that they are not obligated to answer questions they don't want to, and that all data will remain confidential.

9. Benefits:

There are no direct benefits from participating in these studies, other than possibly the satisfaction of contributing to build a program aimed at improving health and wellbeing of children.

10. Payment:

Not applicable.

11. FDA regulated studies:

Not applicable.

12. Safety monitoring:

Not applicable.

13. Plan for reporting unanticipated problems/adverse events:

The PI will be notified immediately of any unexpected event. The PI will report these to the IRB according to guidelines.

14. Other IRBs:

The study will be reviewed by INCAP's IRB, which is headed by Valentina Santa-Cruz (Calzada Roosevelt 6-25 Zona 11, Guatemala City, Guatemala. Tel.: 502 2440-9862. Email: vsantacr@incap.ops-oms.org).

15. Outside collaborations:

The Institute of Nutrition of Central America and Panama (INCAP) and JHSPH will be the participating sites. The study will take place in Guatemala.

Manuel Ramirez-Zea, M.D., Ph.D. (Exercise Physiology). Co-PI (INCAP). He will be responsible for the study design, overseeing all aspects of the core project implementation, hiring, training, and supervision of study personnel; development of data collection protocols, administration of study budget, data analysis and interpretation.

Benjamin Caballero, M.D., Ph.D. (Nutrition). Co-PI (JHSPH). He will also be responsible for the study design, development of data collection protocols, data analysis and interpretation.

Paola Letona M.Sc. (Neurophysiology of Behavior). She will be the project director, supervising and coordinating all project activities, and will be responsible for development of manuals of operations, support on data management and analysis, and supervision of all personnel.

16. Oversight plan for student studies:

Not applicable

17. Oversight plan for studies conducted at non-JHSPH sites, including international venues, for which the JHSPH investigator is the responsible PI:

The Co-PI at INCAP (Dr Ramirez-Zea) is a U.S.-trained PhD in human nutrition and exercise physiology with 12 years of experience in clinical trials and prevention interventions. He will be responsible of overseeing all aspects of the project's implementation on a daily basis. The JHU PI, Dr Caballero, has himself worked as a researcher and lived in Guatemala for a number of years, so he knows the environment for these studies very well. He has known and worked with Dr. Ramirez-Zea for

over 10 years, and the communication among these colleagues has always been fluid, friendly and open. The funding agency, NIH, holds monthly conference calls with PI's to discuss progress and issues with all activities of the Center of Excellence, including this particular study.

18. Creation of a biospecimen repository:

There are no plans to create a biospecimen repository.

19. Data Coordinating Center: n/a

JHSPH Institutional Review Board

RESEARCH PLAN

PI: Benjamin Caballero, Manuel Ramirez-Zea, Paola Letona, Dina Roche, Fernanda Kroker

Study Title: Obesity and cardiovascular disease risk factors in school-age children living in poor urban areas of Guatemala

IRB No.: IRB00002914

PI Version Number/Date: Version 4 (July 25, 2010)

1. Aims/objectives/research question/hypotheses:

We hypothesize that the occurrence of cardiovascular disease (CVD) risk factors in overweight school-age children are already increased compared to normal weight peers. Our primary aim is to quantify the occurrence of key cardiovascular disease (CVD) risk factors in normal weight and overweight school-age children, including body composition, central and peripheral systolic augmentation indexes, central and peripheral pulse pressure, aortic pulse wave velocity, serum lipids, insulin, homocysteine, VO₂max, physical activity level, and dietary intake.

2. Background and rationale:

Cardiovascular health is deteriorating since early years of life in the developing world, due to rapid changes in lifestyle patterns. These alterations in the way of life throughout childhood are being reflected by a rapid increase in the prevalence of obesity and being driven by environmental (obesogenic) determinants, such as increase in use of motorized transport, fall in opportunities for recreational physical activity, increased sedentary recreation, greater quantities, variety, and marketing of energy-dense micronutrient-poor foods and beverages available, among others.¹ Obesity and sedentarism are the most prevalent risk factors that are inducing the CVD epidemic. Obesity has been increasing at a remarkable rate in the last decade in the developing world, even in countries like Guatemala, where the double burden of nutritional problems is one of the greatest in the world.² The data on physical activity patterns change is scarce. Unfortunately, most school- and home-based interventions have failed to prevent childhood obesity and few comparable data are available about cardiovascular alterations during childhood.³ To intervene in early ages becomes a critical subject, due to the fact that the direct and indirect costs related to the treatment of CVD are high, and prevention seems to be the only cost-effective alternative. Furthermore, children shape their food and activity preferences and develop essential motor skills before 7 years of age; thus, working before or around this age becomes extremely decisive. Therefore, it is crucial to start actions as early in life as possible, to revert or at least stop the CVD epidemic.⁴ The most important actions that have to be performed are the prevention of childhood obesity and sedentarism.

With this study we hope to gather information on the status of several CVD risk factors and how premature the damage is established. This information will be critical to promote and evaluate lifestyle change interventions in schools. These studies are part of the research activities of the recently created Center of Excellence for the Prevention of Chronic Diseases at the Institute of Nutrition of Central America and Panama (INCAP), supported by NHLBI.

¹ Caballero, B. The global epidemic of obesity: an overview. *Epidemiol Rev.* 2007; 29:1-5.

² Duran, P.; Caballero, B., and de Onis, M. The association between stunting and overweight in Latin American and Caribbean preschool children. *Food Nutr Bull.* 2006 Dec; 27(4):300-5.

³ Summerbell CD, Waters E, Edmunds LD, Kelly S, Brown T, Campbell KJ. Interventions for preventing obesity in children (Review). The Cochrane Collaboration. Published by John Wiley & Sons, Ltd. 2006.

⁴ Caballero, B. Obesity prevention in children: opportunities and challenges. *Int J Obes Relat Metab Disord.* 2004 Nov; 28 Suppl 3:S90-5.

3. Participants:

The main target population consists of children 6-12 years of age enrolled in low-income urban elementary schools from a peri-urban community (San Jose La Comunidad, Municipality of Mixco) in the metropolitan area of Guatemala City. Two of the five schools of this community will be selected by convenience, according to the support of directors and teachers. We will enroll 96 healthy children, 48 with normal weight (BMI z score < +1 of the median reference of WHO standard population) and 48 overweight (BMI z score > +1 of the median reference of WHO standard population). Exclusion criteria: chronically ill children, menarche, siblings of a child already included in the study, and undernutrition (BMI z score < -2).

Sample size calculations were based on differences among cardiovascular risk factors between overweight and normal weight children, and plasma insulin, blood pressure, and VO₂max, with an effect size between 0.59 and 0.88, power to detect desired differences (80%), level of significance (0.05), using data from previous studies.^{5,6} All calculations were computed using PC-Size (shareware, 1990, v.1.01, Andover, MA). For example, for an estimated group means of 1.3 and 2.4 of the insulin resistance index by homeostasis model assessment (HOMA-IR) and a within group standard deviation of 1.85, employing 45 children per group will allow us to detect an effect size of 0.59 or greater with 80% power and a 2-tailed test of alpha = 0.05. The formula used for the calculation was the following:

$$n = (16\sigma^2/\Delta^2)+1 = (16*1.85^2/(2.4-1.3)^2)+1 = 45 \quad [\text{effect size} = (2.4-1.3)/1.85 = 0.59]$$

Accounting for a 5% drop-out rate and loss of data, we will recruit 50 children per group.

4. Study procedures:

Screening: Anthropometric measurements (weight and height) will be taken from children (grades 1 to 6) at 2 public schools located in the municipality of Mixco. We will invite parents to a school meeting to do the consent process and then screen those who agree to be potential participants. Those children who meet the inclusion criteria will be selected for performing the entire set of measurements. The screening process will continue until the sample needed is completed.

Measurements will be done at INCAP's Laboratory of Physiology and Body Composition, and will require a half-day visit. All tests will be performed by the study's PI and co-investigators, who have several years of experience conducting these tests in children and adolescents. Blood will be drawn by experienced phlebotomists from INCAP's clinical lab. Scheduled children will be asked not to eat anything after dinner the previous evening (at least 12 hour fasting). Two to three children per day will be transported from the school to INCAP early in the morning and back to school when the tests are finished, in a minivan from the institution, clearly identified (the community is familiar with INCAP's vans, which are used in other community health activities). We will ask that a parent, caretaker, or designate person accompany the child. Tests that require fasting (body composition and blood draw) will be done first; a snack will be given around mid-morning and a meal at the end of all tests.

Measurements

Arterial stiffness and pulse-wave velocity (SphygmoCor®): The SphygmoCor (AtCor Medical Pty Ltd, Illinois, US) is a device that measures several cardiovascular parameters such as central and peripheral systolic augmentation indexes, central and peripheral pulse pressure, arterial stiffness, and pulse-wave velocity. All these measurements are performed non-invasively through a 3-lead ECG and

⁵ Sakuragi, S.; Abhayaratna, K.; Gravenmaker, K. J.; O'Reilly, C.; Srikusalanukul, W.; Budge, M. M.; Telford, R. D., and Abhayaratna, W. P. Influence of adiposity and physical activity on arterial stiffness in healthy children: the lifestyle of our kids study. *Hypertension*. 2009, 53(4):611-6.

⁶ Freedman DS, Dietz WH, Srinivasan SR, Berenson GS. The relation of overweight to cardiovascular risk factors among children and adolescents: the Bogalusa Heart Study. *Pediatrics*. 1999, 103(6 Pt 1):1175-82.

transcutaneous tonometry recorded at the radial, carotid, and femoral arteries. These procedures have been widely used in adults and children.^{7,8}

Physical activity patterns (actigraph, NL1000 pedometer): The actigraph (Actigraph, Florida, US) is a device that is worn on the waist and measures the amount and intensity of activity every 15 seconds through accelerometry.⁹ The NL1000 pedometer (Newlifestyles Inc, Montana, US) is also worn on the waist and is a validated device that accurately counts steps and detects the intensity of each step displaying intensity as moderate-to-vigorous physical activity (MVPA) time accumulation in a day.¹⁰ An accelerometer, a pedometer, and a heart rate monitor (Polar Electro Inc, New York, USA) will be worn by each child for the following 3 to 7 days and a field worker will remove the devices at the school (See Table 1). We will instruct children and parents on how to take care of the devices that the child will wear for few days. The accelerometer and the pedometer should be worn during waking hours, but the heart rate monitor all day and night. All devices should be removed during shower or any similar activity (devices are not waterproof). Specific instructions will be given on how to detached and attached again each device.

Table 1. Devices the children will wear.

Device	Usage	How long should the child wear the device?
Actigraph (Actigraph, Florida, US)	This is a device that is worn on the waist and measures the amount and intensity of activity every 15 seconds through accelerometry.	Each child will wear the accelerometer for 7 days. A field worker will remove the device at the school. The accelerometer should be worn during waking hours and removed during shower or any similar activity (the device is not waterproof).
NL1000 pedometer (Newlifestyles Inc, Montana, US)	The pedometer is worn on the waist and is a validated device that accurately counts steps and detects the intensity of each step displaying intensity as moderate-to-vigorous physical activity (MVPA) time accumulation in a day.	Each child will wear the pedometer for 7 days. The pedometer should be worn during walking hours and removed during shower or any similar activity (the device is not waterproof).
Heart rate monitor (Polar Electro Inc, New York, USA)	The heart rate monitor measures heart rate with ECG accuracy, in intervals of 60 seconds. The heart rate monitor has a transmitter with elastic belt to be worn around the chest against the skin and a wrist monitor which should be used as an ordinary watch.	Each child will wear the heart rate monitor for the following 3 days. This heart rate monitor should be worn during day and night, and should be removed during shower or any similar activity (the device is not waterproof).

⁷ Sakuragi S, Abhayaratna K, Gravenmaker KJ, O'Reilly C, Sriksalanukul W, Budge MM, Telford RD, Abhayaratna WP. Influence of adiposity and physical activity on arterial stiffness in healthy children: the lifestyle of our kids study. *Hypertension*. 2009 Apr;53(4):611-6.

⁸ Urbina EM, Williams RV, Alpert BS, Collins RT, Daniels SR, Hayman L, Jacobson M, Mahoney L, Mietus-Snyder M, Rocchini A, Steinberger J, McCrindle B; American Heart Association Atherosclerosis, Hypertension, and Obesity in Youth Committee of the Council on Cardiovascular Disease in the Young. Noninvasive assessment of subclinical atherosclerosis in children and adolescents: recommendations for standard assessment for clinical research: a scientific statement from the American Heart Association. *Hypertension*. 2009 Nov;54(5):919-50.

⁹ De Vries SI, Van Hirtum HW, Bakker I, Hopman-Rock M, Hirasings RA, Van Mechelen W. Validity and reproducibility of motion sensors in youth: a systematic update. *Med Sci Sports Exerc*. 2009 Apr;41(4):818-27.

¹⁰ Rowe DA, Mahar MT, Raedeke TD, Lore J. Measuring physical activity in children with pedometers: Reliability, reactivity and replacement of missing data. *Ped Exerc Sci*. 2004;16:1-12.

Physical fitness (Peak oxygen uptake, 6-min walk test): Peak oxygen uptake will be determined through an incremental treadmill exercise protocol to exhaustion (15-20 min).¹¹ During the exercise test, each child will wear a face mask to measure expired air volume and oxygen concentration (Moxus Metabolic Cart, AEI Technologies Inc, Illinois, US) and a chest belt to measure heart rate (Polar Electro Inc, New York, USA). The 6-min walk test is a validated field method to measure fitness in children.¹²

Behavioral factors: The diet of each child will be evaluated by a food frequency questionnaire (mother's interview) and a qualitative questionnaire (child interview) adapted from previously used instruments. The questionnaire will also include questions about physical activity, health, and socio-demographics (parents' occupation and literacy, type of housing) to assess socio-economic status.

Body composition: The 4-compartment model of body composition gives the best estimate of percent body fat in children and requires the measurement of body fat, bone mass, and total body water.¹³

--Body volume and thoracic gas volume will be measured at least twice using air-displacement plethysmography (Bod-Pod), which requires that the subject is seated within the Bod-Pod chamber, each time lasting less than a minute, and is connected to the breathing circuit of the system via a disposable air filter and breathing tube.¹⁴ The computer software calculates then body density and %fat.

--The dual energy X-ray absorptiometry (DEXA) is a technique validated in children to measure bone mineral density and total fat.¹⁵ The amount of radiation received during this test is minimal (comparable to the exposure get in a sunny day).

--The deuterium dilution technique is a well established method for the measurement of total body water in children.¹⁶ It is safe (non-radioactive), non-invasive technique in which deuterium (a stable isotope of hydrogen) enriched water is drunk by each subject (12-18 ml). A 4 ml saliva sample will be obtained using cotton swabs before and 3 hours after drinking the dose of deuterium water. Samples will be kept frozen until analysis of deuterium enrichment using a Fourier Transformed Infrared Spectrometer.

--Anthropometry (weight; height; sitting height; waist, middle arm, and calf circumferences; tricipital and subscapular skinfold thicknesses), and bioelectric impedance analysis (BIA), using standard techniques.

Justification for use of the 4-compartment model: simpler techniques, such as bioelectrical impedance or skinfold equations can be used only under a 2-compartment model. The assumptions and constants in this model have been derived from healthy children of developed countries, and are unlikely to be valid for children from low-income developing countries, who typically have low stature for their age and a relative excess weight for their height. The data generated by our study will contribute to the future development of simplified approaches for body composition assessment in chronically undernourished populations.

Total energy expenditure (minute-by-minute heart rate monitoring technique): The minute-by-minute heart rate monitoring technique has been validated and used in children.¹⁷ This technique requires measuring the individual relation between heart rate and oxygen uptake at rest (lying supine, sitting,

¹¹ Sheehan JM, Rowland TW, Burke EJ. A comparison of four treadmill protocols for determination of maximum oxygen uptake in 10- to 12-year-old boys. *Int J Sports Med.* 1987 Feb;8(1):31-4.

¹² Calders, P.; Deforche, B.; Verschelde, S.; Bouckaert, J.; Chevalier, F.; Bassle, E.; Tanghe, A.; De Bode, P., and Franckx, H. Predictors of 6-minute walk test and 12-minute walk/run test in obese children and adolescents. *Eur J Pediatr.* 2008 May; 167(5):563-8.

¹³ Bray, G. A.; DeLany, J. P.; Volaufova, J.; Harsha, D. W., and Champagne, C. Prediction of body fat in 12-y-old African American and white children: evaluation of methods. *Am J Clin Nutr.* 2002 Nov; 76(5):980-90.

¹⁴ Vescovi JD, Zimmerman SL, Miller WC, Hildebrandt L, Hammer RL, Fernhall B. Evaluation of the BOD POD for estimating percentage body fat in a heterogeneous group of adult humans. *Eur J Appl Physiol.* 2001 Aug;85(3-4):326-32.

¹⁵ Helba M, Binkovitz LA. Pediatric body composition analysis with dual-energy X-ray absorptiometry. *Pediatr Radiol.* 2009 Jul;39(7):647-56.

¹⁶ Wells JC, Fewtrell MS, Davies PS, Williams JE, Coward WA, Cole TJ. Prediction of total body water in infants and children. *Arch Dis Child.* 2005 Sep;90(9):965-71.

¹⁷ Spurr GB. Physical activity and energy expenditure in undernutrition. *Prog Food Nutr Sci.* 1990;14(2-3):139-92.

and standing quietly) and during exercise (same protocol described above for peak oxygen uptake). Measurements of heart rate and oxygen uptake at rest will last 15 min and will be measured the same way as in exercise. A 3-day minute-by-minute heart-rate recording is the other measure that is needed to estimate total energy expenditure, following an algorithm described elsewhere.¹⁷

Other cardiometabolic factors (serum insulin, plasma glucose, serum homocysteine, serum lipid profile, blood pressure) will be measured in all participants. Blood pressure will be taken using a standard technique and an appropriate arm cuff size. A blood sample (8 ml) will be collected through forearm venipuncture by the study physician. Plasma and serum samples will be kept frozen until analysis. Blood chemistry will be done through enzymatic colorimetric methods (glucose, homocysteine, and lipid profile using a Cobas C111 analyzer, ROCHE, Indiana, US) and electrochemiluminescence immunoassay (Elecsys, ROCHE, Indiana, US).

Data analysis

All results will be either written in a form or directly downloaded to a computer. All data forms will be checked daily for accuracy and completeness by the study coordinator, and 100% of the data double-entered into the project's computer files using Epi-Info, Version 6. Any incorrect entries or missing data will be checked and verified on a systematic basis using range checks for values beyond permissible values and missing values. Means and standard deviations will be calculated for all continuous variables, and proportions for categorical ones for normal weight and overweight children. Differences between groups will be compared by using Student's *t* tests (continuous variables) and Chi-square tests (categorical variables). A second level of analysis will involve controlling for possible factors that may confound the main outcome differences, such as total body fat, physical activity, and fitness (analysis of co-variance). One or two manuscripts with the main results will be sent to peer-review journals.

5. Data Security and Protection of Subject Confidentiality

Hard Copy of data collection form: Indicate your choice but typing an X in the appropriate box on the left:	
X	Hard copies of data collection materials <u>have identifiers</u> and are locked in a secure cabinet or room with limited access by specified individuals. When possible, redacted (de-identified) versions of the data collection sheets will be used for coding and analysis.
	Hard copies of data collection materials include an ID code and <u>do not have personal identifiers</u> . However, a code linking the data to the subject's personal information is stored separately from the data collection sheets, and is locked in a secure cabinet or room with limited access by authorized individuals.
	Data are not collected on paper.
	Other (describe):
Electronic Databases: Indicate your choice but typing an X in the appropriate box on the left: : <i>Note: A de-identified version of the database should be used for data analysis except in instances in which identifying information is prerequisite for coding or analysis. Databases that retain identifying information require a higher degree of electronic security.</i>	
X	The study is minimal risk and data collected are not sensitive in nature. <u>No personal identifiers</u> are included in the electronic database. Any electronic documents that link IDs to identifying information are stored on a computer in accordance with JHSPH Data Security guidance.
	<u>Personal identifiers</u> are included in the database. The data are stored on a computer that is password protected with a secure server. Transfer or storage on portable devices (e.g., laptops, flashdrives) is encrypted. The devices on which this information is stored are accessible only to individuals who need access to these data.

	<p><u>No personal</u> identifiers are included in the database but linkable identifiers exist separately and the data are sensitive in nature (e.g., <i>substance use, mental health, genetic propensities, sexual practices or activities</i>) such that disclosure could provide a risk to the individual. The codes are stored on a computer that is password protected with a secure server. Transfer or storage on portable devices (e.g., laptops, flashdrives) is encrypted. The devices on which this information is stored are accessible only to individuals who need access to these data.</p>
	<p>Other (describe):</p>

Hard copies of data collection forms have subjects' identifiers, and will be stored in a secure room, in locked cabinets. Data will be entered in an electronic study database without identifiers. A linkable identifier list will be kept by the PI in a locked cabinet, and will be destroyed once the electronic dataset is considered clean and final. All subsequent analyses will be performed in this database and will not include identifiable information.

6. Recruitment process:

We will recruit the study populations at public schools We will invite parents to a school meeting to explain the purpose and procedures of the study, using a slide presentation with pictures of all tests. Parents will be allowed to take the informed consent form to their home and return the form in the following week. We will screen those children who assent and whose parents consent to participate and recruit those who meet the inclusion criteria.

7. Consent process and documentation:

Written informed consent will be sought from parents and assent from all children recruited. The consent form will be read and explained to the parents of every participant in a school meeting and time for answering question will be given publicly and privately. The consent form will be administered in Spanish and a signed copy will be given to each participant. The project director and a research assistant will be in charge of explaining the study procedures and consent information to potential subjects before the study starts, both at a school meeting and privately with each subject. The study staff will clarify that the participation in the research by them and/or their children is voluntary, that refusal to participate will bring no negative consequences, and that they can stop participating at any point after consent is granted without any penalty. Parents will be allowed to take home the consent form and decide later if they wish their child to participate, in which case we will offer to pick up the consent at school or from their home. After the parents have given their consent from their children's participation, the study staff will individually do the oral assent with each child, with the presence of his/her parent or legal guardian and using pictures to show the tests to be done. Results will be given to all parents in a written report, and basic counseling on diet and physical activity will also be provided to all.

8. Risks:

Ocasionally, a small bruise may appear on the site of the arm veinpuncture. The motion sensors and heart rate monitors worn for 3 to 7 days may cause minimal discomfort. The treadmill test will cause muscle fatigue and shortness of breath for a brief period, which might cause discomfort. There may also be some slight discomfort or feeling of confinement when conducting measurements such as air-displacement plethysmography or DXA. DXA scans will be performed on a new, state-of-the art instrument by qualified technicians. The radiation exposure of one scan is small: 1 to 4 μ Sv. For

comparison, one-day exposure to natural background radiation yields an effective dose of 5-10 μSv ¹⁸. The dose constraint for children participating in research involving radiation has been defined as 5 mSv (5,000 μSv) over the first 18 years of life¹⁸. There is no risk related to drinking deuterated water at the doses used.

9. Benefits:

Direct benefits to the subjects will include knowing important indicators of their health status including their glycemia, lipid profile, and blood pressure, and receiving behavior counseling related to healthy diet and physical activity. All these results will be given in a writing report to parents and a meeting will be organized at the school to explain them. Subjects with abnormal values in any indicator will be given a referral note to consult a doctor. This study will help characterize key risk factors for overweight early in life. Because there are very few studies in Hispanic populations and in developing countries, this study will contribute valuable information that will help identify overweight risk factors in these populations.

10. Compensation

Monetary compensation is not common in studies done in Guatemala. This “tradition” may have developed (at least at INCAP) in part because of the difficulty in avoiding the appearance of coercion when dealing with impoverished populations. Instead, participants in our study and anyone accompanying them will receive a snack and lunch during their visit to the INCAP laboratory. Each child will also receive a photo of him/herself (which is usually much appreciated), a soccer ball and a wristband.

We anticipate that virtually every participant will be transported by us to the study laboratory, since car ownership is very rare. Nevertheless, a compensation of Q10 (about 1.4 USD) will be provided to those who come to the study laboratory on their own.

11. Devices:

All devices that children will wear (pedometers, accelerometers, and heart rate monitors) are non-invasive, commercially available, and have been in use in children and adults for many years by clinics, health clubs, and fitness centers. We selected the best and more established brand in their category, as expected by reviewers of a competitive NIH application. We will not use any investigational device.

12. Safety Monitoring

A physician experienced in the procedures included in this study (the Co-PI at INCAP or another INCAP physician) will be present at all times during the day that a group of 2 to 3 children come to INCAP's clinical laboratory. All personnel will be trained and standardized in all tests that are included in this study to minimized risks. The physician will ensure that personnel performing each test adhere to standardized protocols and procedures every day that tests are done. No adverse events are expected with the use of the devices each child will use during few days after his/her visit to INCAP; however, a field worker will fill a report of any adverse event during the days the devices are worn. The IRBs at JHSPH and at INCAP, will be notified immediately of any adverse events thought to be directly related to the research or other aspects of study participation. Any adverse events will be tabulated and submitted to the IRBs annually.

13. Plan for reporting unanticipated problems/adverse events:

¹⁸ Use of dual-energy x-ray absorptiometry (DXA) and computed tomography (CT) for the assessment of body composition. Report of a Consensus Conference. International Atomic Energy Agency, Vienna, June 2007,

The PI will be notified immediately of any unexpected event. The PI will report these to the IRB according to guidelines.

14. Other IRBs:

The study will be reviewed by INCAP's IRB, which is headed by Valentina Santa-Cruz (Calzada Roosevelt 6-25 Zona 11, Guatemala City, Guatemala. Tel.: 502 2440-9862. Email: vsantacr@incap.ops-oms.org).

15. Outside collaborations:

The Institute of Nutrition of Central America and Panama (INCAP) and JHSPH will be the participating sites. The study will take place in Guatemala.

Manuel Ramirez-Zea, M.D., Ph.D. (Exercise Physiology). Co-PI (INCAP). He will be responsible for the study design, overseeing all aspects of the core project implementation, hiring, training, and supervision of study personnel; development of data collection protocols, administration of study budget, data analysis and interpretation.

Benjamin Caballero, M.D., Ph.D. (Nutrition). Co-PI (JHSPH). He will also be responsible for the study design, development of data collection protocols, data analysis and interpretation.

Paola Letona M.Sc. (Neurophysiology of Behavior). She will be the project director, supervising and coordinating all project activities, and will be responsible for development of manuals of operations, support on data management and analysis, and supervision of all personnel.

The two schools from San Jose La Comunidad, Mixco that will participate in the study are School Perez Guizasola and School Berlin. Letters of collaboration will be obtained from School Directors.

16. Oversight plan for student studies:

Not applicable

17. Oversight plan for studies conducted at non-JHSPH sites, including international venues, for which the JHSPH investigator is the responsible PI:

The Co-PI at INCAP (Dr Ramirez-Zea) is a U.S.-trained PhD in human nutrition and exercise physiology with 12 years of experience in clinical trials and prevention interventions. He will be responsible of overseeing all aspects of the project's implementation on a daily basis. The JHU PI, Dr Caballero, has himself worked as a researcher and lived in Guatemala for a number of years, so he knows the environment for these studies very well. He has known and worked with Dr. Ramirez-Zea for over 10 years, and the communication among these colleagues has always been fluid, friendly and open. The funding agency, NHI, holds monthly conference calls with PI's to discuss progress and issues with all activities of the Center of Excellence, including this particular study.

18. Creation of a biospecimen repository:

There are no plans to create a biospecimen repository. All samples will be analyzed at INCAP's laboratories or one of its collaborating centers. Samples will be analyzed without personal identifiers, and remaining specimens will be properly discarded after completion of analyses.

19. Data Coordinating Center: n/a

Institutional Ethics Committee of INCAP

RESEARCH PLAN

PI: Benjamin Caballero, Manuel Ramirez-Zea, Paola Letona, Giovanna Gatica

Study Title: Feasibility of a community-based pilot intervention to prevent chronic cardiometabolic diseases in school-age children

Version Number/Date: Version 2/March 28, 2011

1. AIMS

*Primary:*²

- To assess the feasibility and acceptability of *iPilas!*, a community-based intervention aimed to promote a healthy lifestyles to reduce risk factors for chronic cardiometabolic diseases in children.

Secondary:

- To evaluate the outcomes of the intervention program in the children's and the mothers' knowledge, attitudes and practices of a range of modifiable risk factors associated with chronic diseases (unhealthy diet, physical inactivity, secondhand smoke, tobacco use, and alcohol consumption).
- To evaluate the degree to which the components of the intervention are delivered and administered as planned.

2. BACKGROUND AND RATIONALE

Chronic non-communicable diseases are the largest cause of death in the world. In 2002, cardiovascular disease, cancer, chronic respiratory disease, and diabetes caused 29 million deaths worldwide.¹ Some modifiable behaviors are contributing to their development.² The World Health Organization identified several leading risk factor causes of death in middle-income countries (e.g., Guatemala): high blood pressure (responsible for 17% of deaths), tobacco use (11%), overweight and obesity (7%), physical inactivity (7%) and alcohol consumption (6%).³

Health starts deteriorating early in life in the developing world, due to rapid changes in lifestyle patterns. These alterations in the way of life throughout childhood are reflected in the rapid increase in the prevalence of obesity, driven by environmental (obesogenic) determinants, such as increase in use of motorized transport, limited opportunities for recreational physical activity, increased sedentary recreation, greater quantities, variety, and marketing of energy-dense nutrient-poor foods and beverages.⁴ Obesity has been increasing at a remarkable rate in the last decade in the developing world, even in countries like Guatemala, where the double burden of nutritional problems is one of the greatest in the world.⁵ Data on physical activity patterns change, secondhand smoke, tobacco use, and alcohol consumption in children is scarce. This is a critical period, since food preferences and lifestyle begin to form before 7 years of age. The aim of this project is to generate evidence on feasibility and short-term impact of early preventive interventions.

INCAP has been developing a community-based intervention for four years, recently naming it Pilas! This program integrates the effort of the educational community (principals, teachers, parents and students), community leaders, and institutions for the promotion of behaviors that reduce the risk factors associated with cardiometabolic diseases in children.

¹ Yach, D., Hawkes, C., Gould, C. L., and Hofman, K.J. The Global Burden of Chronic Diseases: Overcoming Impediments to Prevention and Control. *Journal of the American Medical Association*, 2004, 291 (21), 2616-2622.

² Schroeder, S.A. We Can Do It Better – Improving the Health of the American People. *New England Journal of Medicine*, 2007, 357, 1221-1228.

³ World Health Organization. Global Health Risks: Mortality and Burden of Disease Attributable to Selected Major Risks, 2009.

⁴ Caballero, B. The global epidemic of obesity: an overview. *Epidemiol Rev.* 2007; 29:1-5.

⁵ Duran, P.; Caballero, B., and de Onis, M. The association between stunting and overweight in Latin American and Caribbean preschool children. *Food Nutr Bull.* 2006 Dec; 27(4):300-5.

With the aim of Pilas! being a feasible and sustainable program, accepted by the people from the community, two formative research studies have been performed, were observations within the school and community, focus groups with teachers, parents and students; interviews with principals, school food vendors, religious leaders, authorities (municipal, education, and health) and program directors were made. This helped us to explore knowledge, beliefs and needs the community had about health and promotion of healthy lifestyles, and on that basis develop this program.

In 2008 a pilot study of two months was conducted in four schools, where the educational community was responsible of applying. The program and the feasibility and acceptability of the activities inside the classroom were assessed; as well, as the short-term impact in the knowledge, attitudes and practices in the students from the first to third grade. The activities were feasible and accepted by the educational community and improvements in knowledge, attitudes and practices were observed. For this reason, it was decided to finish the activities for the rest of elementary and strengthen the intervention the program by including by including people and institutions of the community identified as resources in the formative research done before.

Specifically, we propose to develop a community-based intervention that is feasible, culturally acceptable and effective in promoting healthy behaviors in school-age children (4th – 6th grade), for the prevention of cardiometabolic diseases.

This study is part of the research activities of the Center for the Prevention of Chronic Diseases in Mesoamerica and the Dominican Republic (CIIPEC), located in Guatemala City, and funded by the National Heart, Lung and Blood Institute (NHLBI).

3. PARTICIPANTS

The target population consists of elementary school children (4th- 6th grade), who are enrolled in two low-income urban public schools located within the Municipality of Mixco (Guatemala City), and their mothers. All the students of these grades and their parents will be exposed to the intervention. However, only a randomly selected sample of 120 child/mother pairs (60 per school) will be evaluated at the beginning and at the end of the intervention. Exclusion criteria include:

- Children with long-term restrictions for physical activities
- Siblings of a child already included in the study
- A child with a pregnant mother

Pregnant women will be excluded because physical activity and dietary habits change during pregnancy. If she becomes pregnant after enrolling in the study, she will be excluded from the post-intervention assessment. However, she still will be able to participate in the activities offered by iPilas! (just as any other parent, not enrolled in the study). Children with chronic medical conditions/medication who are under long-term medical care will be asked to bring a letter from their doctor approving their participation in the study.

We plan to enroll approximately 20% of the students of the selected grades in this exploratory study. As a result, we will obtain critical information for the planning of a randomized control trial, including effect size estimates for a better sample size calculation. Accounting for a 10% dropout rate and loss of data, we will recruit 66 child/mother pairs in each school, for a total of 132.

A file on each child/mother pair that includes personal identifiers (e.g., child's and mother's name, sex, date of birth, address, telephone number, school, grade and links to the study ID) will be held in a secure location. All data entered in the forms and questionnaires will identify the individual only by the study ID.

4. STUDY PROCEDURES

The 10-month study is a pre-test/post-test, single sample design that will be conducted in two low-income elementary schools. The study will be divided in four phases: Phase 1: Recruitment and training of the intervention agents. Phase 2: Baseline assessment. Phase 3: Implementation of the ¡Pilas! pilot intervention. Phase 4: Post-intervention assessment and data analysis.

Month	1	2	3	4	5	6	7	8	9	10
1 – Recruitment and training										
2 – Baseline assessment										
3 – ¡Pilas! Pilot intervention										
4 – Post-intervention assessment and data analysis										

The program strategies are directed to:

1. Increase fruit and vegetable intake.
2. Decrease intake of sugar-sweetened beverages and increase water intake.
3. Decrease consumption of high-fat, high-sodium, and high-sugar foods (snacks, traditional foods, fast food, cookies, and candies).
4. Increase the amount of time spent in moderate-to-vigorous physical activity (MVPA) during and out of school.
5. Decrease the amount of time spent watching television and playing electronic games.
6. Decrease exposure to secondhand smoke and prevent initiation of tobacco use and alcohol consumption.

The design of this intervention was developed within the context of a **socio-ecological multilevel model**. This brings together the efforts of the educational community (principals, teachers, children health promoters, parents and, school food vendors), institutions (Health Center, Open Schools Program and the Municipality) and leaders from the community (religious leaders) that can influence the environment and behaviors of children. Another main foundation will be the **social cognitive theory**. This theory emphasizes on how social, institutional and cultural context impact behavior; highlights the determinants that are capable of predicting behavior (knowledge, self-efficacy, result expectations, goals, facilitators, and obstacles) and identifies principles on how to inform, facilitate, guide and motivate people to adopt new health habits. **¡Pilas!** program consists of three integrated components: School, Food Kiosk, and Community.

Phase 1: Recruitment and training of the intervention agents (two months)

To ensure program sustainability, key persons of the school and selected community institutions will be recruited and trained as intervention agents for the application of the program components. The following institutions and individuals will be informed about the program and will be invited to participate:

- **Schools.** Two low-income urban elementary public schools located in different communities that meet the following inclusion criteria will be selected:
 - The school principal, teachers and food vendors are supportive and agree to participate in the study
 - The school has children in the Child Health Promoters Program organized by the Health Center of Mixco or is willing to support the training of their own child health promoters inside the school.
 - The school belongs to the Open Schools Program

As soon as the schools are identified, we will request approval by the Ministry of Education Supervisor of both schools.

- *Teachers.* Approximately 24 teachers from 4th to 6th grade (12 per school) will be recruited and trained as agents of the classroom-based program of the School Component.
- *Food kiosk staff.* At the most, eight food kiosk staff members (4 per school) will be recruited and trained. They will be an integral part of the Food Kiosk Component.
- *Health Center and child health promoters.* In 2011, the Health Center of Mixco will initiate the first Child Health Promoters Program (CHPP). This Center will recruit around ten children (9 to 13 years old) in three different schools (selected for their own pilot study), including one school selected for this project. The children will be trained in health issues, human rights, public safety, and natural disasters. The Health Center Director has invited the Institute of Nutrition of Central America and Panama (INCAP) to participate in this program, and has offered us the opportunity to teach the children about healthy lifestyles. The Health Center will be part of the Community Component.
- *Religious leaders.* After selecting the schools, we will recruit and train eight of the most popular religious leaders (various religions) among the students' families (4 from each community) for the application of the Community Component.
- *Open Schools Program.* Open School Program (OSP) is a Presidential program that consists in maintaining several public schools open during weekends (8:00 – 17:00) to offer children and adults the opportunity to attend and participate in artistic, community, cultural, and sports workshops, free of cost. Some of the workshops include break-dance, singing, theater, circus activities, arts and crafts, reading and writing, and a variety of sports. We will seek the participation of the OSP in the Community Component, with the aim of offering the students and their parents a safe place where they can learn and practice healthy lifestyles activities during the weekend. The OSP's instructors will also be trained.
- *Municipality of Mixco.* The Municipality of Mixco organizes several activities that promote healthy lifestyles among its residents. We will ask for their support in transmitting general information (e.g., date, place and description) about these activities to the principals of the selected schools.

In order to become an intervention agent of each community (teachers, food kiosk's staff, OSP's instructors and religious leaders) each person will need to participate in a 5-hour ¡Pilas! Training Workshop to be held at each selected school. The first three hours will be used to provide information and skills needed to promote healthy lifestyles (healthy diet, physical activity and the prevention of secondhand smoke, tobacco use and alcohol consumption) and understand the psychosocial factors that influence these behaviors. Subsequently, the group will be divided in subgroups and during the last two hours, teachers and OSP's instructors will be trained in auto-efficacy improvement strategies; physical education teachers in increasing amount of time students spend in MVPA during PE class; food kiosk staff in healthy food preparation and marketing strategies; and, religious leaders in motivational strategies related to the healthy lifestyles to be promoted.

The 5-hour ¡Pilas! Training Workshop will be introduced into the CHPP organized by the Health Center, so the child health promoters will be trained at a location assigned for this program with the support of the health center's staff. Even though our pilot intervention will take place in two schools, the child health promoters who attend other schools not included in this study will also benefit from this training. With the support of the center's staff, we will educate and train the children to become promoters, communicators, and positive role models of a healthy lifestyle. The child health promoters from the school that still does not belong the CHPP, will be trained in the school with the support of the teachers.

All training conducted in adult and child intervention agents will be based on a Trainer's Manual and will be conducted by the project coordinator and the research assistants. We will coordinate with all intervention agents and their superiors the best dates that fit their work schedule. Additionally, each group will receive the materials needed for the implementation of the intervention.

Each community group will be asked to select six representatives (teacher, child health promoter, food vendor, religious leader, and OSP's instructor) to be part of the Community Intervention Board, which will meet at month three, five, and seven of the study. The meetings will take place at a selected school, Municipality, or at INCAP's facilities and will last approximately two hours each. The purpose of this board is to create a discussion forum where the intervention agents can share their opinions, experiences, advances, difficulties and solutions about the intervention program. The project coordinator and research assistants will also conform this board.

Phase 2: Baseline assessment (two weeks)

Mothers and children will be individually evaluated at the school in three different days during school hours (table 1). Children will be taken out from the classroom with their teacher's authorization. In the case of mothers, a written notification will be sent three days before their appointment date and a phone call will be made one day before to remind them about their appointment. Trained INCAP staff will be responsible for all measurements and administering the questionnaires to each participant. The following assessments will be made:

- *Family characteristics.* A short questionnaire (form 2) will be administered to each mother to collect information on sociodemographic characteristics (e.g., parent's age, occupation, and education level; religion; household composition) and family history of chronic diseases.
- *Anthropometric status.* Well trained and standardized staff will measure height and weight in each child and his/her mother, using standard techniques.
- *Physical activity patterns.* The child will wear a pedometer (NL1000, Newlifestyles Inc, Montana, US) on the waist for 7 days during walking hours and removed during the shower or any similar activity (the device is not waterproof). Specific care instructions and indications of how to detach and attach the device will be given. The pedometer is a device validated in children, which accurately counts steps and detects the intensity of each step displaying intensity as moderate-to-vigorous physical activity (MVPA) time accumulation in a day.⁶

Additionally, 12 Physical Education (PE) classes will be observed (6 per school, 2 per grade) using a standardized protocol (SOFIT protocol) to evaluate the students' physical activity levels. Furthermore, structured observations will be made during each recess (BEACHES protocol) at both schools during six weeks. The observers will not interact with the PE teachers or students during this assessment.

- *Dietary intake.* A 24-hour food checklist will be conducted with each child and mother or father (form 5) to assess food and beverage intake before the pilot intervention. The checklist will be administered three times over a week, including a Monday to have information about food eaten the previous Sunday.
- *Psychosocial factors.* A questionnaire about knowledge, attitudes and behaviors (KAB) related to diet, physical activity, secondhand smoke, tobacco use, and alcohol consumption will be applied to both the mother/father and the child (form 3).

Table 1. Measurements, questionnaires, and duration of baseline assessments.

Assessment day (duration)	1st day (60 minutes)		2nd day (10 minutes)		3rd day (10 minutes)	
<i>Participant</i>	<i>Child</i>	<i>Mother</i>	<i>Child</i>	<i>Mother</i>	<i>Child</i>	<i>Mother</i>
Demographics questionnaire		x				
Anthropometry	x	x				
Place the pedometer	x					
24-hour food checklist	x	x	x	x	X	x
KAB questionnaire	x	x				

⁶ Rowe DA, Mahar MT, Raedeke TD, Lore J. Measuring physical activity in children with pedometers: Reliability, reactivity and replacement of missing data. *Ped Exerc Sci.* 2004; 16: 1-12.

Phase 3: Implementation of the *iPilas!* program pilot intervention (five months)

The intervention consists of three integrated components: School, food kiosk and community. The intervention agents previously trained will be responsible for their simultaneous implementation.

- I. **School Component.** The teachers who participated in the training workshop and were given the intervention's materials (table 2) will be responsible for the implementation of this component. Several strategies will be used to seek influence the children's and their parents' health knowledge, attitudes and behaviors:
 - A guideline for the achievement of a healthy school environment will be given to the principal, who will need to organize the evaluation team. This tool will help identify the school's strengths and weaknesses, develop a work plan and join efforts for the improvement of the school environment and promotion of healthy lifestyles.
 - A classroom-based program will target knowledge, auto-efficacy, outcome expectations, and goals for behavioral change. During the *iPilas!* pilot intervention, the students will participate in 10 activities (two 45-minute session per month) delivered by their teacher. Each activity includes a simple homework assignment that will promote the interaction between parents/family members and the students. We will distribute 10 different activities of the 16 available per grade to each teacher; hence, all activities will be administered throughout the intervention.
 - A set of PE norms to promote order in class, and accomplishment of class schedules and recommended MVPA in children will be implemented during the pilot intervention.
 - Five-episode radio mini-series (five minutes each) will be played throughout the school during the parent's meetings that are organized by the school principals every year. The aim is to enhance parental support of behavior change.

The school component will be the only one not fully implemented. The classroom-based program is originally scheduled to take place over a period of eight months (16 activities). Therefore, 10 lessons will be selected and applied by the teachers during the pilot intervention.

Table 2. Strategies and materials needed in the School Component. For a detailed explanation of materials see Annex 1.

Strategy	Intervention agents	Materials needed
Guideline for a healthy school environment	Teacher and promoters	<ul style="list-style-type: none"> ▪ Handbook: Improvement of the School Environment
Classroom-based program	Teachers	<ul style="list-style-type: none"> ▪ <i>iPilas!</i> Promoter's Handbook ▪ Handbook: 4th-Grade Classroom Activities ▪ Handbook: 5th-Grade Classroom Activities ▪ Handbook: 6th-Grade Classroom Activities
Implementation of PE norms	School principal	<ul style="list-style-type: none"> ▪ Booklet: Establishment of PE norms
Radio mini-series	School principal	<ul style="list-style-type: none"> ▪ Radio mini-series: Familia Pilas

- II. **Food kiosk component.** This component will target the quantity and nutritional quality of foods and beverages offered at the school food kiosk. Principals and previously trained kiosk staff will be responsible of its implementation. The following strategies will be used (table 3):

- The implementation of food kiosk norms that limit or eliminate the availability of unhealthy foods and beverages.
- A series of guidelines to assist their successful establishment will be given to the principals and a booklet with healthy recipes to the food vendors.

Table 3. Strategies and materials needed in the Food Kiosk Component. For a detailed explanation of materials see Annex 1.

Strategy	Intervention agents	Materials needed
Implementation of food kiosk norms	School principal Food vendors	<ul style="list-style-type: none"> ▪ Booklet: Strategies for the successful establishment of food kiosk norms ▪ Booklet: Healthy recipes for food kiosks

III. Community component. This component will be implemented with the collaboration of religious leaders, the Health Center, OSP, and the Municipality. Their involvement seeks to increase the number of people who communicate and reinforce messages of a healthy lifestyle to the child, and/or who can also offer opportunities and safe places to families for practicing a healthy diet, sports, and recreational activities. The strategies that will be used are the following (table 4):

- Religious services/activities: The religious leaders will not be given specific assignments, but we will offer them suggestions about activities that they can organize together with their followers.
- Child health promoters: We will train the child health promoters identified by the Health Center and give them specific assignments at school, such as: public announcements, promotion of OSP workshops and game activities during recess.
- OSP: A “healthy lifestyle month” will be planned in the OSP. During this time, the staff will organize simple activities within the usual OSP workshops, to communicate messages about a healthy lifestyle. Furthermore, two cooking courses, one for parents and one for children, will be developed and offered at the OSP. These courses will be scheduled to take place over a period of one month and three months, respectively (one class every week). OSP staff and instructors will be responsible for the organization of the workshops and their implementation.
- Municipality: We will coordinate with the Municipality of Mixco about sending monthly information to the schools on activities they organize for promoting healthy lifestyles in their residents, such as: sport events, health fairs, and aerobics classes.

Table 4. Strategies and materials needed in the Community Component. For a detailed explanation of materials see Annex 1.

Strategy	Intervention agents	Materials
Messages and activities during religious services/activities	Religious leaders	<ul style="list-style-type: none"> ▪ ¡Pilas! Promoter’s handbook ▪ Radio mini-series
Child health promoters	Children	<ul style="list-style-type: none"> ▪ ¡Pilas! Promoter’s handbook ▪ Fun games booklet
OSP Program	OSP’s instructors	<ul style="list-style-type: none"> ▪ ¡Pilas! Promoter’s handbook ▪ Radio mini-series ▪ Cooking workshop for parents ▪ Cooking workshop for kids

Feasibility and acceptability assessment during the intervention:

Two field workers will be assigned to each school/community for the process evaluation (fidelity, dose, and reach), feasibility (schedule constraints, staff’s time and commitment, physical space requirements, materials

needed and individual capacity) and acceptability assessments (intervention's agents and target group's enjoyment and opinions). This information will be collected through structured observations made by the field workers and self-report surveys filled out by the intervention agents. These short surveys will be part of the materials provided to them at the beginning of the pilot intervention, which will be returned to us after an intervention agent delivers an activity. The following assessments will be made during this phase:

- School component
 - Structured observation of classroom-based program activities
 - Short self-report survey about the activity delivered by the teacher
 - Structured observation of PE class
 - Structured observation of school recess
 - Structured observation of radio mini-series

- Food kiosk component
 - Weekly structured observation of changes occurring in the food kiosk
 - Weekly short self-report survey from each food vendor

- Community component
 - Structured observation of religious leader's activities
 - Short self-report survey from each religious leader
 - Structured observation of OSP activities (e.g., cooking classes)
 - Attendance lists to each cooking class
 - Count of how many children participate in the games organized by the child health promoters
 - Structure observation of child promoter's activities
 - Monthly short self-report survey of each child health promoter

The information of the direct observations and the feasibility and acceptability questionnaires used during the pilot intervention will be analyzed comprehensively, categorizing by school, grade, teacher and specific activity. The acceptability of the children and parents will be assessed according to their desire to be recruited and of their participation in the program and experiences in the different activities they participated. The feasibility of the program will be assessed in terms of schedule restrictions, time and personal commitment, physical space, difficulties and challenges identified for the implementation of the study. Also a feasibility of the manuals of the program will be done.

Phase 4: Post-intervention assessment and data analysis (three months)

All child/mother pairs evaluated at baseline will be assessed at the end of the five-month intervention. The assessment to be performed will be the same used at baseline, but the demographics questionnaire. A short questionnaire will be added to explore the activities the child and/or mother participated in and were exposed to during the pilot intervention.

We will organize a recognition meeting at each school for all the intervention agents, where they will receive a diploma and an appreciation letter. At the beginning of the meeting we will ask them to share their experiences with the ¡Pilas! program and recommendations on how to improve it. These recommendations will be very important to finish the feasibility and acceptability assessment.

All results will be written in forms that will be checked for accuracy and completeness by the study coordinator the same day they are filled, and 100% of the data will be double-entered into the project's computer files using Epi-Info, Version 2000. Any incorrect entries or missing data will be checked and verified on a systematic basis

using range checks for values beyond permissible values and missing values. Means and standard deviations will be calculated for all continuous variables and frequency tables for all categorical variables included in the pre-post intervention assessment of children and mothers. Differences between groups will be compared by using Student's *t* tests (continuous variables) and Chi-square tests (categorical variables). All information gathered during process evaluation will be qualitatively analyzed with the purpose to improve each activity and intervention component.

5. DATA SECURITY AND PROTECTION OF SUBJECT CONFIDENTIALITY

A file on each child/mother pair that includes personal identifiers (e.g., child's and mother's name, sex, date of birth, address, telephone number, school, grade and links to the study ID) will be stored in locked cabinets, in a secure room. All data entered in the forms and questionnaires will identify the individual only by study ID and will be entered in an electronic study database without identifiers. A linkable identifier list will be kept by the PI in a locked cabinet, and will be destroyed once the electronic dataset is considered clean and final. All subsequent analysis will be performed in this database and will not include identifiable information.

6. RECRUITMENT PROCESS

The first step in this process will be the recruitment of the intervention agents (teachers, food vendors, religious leaders, child promoters, and Health Center's and OSP's instructors). Individual or group meetings will be held at their institutions to explain the study and request their support. Our expectation about their participation, roles, and responsibilities in the project will be clearly explained.

The recruitment of the study population will be done in two low-income, urban public schools located within the Municipality of Mixco (Guatemala City). Parents will be invited to participate in a school meeting, in which information about the study will be given, with the aid of a Power Point presentation, informed consent will be obtained, and child/mother pairs will be screened. The school principal will provide us with the official lists of students from each grade, in alphabetical order. As we will need 10 girls and 10 boys from each grade, it will be divided by ten the total of boys and girls in each grade, providing the counting number. Then it will be asked that a person not involved in the study select a number between 1 and 10. The number that she and he indicates will be the first participant invited and then the selection will continue according to the number proposed before. Those children and their mothers who meet the inclusion criteria will be included in the intervention. If someone doesn't want to participate, he/she will be substituted by another randomly selected subject. We will program school meetings with parents until the required sample is completed (n=126). Parents will be allowed to take the informed consent form to their home and return it during the following week. After the parents have given their consent form for their child's and their own participation, the study's staff will do the oral assent with each child.

7. CONSENT PROCESS AND DOCUMENTATION

Written informed consent will be sought from all mothers and assent from all children recruited. In the school meeting, the project coordinator will explain the study, procedures and inclusion criteria (e.g., siblings, child's chronic disease, mother's pregnancy) before reading the consent form (administered in Spanish). Time for answering questions will be given publicly and privately. When the parents have given the consent for their child's participation, the study's staff will individually do the oral assent with each child privately, in the presence of his/her parent or teacher. A child/mother pair will be included in the study if both agree to participate.

It will be clarified that all participants (child/mother pairs) involvement in this research study is voluntary, that the refusal to participate will bring no negative consequences, and that they can stop participating at any point after the consent is granted without any penalty. They will be allowed to take home the consent form and

decide later if they wish to participate, in which case we will offer to pick up the consent at the school or their home. A signed copy will be given to each participant.

8. RISKS

Children and mothers enrolled in this study will be subject to minimal risks. All measurements planned for every participant pose no physical risk, but some of the questions may create apprehension in some people. We will reassure children and mothers that they are not obligated to answer questions they don't want to, and that all data will remain confidential.

The study will be conducted at the schools and the assessment will be done during school hours. This will not imply out-of-pocket costs for the child or the mother. It is fairly common that mothers travel with their children to and/or from school.

9. BENEFITS

The activities that are programmed during the intervention will offer children and their mothers the opportunity to learn about a healthy lifestyle, gain healthy cooking skills and practice physical activity in a safe environment, additionally, parents and children who present abnormal values will be offered guidance about the importance of maintaining healthy weight and recommendations of a healthy diet and increase of physical activity will be given. Then they will receive a referral note to consult a doctor.

This study will help develop a community-based intervention that is feasible, culturally acceptable, and effective in promoting healthy behaviors to the prevention of cardiometabolic diseases in children and their families.

10. PAYMENT

Monetary compensation is not common in studies done in Guatemala. This "tradition" may have developed (at least at INCAP) in part because of the difficulty in avoiding the appearance of coercion when dealing with impoverished populations. Instead, participants in our study will receive:

- Children (baseline assessment): School supplies (eraser, sharpener and a pencil)
- Children (post-intervention assessment): A plastic ball.
- Mothers (baseline assessment): A plastic kitchen supply.
- Mothers (post-intervention assessment): Two packages of INCAPARINA (a popular nutrient-dense high-quality flour).

11. RECOGNITION OF INTERVENTION AGENTS

The effort and time spent by the intervention agents during this research study will be appreciated.

- The work of all intervention agents will be recognized at the special school meeting.
- Food vendors will receive a Completion Diploma of the ¡Pilas! Training Workshop, a thank you letter for their participation in the pilot intervention, and the cleaning and painting of their food kiosk.
- Other intervention agents will also receive a Completion diploma of the ¡Pilas! Training Workshop, a thank you letter for their participation in the pilot intervention and a public recognition (if desired) for their support in our website: www.ciipec-incap.org

12. SAFETY MONITORING

A DSMB will be established for the implementation of the ¡Pilas! pilot intervention. It will consist of two members, with expertise covering the areas of nutrition, pediatrics, and CVD. They will meet in person or by

conference calls three times (approximately every two months) during the study. None of them will be part of the research team conducting this project. The discussions and decisions of the DSMB will be summarized in written reports and forwarded to the IRB.

13. PLAN FOR REPORTING UNANTICIPATED PROBLEMS/ADVERSE EVENTS

The PI will be notified immediately of any unexpected event. The PI will report these to the IRB and the DSMB, according to the guidelines.

14. OTHER IRBs/ETHIC REVIEW BOARDS

The study will be reviewed by the Ethics Committee of the Bloomberg School of Public Health at the University of Johns Hopkins.

15. OUTSIDE COLLABORATIONS

The Institute of Nutrition of Central America and Panama (INCAP) and JHSPH will be the participating sites. The study will take place in Guatemala.

Manuel Ramirez-Zea, M.D., Ph.D. (Exercise Physiology). Co-PI (INCAP). He will be responsible for the study design, overseeing all aspects of the core project implementation, hiring, training, and supervision of study personnel; development of data collection protocols, administration of study budget, data analysis and interpretation.

Benjamin Caballero, M.D., Ph.D. (Nutrition). Co-PI (JHSPH). He will also be responsible for the study design, development of data collection protocols, data analysis and interpretation.

Paola Letona M.Sc. (Behavioral Neurophysiology). She will be the project director (INCAP) in charge of supervising and coordinating all project activities, developing the manuals of operations, training and supervision of personnel, and support on data management and analysis.

Giovanna Gatica M.Sc. (Epidemiology). She will be a research assistant (INCAP) and will be involved in the development of instruments and operations manuals, training of personnel, supervision of the pilot intervention activities, and data management and analysis.

ANNEX 1 – EDUCATIONAL MATERIALS DESCRIPTION

Educational material	Description
iPilas! Promoter's Handbook	<ul style="list-style-type: none"> ▪ This handbook is intended for the use of the intervention agents. ▪ It includes all the theoretical concepts needed to promote healthy lifestyles in school-age children. ▪ Strategies for the improvement of auto-efficacy in children and adults will be given. ▪ Ideas will be shared about the activities they can do to promote healthy lifestyles. ▪ The issues addressed in this handbook are: healthy diet, physical activity, secondhand smoke, tobacco use, and alcohol consumption.
Handbook: Improvement of the School Environment	<ul style="list-style-type: none"> ▪ This handbook will be used by teachers, parents and/or health promoters. ▪ It is a tool that will help them to improve the school environment, so it promotes healthy lifestyles in their students. ▪ It includes an assessment tool, low-cost solutions and suggestions for the establishment of school norms.
Handbooks: 4 th , 5 th and 6 th Grade Classroom Activities	<ul style="list-style-type: none"> ▪ The classroom activities handbooks are aimed at fourth, fifth, and sixth grade teachers. ▪ Each handbook describes in detail 16 classroom activities (30-45 minutes each) that the teacher can do every other week with their students to promote healthy lifestyles. ▪ The topics addressed in each handbook are: healthy diet, physical activity, secondhand smoke, tobacco use, and alcohol consumption.
Booklet: Establishment of PE norms	<ul style="list-style-type: none"> ▪ This booklet will be used by the school principals. ▪ A series of norms will be suggested with the goal of increasing the time children spend in MVPA during PE classes.
Booklet: Fun games	<ul style="list-style-type: none"> ▪ The Fun games booklet will help child health promoters with the promotion of physical activity during recess. ▪ The booklet includes 36 games and is intended for the child promoters to teach other children one game a week.
Radio mini-series	<ul style="list-style-type: none"> ▪ The radio mini-series is aimed at parents who will listen to them at school, OSP, health center and/or religious services or activities. ▪ The mini-series will include 5 (5-minute) episodes that will address the issues of a healthy diet, physical activity, and secondhand smoke; as well as, prevention of tobacco use and alcohol consumption in children. ▪ The mini-series will focus not only on conveying information about these issues but also to help parents identify and change family customs that are promoting unhealthy lifestyles.
Booklet: Strategies for the successful establishment of food kiosk norms	<ul style="list-style-type: none"> ▪ The booklet of strategies for the establishment for food kiosk norms is intended for school principals. ▪ A series of norms will be suggested for the improvement of the quality of foods and beverages offered in the food kiosk. Detailed guidelines for the successful establishment of each norm will be included.

Educational material	Description
Booklet: Healthy recipes for food kiosks	<ul style="list-style-type: none"> ▪ This booklet will be used by food vendors at school. ▪ The booklet will give the food vendors ideas to increase the amount of healthy foods and beverages that they offer at the kiosk. ▪ It will include 20 healthy and delicious recipes previously validated and advertising ideas on how to promote their healthy snacks.
Cooking workshop for parents	<ul style="list-style-type: none"> ▪ This healthy cooking workshop will be conducted by the OSP instructors. ▪ It will include 24 healthy recipes previously validated. ▪ This workshop is to be carried out in three months (one class every week).
Cooking workshop for kids	<ul style="list-style-type: none"> ▪ This healthy cooking workshop will be conducted by the OSP instructors. ▪ It will include 10 healthy recipes previously validated. ▪ This workshop is to be carried out in 5 weeks (one class every week).