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TITLE: BREAST CANCER EPIDEMIOLOGY IN PUERTO RICO

PRINCIPAL INVESTIGATOR: DR. CRUZ NAZARIO

CONTRACTING ORGANIZATION:

University of Puerto Rico
San Juan PR 00936-5067

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14. ABSTRACT

This second year report provides evidence that the training of investigators phase has been develop successfully. The infrastructure for developing the pilot case-control study has been established. The work, communication and coordination between mentor institution, minority institution and the project agency sponsoring has been effective and this has stimulated the first phase of the study has been completed successfully (initial training in the study protocol, obtaining all institution and DOD IRBs and HIPAA approvals, designing the questionnaire, etc.)

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INTRODUCTION:

This project has two mayor goals: to design and conduct a pilot case-control breast cancer study among Puerto Rican women and to train and develop researchers in breast cancer at the minority institution. The case control study will enroll women 30-79 years of age who are residents of the San Juan metropolitan area. Cases will be women with incident, primary, pathologically confirmed breast cancer with no history of previous cancer other than non-melanoma skin cancer; controls will be frequency-matched by age and randomly selected from female residents of the same geographical area. We will examine adult and childhood factors in relation to risk of breast cancer in this understudied population of Puerto Rican women. The specific aims are: to examine dietary risk factors in relation to breast cancer and also in relation to tumor characteristics (e.g., estrogen and progesterone receptor status); to examine other established risk factors such as lifetime weight gain, physical activity, alcohol consumption, reproductive history among Puerto Ricans in relation to breast cancer risk; and to examine factors related to early life exposure including birth weight, adult height, childhood diet, physical activity, environmental factors and residential history as a proxy for environmental exposure in relation to proxy risk. The overall training goal is to develop a team of independent investigators with the necessary skills to develop a program of breast cancer research in Puerto Rico and to obtain funds and support for that research. To accomplish this goal, researchers from the University of Puerto Rico will obtain formal training in cancer epidemiology and participate in the design and conduct of the population-based case control study.

BODY:

STATEMENT OF WORK

Task 1. Training researchers from the University of Puerto Rico

The training of investigators at the minority institution has been an important goal in this project. Investigators have been involved in activities directed toward achieving the following goals: 1) to develop expertise in breast cancer epidemiology, especially in the areas of interest for the planned study, 2) to understand cutting edge developments in breast cancer in order to design future studies to test new hypothesis and 3) to develop needed expertise specially for the planned as well as for future studies.

In order to meet the training goals, during the second year of the award, the investigators have participated in the following scientific activities. Dr. Michele Schelske-Santos took a graduate course in epidemiologic method at Graduate School of Public Health, University of Puerto Rico and other two of the investigators from the minority institution, Dr. Imar Mansilla-Rivera and Dr. Rosa Rosario-Rosado registered to take summer courses in Cancer Epidemiology (June-July 2009).

Drs. Mansilla and Cruz M Nazario attended the Joint Annual Conference of the International Society for Environmental Epidemiology (ISEE) and International Society of Exposure Analysis (ISEA), in October 2008. In this conference Dr. Mansilla-Rivera participated in a pre-conference workshop on "Ultrafine and fine particulate matter monitoring in exposure and epidemiological studies". Dr Mansilla-Rivera presented a poster which gave an opportunity to receive comments regarding the measurement of contaminants in biological samples. They also had the opportunity to discuss the results of a study on breast cancer and environmental contamination being conducted in Spain, since some of the methods to test exposure to estrogens were pertinent to our study hypothesis. Dr. Nazario met with epidemiologists participating in the meeting to discuss ideas for future studies on environmental contamination and breast cancer risk. The Program coordinator attended the American Public Health Association's 136th Annual Meeting & Exposition: Public Health without Borders, in October 2008. She participated in the following cancer and epidemiology section: Cancer and its Impact on Women's Lives and Health, Cancer Epidemiology I and II and Social Epidemiology: Effects of Race/Ethnicity and Socioeconomic Disparities on Health.

Drs. Nazario, Rosario and the program coordinator participated in the Science of Cancer Health Disparities in Racial/Ethnic Minorities and the Medically Underserved workshop sponsored by AACR. (Carefree, Arizona February 3-6). Dr Nazario was invited to present *Breast Cancer Research in Puerto Rico* at the pre-meeting workshop Latino Breast Cancer Consortium. Dr Nazario applied to the Pathobiology of Cancer Workshop, an AACR workshop, but was not accepted.

Dr. Farah Ramirez attended the Experimental Biology 2009Annual Meetings (New Orleans, Louisiana April 18-22). In this meeting Dr. Ramirez participated in an Obesity, Aging and Cancer section and visited several poster related to the themes of genes, estrogen receptors and breast cancer, to discuss with presenters about methodological issues on breast cancer research. Dr. Ramirez also attended to the American College of

Sports Medicine meeting (Seattle, Washington May 27-30) to participate in several sections related to physical activity and breast cancer risk, being this an important hypothesis in our research.

In addition, Dr. Schelske-Santos and Dr. Ramirez attended the 2009 International Conference on Diet and Activity Methods in Washington, DC (June 5-7). In this conference Drs. Schelske and Ramirez participated in several section directly related to dietary, physical activity patterns, lifestyles and Breast Cancer. Those activities were important for the second and third goal of understanding cutting edge science and develop needed expertise especially for the planned as well as for future studies on breast cancer.

Task 2. Develop and maintain communications among participating investigators.

During the second year of the award, the minority and the mentoring institutions have been in close communication to revise the study protocol, the study questionnaire and the informed consent. This communication has included weekly teleconference calls of at least one hour, as well as frequent communications by email.

Task 3. To design, implement and analyze a case control study of breast cancer in Puerto Rico.

During these past months both institutions have been in close communication to revise the study protocol and incorporate requests regarding the second DOD-CDMRP IRB review. We have also been working closely with the Human Subjects Protection Scientist of the CDMRP IRB office to assist and answer questions about the study protocol or informed consent documents. The study protocol (identification of incident cases at hospitals, identification of control and laboratory protocol for the collection and management of biological samples) and informed consent documents has been revised and submitted along with informed consent documents to the local IRB (University of Puerto Rico). Local IRB approval was obtained on March 2009 and DOD-CDMRP IRB approval was received on June 2009. Also, the Informed Consent and the study protocol were submitted to the appropriate IRB Committee in the mentoring institutions (University at Buffalo, and Roswell Park Cancer Institute) for their revision and approval.

The Principal Investigator and project coordinator met with Puerto Rico Central Cancer Registry Director to discuss the proposed method for this study of case ascertainment from the Puerto Rico Central Cancer Registry data base. The approval letter of the Puerto Rico Central Cancer Registry Director for the study case ascertainment protocol was received in January 2009. The program coordinator began to contact primary physicians (or referring physicians) who refers breast cancer patients to the Puerto Rico Central Cancer Registry according to the cancer registry data base to identify sources of cases.

The geographical area from which cases and controls will be drawn has been defined to include the following municipalities: San Juan, Bayamón, and Guaynabo. The list of

potential population controls from the above mentioned area is available to identify eligible controls.

The survey instrument to be use in this study has been revised. The questionnaire will collect information on diet, lifetime physical activity, smoking, sun exposure, demographic characteristics, personal and familial history of chronic diseases, residential history, vitamin and medications, menstrual, reproductive and weight history, cancer diagnosis and treatment, among others. The instrument has been pre-tested on people with backgrounds similar to the target population to evaluate its ability to handle cultural and social sensitive issues, vocabulary, and the questions' sequence. The resulting observations have been incorporated into the final instrument version (Appendix C, questionnaire). The final version of the questionnaire was translated into English to be available for English speaking participants. An electronic version of the questionnaire is being developed using Microsoft Access. At this moment we are working on revision of the Spanish version of the questionnaire and we hope to complete the revision and the electronic versions /English and Spanish versions) by early August 2009.

We have identified personnel for all stages of the study (Interviewers, nurses, laboratory personnel, and community outreach personnel). We plan to recruit and re-trained study personnel in the next months.

Task4. Study participants' recruitment and data collection to conduct a breast cancer case-control study in Puerto Rico.

Recruitment and data collection for this study will start in early September. We hope to obtain IRB approval from the mentoring institutions (University at Buffalo, and Roswell Park Cancer Institute) on August 2009.

At this moment, no problems have been anticipated that could impede the progress of this project. Investigators will develop plans for the next several years for their training. They will participate in summer courses in epidemiology (basic and applied) courses. We plan to finalize the questionnaire electronic version revision, and to test it with using a formal interview format. We hope to begin interviewing in September 2009.

KEY RESEARCH ACCOMPLISHMENTS:

- Training of study investigators in basic epidemiology and nutrition epidemiology (Appendix A)
- Establishment of collaboration links with Puerto Rico Cancer Registry, hospitals and physicians to identify sources of cases (Appendix B).

REPORTABLE OUTCOMES:

- Survey instrument final revision "CASE CONTROL STUDY OF BREAST CANCER IN PUERTO RICO" (Appendix C).

- Protocol study and Informed Consent fully approved in local (UPR-Medical Sciences Campus) and DOD-CDMRP IRB Committee (Appendix D).

CONCLUSION:

This second year report provides evidence that the training of investigators phase has been develop successfully. The infrastructure for developing the pilot case-control study has been established. The work, communication and coordination between mentor institution, minority institution and the project agency sponsoring has been effective and this has stimulated the first phase of the study has been completed successfully (initial training in the study protocol, obtaining all institution and DOD IRBs and HIPAA approvals, designing the questionnaire, etc.)

REFERENCES: N/A

APPENDIX:

- Progress and Training Report of study investigators in basic epidemiology and nutrition epidemiology (Appendix A)
- Establishment of collaboration links with Puerto Rico Cancer Registry, hospital and physician (to identify sources of cases) (Appendix B).
- Survey instrument final revision "CASE CONTROL STUDY OF BREAST CANCER IN PUERTO RICO" (Appendix C).
- Protocol study and Informed Consent fully approved in local (UPR-Medical Sciences Campus) and DOD-CDMRP IRB Committee (Appendix D).

Breast Cancer Epidemiology in Puerto Rico
Annual Report
June 2008 to June 2009

Appendix A
Progress and training report of study investigators

Dr. Cruz M. Nazario (Principal Investigator)

1. Weekly conference call with investigators from Puerto Rico and Dr. Jo Freudenheim from the University at Buffalo.

2. Weekly meeting with project coordinator.

3. Training

    a. Attendance to Annual Meetings:

        i. The Joint Annual Conference of the International Society for Environmental Epidemiology (ISEE) and International Society of Exposure Analysis (ISEA), held in Pasadena, California, from October 12-16, 2008.
            1. I met with epidemiologists participating in the meeting to discuss ideas for future studies on environmental contamination and breast cancer risk.

        ii. Science of Cancer Health Disparities in Racial/Ethnic Minorities and the Medically Underserved workshop sponsored by AACR. (Carefree, Arizona February 3-6, 2009).
            1. I was invited to present about *Breast Cancer Research in Puerto Rico* at the pre-meeting workshop Latino Breast Cancer Consortium.

Dr. Farah A. Ramírez-Marrero (Co-investigator)

1. Weekly conference call with investigators from Puerto Rico and Dr. Jo Freudenheim from the University at Buffalo.

2. Training

    a. Attendance to Annual Meetings:

        i. **Experimental Biology 2009** in New Orleans, Louisiana (April 18-22)
            1. Monday, April 19:
                a. Session: Obesity, Aging and Cancer
            2. Tuesday, April 20:
                a. Poster: Effects of herbal enzyme bromelain against breast cancer cell line. Paroulek, Jaffe and Rathinavelu. Nova Southeastern Univ.
            3. Wednesday, April 21:
                a. Poster: Molecular mechanism effect of taxotere and ukrain in cell cycle regulating genes in positive and negative breast cancer cell lines. Alokail, Al-Mansouri, Bin Amer. King Saud University, Saudi Arabia.
                b. Poster: Study of the activity of estrogen response elements present on Pax-5 promoter and their potential role on regulating Pax-5 expression in breast cancer. Faye and Ouellette. University of Moncton, Canada.

        iii. **American College of Sports Medicine 2009** in Seattle, Washington (May 27-30)
            1. Thursday, May 28:
                a. Session: Exercise interventions in Cancer Survivors: A diverse Perspective
                    i. Effect of physical activity on weight and body composition in breast cancer survivors. Cadmus, Yu, Wiley, Chung, Irwin. Yale University, CT.
                    ii. One-year randomized, controlled trial of strength training in older breast cancer survivors: preliminary findings. Winters-Stone, Reiner, Dobek, Nail, Bennet, and Naik. Oregon Health and Science University, Portland, OR.
                b. Session: Physical activity, fatigue and pain
                    i. The effects of 12-weeks cross training on fatigue and mood in recent breast cancer survivors. Poudevigne, Wojcik, Lane, Polovich and Simonishvili. Emory University, Atlanta.

2. Friday, May 29:
    a. Session: Exercise with Cancer Patients
        i. The relationship of physical activity across the lifespan and breast health. Sprod, Hsieh, Carter, Hayward, Schneider. Univ. Northern Colorado, CO.
3. Saturday, May 30:
    a. Session: Clinical Medicine II – Medical
        i. Effect of comprehensive exercise on lymphedema in breast cancer survivors: a pilot study. Oki, Troumbley, Walker, Plante, Hansen. Univ. of Utah, UT.

iv. **2009 International Conference on Diet and Activity Methods** in Washington, DC (June 5-7)
1. Friday, June 5:
    a. Conference: Climate change: interrelationships with diet and physical activity.
    b. Conference: An integrated assessment of health and climate change impacts of community design.
    c. Symposium: Measures of the physical activity built environment
    d. Symposium: Measuring change in diet and physical activity in intervention studies
2. Saturday, June 6:
    a. Conference: The magnitude of the energy gap and its determinants
    b. Conference: The energy gap for obesity
    c. Symposium: Physical activity measurement of individuals
    d. Symposium: Energy balance: synchronized measures of energy intake and output, and data complexity in physical activity measures
    e. Meeting with Dr. Jo Freudenheim.
3. Sunday, June 7:
    a. Symposium: Lessons learned from national surveillance studies on physical activity

Dr. Michelle Schelske-Santos **(Co-investigator)**

1. Weekly conference call with Dr. Jo Freudenheim (University at Buffalo) and Puerto Rico investigators team.

2. Training
    a. Courses:
        i. **Epidemiological Methodology at Graduate School of Public Health, University of Puerto Rico**. The course presents epidemiological principles and methods as an approach to the study of the phenomena of health and disease. The scientific method, the epidemiological method, the concept of causality, descriptive epidemiology, and hypothesis formulation, case studies, cohort studies, intervention studies, screening, outbreaks, and research design are among the topics covered.

    b. Attendance to Annual Meetings:

        i. Seventh International Conference on Diet and Activity Methods
        Hyatt Regency Capitol Hill Washington DC June 4 – 7, 2009
        1. Pre-Conference Workshop: Understanding Measurement Error in Diet and Activity Assessment (This conference was very useful in presenting systematic and random measurement errors common to food frequency questionnaires, 24h dietary recalls, physical activity questionnaires and accelerometer measures, in addition to how a variety of statistical models can be applied to collected data to help correct for these errors during data analyses.)
        2. Conference: Climate Change: Interrelationships with Diet and Physical Activity
        3. Conference: Technological Advances in Measuring Diets of Individuals, Dietary Biomarkers: Novel Techniques
        4. Conference: Advances in Coping with Measurement Error in Diet and Physical Activity Measures
        5. Conference: Diet Measurement of Individual Intake: Development of Instruments
        6. Conference: technology demonstration of on line 24h dietary recalls
        7. Conference: Dietary Patterns: Research Challenges and New Methodological Directions, Energy Gap that Causes Obesity;

    This three-day conference was very informative and useful in helping with the concepts of instrument development, validation, and analysis in order to obtain the data necessary to link dietary and physical activity patterns and lifestyles to disease outcomes and prevention. These techniques and potential applications are directly related to the development and validation of our food frequency questionnaire to assess potential exposures related to breast cancer incidence and prevention in our Puerto Rican population.

Dr. Imar Mansilla-Rivera **(Co-investigator)**

1. Weekly conference call.

2. Training
    a. Courses:
        i. Attended a short course on measurements of environmental exposures and their association with chronic diseases. (International Society for Environmental Epidemiology meeting in Pasadena, California October 12-16, 2008).

    b. Attendance to Annual Meetings:
        i. The Joint Annual Conference of the International Society for Environmental Epidemiology (ISEE) and International Society of Exposure Analysis (ISEA), held in Pasadena, California, from October 12-16, 2008.
            1. Attended a pre-conference workshop on "Ultrafine and fine particulate matter monitoring in exposure and epidemiological studies".
            2. Attended several oral and poster presentations on topics such as environmental exposures at different stages of development, health effects of various groups of contaminants (e.g. air pollutants, trace metals, persistent chlorinated and brominated compounds).
            3. Also, I met with other researchers working in the area of endocrine disruptor exposures and their health effects (e.g. breast cancer) to exchange ideas for future collaborations and potential studies.

Johan Hernandez, MPH (Project Coordinator)

1. Weekly meeting with principal investigator and other staff project.

2. Training:
    a. Attendance to Annual Meetings:
        i. The American Public Health Association's 136th Annual Meeting & Exposition: Public Health without Borders, in October 2008.
            1. Attended several conferences and workshop on cancer and epidemiology (Cancer and its Impact on Women's Lives and Health, Cancer Epidemiology I and II and Social Epidemiology: Effects of Race/Ethnicity and Socioeconomic Disparities on Health).

        ii. Science of Cancer Health Disparities in Racial/Ethnic Minorities and the Medically Underserved workshop sponsored by AACR. (Carefree, Arizona February 3-6, 2009).
            1. Attended a pre-meeting workshop Latino Breast Cancer Consortium.
            2. Attended several conferences on cancer epidemiology.

CENTRO COMPRENSIVO DE CÁNCER
UNIVERSIDAD DE PUERTO RICO

Puerto Rico Central Cancer Registry

January 13, 2008

Cruz María Nazario, Ph.D.
Principal Investigator
Breast Cancer Epidemiology in Puerto Rico
University of Puerto Rico
Graduate School of Public Health
Box 365067, San Juan Puerto Rico, 00936-5967

RE: DOD CDMRP #BC060131

Dear Dr. Nazario:

The Puerto Rico Central Cancer Registry was established in 1951 through a state law and receives support from the National Program Cancer Registries since 1997. Therefore the Registry follows the standards of the National Program Cancer Registries as established since 1992 by CDC through the Cancer Registries Amendment Act. CDC provides support for the administration and audits compliance of cancer data collection with the approved quality standards. Its objectives include cancer surveillance, evaluation of incidence and mortality patterns, guidance in the planning and evaluation of prevention and control strategies, collaboration of other agencies in the establishment of cancer control and prevention strategies, participation and collaboration in clinical and epidemiological research, and maintenance and publication of updated cancer information.

The Registry collects the information to establish the occurrence of cancer, and the types, extension, and treatment of cancers. Health professionals use the information to better understand the burden of cancer in the population, to study cancer incidence and mortality trends, to evaluate the effect of cancer prevention and cancer control strategies, and to participate and collaborate in cancer research investigations. The Puerto Rico Central Cancer Registry also has the duty to provide cancer information to the public.

Within the Registry, the Statistics and Data Analysis Unit of the Registry has the main responsibility to collaborate in epidemiological research designed to evaluate factors

related to the risk of developing cancer as well as factors that modify the risk of dying from this disease by providing specific registry information needed to test etiologic hypothesis. The Puerto Rico Central Cancer Registry follows the US Department of Health and Human Services guidelines and the NPCR standards to protect the confidentiality of collected information and thus requires that researchers follows and comply with the established procedures.

All physicians that diagnose and treat cancer patients, pathology laboratories, cancer treatment centers and hospitals are required by Law to report cancer information to the Puerto Rico Central Cancer Registry. They must follow HIPPA rules and regulations.

The usual procedure for the identification of potential participants in a research study requiring contact with patients is summarized as follows: The Principal Investigator (PI) of the study will send a letter to qualified registered oncologists in the Puerto Rico Department of Health database explaining the study objectives and procedures. Then, the Registry will identify breast cancer patients that potentially comply with the research inclusion criteria and will notify the principal investigator. The PI will then contact the primary or referring physicians of the potential inclusion of the patient in the research study and will ask for their written permission to contact the patient. If the primary or referring physician does not agree for the patient to be contacted, the patient will then be excluded for participation. If the physician agrees, the principal investigator will contact the patient to present the study and invite him/her to participate. The complete protocol is available upon request.

We look forward to this collaboration with your project.

Sincerely,

Nayda R. Figueroa-Vallés, MD, MPH
Director
Puerto Rico Central Cancer Registry

PO Box 70184 San Juan, Puerto Rico 00936-8184
Tel.:(787) 756-6363; 756-6389 ext. 223, 224 Fax: (787) 756-6372

4

PARTICIPANT'S CONTROL SHEET

CONFIDENTIAL
Control Number: | | | | |
1 2 3 4

Name: _____
Last Name First Name Initial

Address: (1) _____

_____

Address: (2) _____

_____

Telephone Number: | | | | - | | | | - | | | | |

Other Tel. Number: | | | | - | | | | - | | | | |

Interview schedule:

DATE				
Day	Month	Year	HOUR	COMMENTS

UNIVERSITY OF PUERTO RICO - RIO PIEDRAS
MEDICAL SCIENCES CAMPUS

CONTROL NUMBER			
1	2	3	4

STUDY:

BREAST CANCER EPIDEMIOLOGY IN PUERTO RICO

QUESTIONNAIRE
(Atabey)

2009

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DEPARTMENT OF DEFENSE
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BREAST CANCER RESEARCH PROGRAM
CDMRP- USAMRMC-DOD #BC060131

STUDY INTRODUCTION

Read to participant

We are conducting a study on women in Puerto Rico to learn about their health and the health of their family, including diet and level of exercise. Your participation in this investigation is very important to us and your answers will enhance our knowledge about the health of women in Puerto Rico. We would like to count on your cooperation. Your participation is voluntary and all of the information provided will be kept in strict confidentiality.

If you agree to participate in this study, you will be required to carefully read and sign the document that contains the consent form. This document contains detailed information with the names and telephone numbers of the people leading this survey in case you need to ask any questions. Before beginning, it is important that you have signed the forms to evidence that you have accepted to participate in this study.

First we will take the blood sample, then we will take the body measurements and lastly we will be asking the survey questions. If you cannot donate blood, a saliva sample can be analyzed in its place. After taking the blood or saliva sample and your body measurements, we will be performing an interview that will take approximately 45 minutes. After completing this process, we will be providing a monetary incentive ($15) in appreciation of your cooperation.
(***At this point, the consent form is reviewed with the participant and signed.***)

I. Signed consent form? Yes _____ No _____ (Do not proceed)

II. Blood sample: Completed ____ Not completed _____
Saliva sample: Completed ____ Not completed _____

III. Body measurements
Weight: _____ (pounds); Height: _____ (inches); Height sitting: _____ (inches)

(1) Waist: _____(inches); (2) Waist: _____(inches); (3) Waist: ___(inches); (4)Average_____(inches)*

(1) Hips: _____(inches); (2) Hips: _____(inches); (3) Hips: ____(inches); (4)Average_____(inches)*

Access program: calculate the average of body measurements* and BMI*

Skin-fold measurements:

(1) Triceps :_____(mm); (2)Triceps:_____(mm); (3)Triceps:_____(mm); (4)Average_____(mm)**

(1) Subscapular :___(mm); (2) Subscapular:___(mm); (3) Subscapular:___(mm); (4)Average__(mm)**

(1) Suprailiac :____(mm); (2)Suprailiac:____(mm); (3) Suprailiac:____(mm); (4)Average__(mm)**

(1) Abdomen :_____(mm); (2)Abdomen:_____(mm); (3) Abdomen:_____(mm); (4)Average__(mm)**

(1) Thigh :_____(mm); (2)Thigh:_____(mm); (3) Thigh:_____(mm); (4)Average__(mm)**

Access program calculate the Skin-fold measurements average**

IV. Colorimeter: Under side of upper arm:_____; Dorsal hand:_____

UNIVERSITY OF PUERTO RICO
MEDICAL SCIENCES CAMPUS

SURVEY

Control Number: ☐☐☐☐	Date of interview ☐☐ ☐☐ ☐☐☐☐
1 2 3 4	Day Month Year
	Start time: ____ ____:___ ___ a.m.___ p.m.___

Part I. SOCIO-DEMOGRAPHIC INFORMATION

***Read to participant*:**

As I mentioned previously, the last part of the study will be questions in reference to your and your family's health and some additional questions about your diet and levels of exercise throughout your life. I can repeat the question as many times as you need in order for you to provide the best answer. First I would like to ask you …

1. What is your date of birth? Date: ☐☐ ☐☐ ☐☐☐☐
   Day Month Year

2. Which one of the following options best defines your marital status?(**Read options**) ☐
   Married / Living with partner.....1
   Single (never married).....2
   Separated / Divorced….....3
   Widow (not living with partner)…….....4
   Does not want to answer.……..…….…….……... 9

3. What is **the highest level** or year of schooling that you **completed**? ☐☐
   00 Didn't go to school
   01-12 (**Code from 01=first grade until 12=grade 12th**)
   13 Technical or Vocational
   14 Associate Degree
   15 Bachelor's Degrees
   16 Graduate Studies
   17 Other _____
   99 Does not want to answer / can't remember

4. What has been the job or occupation that you have held for the longest time? _____

   _____

   4b. How long? _____(Years) _____(Months)

We'd like to know about you and your mother at the time you were born.

5. Please tell me where **your mother lived** when you were born? (*If you do not know the complete address please provide as much information as possible*.)
a. Country: _____

b. Address: County or district (barrio): _____

_____

c. Municipality: _____d. Zip code: _____

e. How long did you live there after you were born? Months _____ Years _____

f. How much did you weigh at birth?_____ (pounds and ounces)
__ Less than 5 lbs.
__ Between 5 lbs. and 6 lbs.
__ Between 6.1 lbs. and 7 lbs.
__ Between 7.1 lbs. and 8 lbs.
__ Between 8.1 lbs. and 10 lbs.
__ More than 10 lbs.

[**If the participant cannot remember the exact weight tell her the following:** *If you do not know your exact birth weight, could you at least tell us if you were ….*]
__ Average; ___less than average (skinny), ___more than average (chubby)
__ Don't know / cannot estimate.

g. Did your mother breastfeed you when you were born?
(0)No; (1) Yes, How long? ____(months) ____(years); (9) Don't know ☐

h. Did your mother suffer from high blood pressure or preeclampsia while she was pregnant with you?
(0)No; (1) Yes; (9) Don't know ☐

i. Did your mother suffer a very stressful event (death of a close relative, divorce, or other traumatic event) while she was pregnant with you? ☐
(0)No; (1) Yes; (9) Don't know

j. When you were born, what was your mother's occupation? _____

k. When you were born, what was your father's occupation? _____

Now I would like to know about your life as an adolescent, more or less when you were between 12 and 13 years old. Although some time has passed please try to remember about that time of your life when you were in middle school or sixth grade or seventh grade. Please provide as much information as possible.

6. Where did you live when you were between **12 and 13 years old**? (If you lived in more than one address during those years, please give us the address where you lived the longest.)

a. Address: County or district (barrio): _____

_____

b. Municipality: _____ c. Zip code: _____

d. Country: _____

e. How long did you live there? _____Months Years _____

7. When you were **12-13 years old**, the drinking water you had came from… ☐

1. Deep well
2. River
3. Surface well
4. Autoridad de Acueductos (AAA)
9. Don't know

8. When you were 12-13 years old, was your house close to a….

a. Gas station	No (0) , Yes (1), Don't know (9)	☐
b. Pharmaceutical industry	No (0) , Yes (1), Don't know (9)	☐
c. Other industries (textiles, electronics, etc.)	No (0) , Yes (1), Don't know (9)	☐
d. Power plant	No (0) , Yes (1), Don't know (9)	☐
e. Sugar industry / refinery	No (0) , Yes (1), Don't know (9)	☐
f. Waste dump	No (0) , Yes (1), Don't know (9)	☐
g. Laundry, dry cleaners	No (0) , Yes (1), Don't know (9)	☐
h. Paint and car repair shops	No (0) , Yes (1), Don't know (9)	☐
i. Commercial agricultural areas	No (0) , Yes (1), Don't know (9)	☐
j. Waste water plant	No (0) , Yes (1), Don't know (9)	☐
k. Others: _____		☐

9. Now we would like you to remember the foods that you consumed when you were **12-13 years old**. Answer as accurately as possible.

9a. When you were **12-13 years old** did you eat/ drink**…?**	**9b. How frequent were you accustomed to eat/ drink…?**
Bread or Soda Crackers (white or whole grain) ☐ Yes (1) No (0), (**Jump to next item)**	(0) Rarely (1) Weekly (2) Daily ☐
Hot cereals (oatmeal, cream of wheat, etc.) ☐ Yes (1) No (0), (**Jump to next item)**	(0) Rarely (1) Weekly (2) Daily ☐
Milk, including when consumed in the cereal ☐ Yes (1) No (0), (**Jump to next item)**	(0) Rarely (1) Weekly (2) Daily ☐
Coffee with milk ☐ Yes (1) No (0), (**Jump to next item)**	(0) Rarely (1) Weekly (2) Daily ☐
Fruits, natural fruit juice or real fruit snow cones (no syrup or artificial fruit drinks) ☐ Yes (1) No (0), (**Jump to next item)**	(0) Rarely (1) Weekly (2) Daily ☐
Eggs ☐ Yes (1) No (0), (**Jump to next item)**	(0) Rarely (1) Weekly (2) Daily ☐
Rice ☐ Yes (1) No (0), (**Jump to next item)**	(0) Rarely (1) Weekly (2) Daily ☐
Beans/legumes (Red=1, Pink=2, Pinto=3, Black=4, White=5, Chick peas=6, pigeon peas=7, lentils=8) ☐ Yes (1+ *bean code that she consumed the most*.) No (0), (**Jump to next item)**	(0) Rarely (1) Weekly (2) Daily ☐
Chicken, turkey ☐ Yes (1) No (0), (**Jump to next item)**	(0) Rarely (1) Weekly (2) Daily ☐
Beef steak, pork, pork chops ☐ Yes (1) No (0), (**Jump to next item)**	(0) Rarely (1) Weekly (2) Daily ☐
Cured ham, sausage, luncheon meat, bacon ☐ Yes (1) No (0), (**Jump to next item)**	(0) Rarely (1) Weekly (2) Daily ☐
Fish (fried filet, tuna, sardines -not salt-cured codfish) ☐ Yes (1) No (0), (**Jump to next item)**	(0) Rarely (1) Weekly (2) Daily ☐
Starchy tubers/Plantain, tannier (not fried)) ☐ Yes (1) No (0), (**Jump to next item)**	(0) Rarely (1) Weekly (2) Daily ☐
Salad (lettuce & tomatoes) ☐ Yes (1) No (0), (**Jump to next item)**	(0) Rarely (1) Weekly (2) Daily ☐

9a. When you were **12-13 years old** did you eat/ drink**…?**	**9b. How frequent were you accustomed to eat/ drink…?**
Vegetables (not starchy tubers or "viandas") Yes (1) (go to 9b, ask frequency) No (0), (**Jump to next item)** ☐	(0) Rarely (1) Weekly (2) Daily ☐
Sodas, sugar-sweetened beverages (colas/soda, sugarcane juice) Yes (1) (go to 9b, ask frequency) No (0), (**Jump to next item)** ☐	(0) Rarely (1) Weekly (2) Daily ☐
Fried Foods (fried plantain, tannier fritters, fried dumplings, turnover, -not French fries) Yes (1) (go to 9b, ask frequency) No (0), (**Jump to next item)** ☐	(0) Rarely (1) Weekly (2) Daily ☐
Salted Codfish salad with tubers Yes (1) (go to 9b, ask frequency) No (0), (**Jump to next item)** ☐	(0) Rarely (1) Weekly (2) Daily ☐
Heavy soup (rice based with chicken, pigeon peas, or shrimp) Yes (1) (go to 9b, ask frequency) No (0), (**Jump to next item)** ☐	(0) Rarely (1) Weekly (2) Daily ☐
Meat Stew Yes (1) (go to 9b, ask frequency) No (0), (**Jump to next item)** ☐	(0) Rarely (1) Weekly (2) Daily ☐
Fast Food (Hamburger, French fries, fried chicken, Tacos) Yes (1) (go to 9b, ask frequency) No (0), (**Jump to next item)** ☐	(0) Rarely (1) Weekly (2) Daily ☐
Cake, Flan, cookies, donuts Yes (1) (go to 9b, ask frequency) No (0), (**Jump to next item)** ☐	(0) Rarely (1) Weekly (2) Daily ☐
Ice Cream /milkshakes Yes (1) (go to 9b, ask frequency) No (0), (**Jump to next item)** ☐	(0) Rarely (1) Weekly (2) Daily ☐

9c. How much did you weigh when you were **12 -13 years old**: __lbs. ___kg ___don't remember [**If the participant cannot remember the exact weight tell her the following:** *Even if you do not remember your exact adolescent weight, could you say that you where about* …]
__ Average, ___less than average (skinny), ___more than average (chubby)
__ Don't know / cannot estimate.

10. Where have you <u>lived the longest</u> in your lifetime? [*In which place did you live or have lived most of your <u>adult life</u>.*] (**If you do not know the complete address please provide as much information as possible**.)

a. Address: County or district (barrio): _____
_____
b. Municipality: _____c. Zip Code: _____
d. Country: _____
e. How long did you live there? _____ Years; _____Months
f. Years at that address? From 19_____ to _____

11a. Have you taken vitamins, minerals, or drugs during the last 12 months? ☐
(0) No **(Jump to question 12)**; (1) Yes; (9) Do not remember **(Jump to question 12)**.

If the answer is Yes, ask with what frequency does she take them (every day or less) and the quantity (number of pills or units)? (*Use pictures / models to help interviewee to remember*)

11b. In the last 12 months have you taken any of the following: **Medicines, vitamins or minerals?**	**(0) No (9)Don't know (1)Yes→**	**Frequency**		**Quantity you take**	
		Every day (2)	**Sometimes (3)**	**# pills**	**Units (mg, µg, IU.)**
Multi-vitamins: _____ (Centrum, One-a-Day, Stress tab, Therapeutics, Theragran-M)					
Vitamin C					
Vitamin E					
Vitamin B12					
Calcium					
Vitamin D					
Folic acid					
Other vitamins minerals or supplements: (Vitamin A, Beta Carotene, Iron, Selenium, Zinc, Magnesium, Omega 3)					
Aspirin (Bufferin, Ecotrin, Bayer)					
Acetaminophen (Tylenol, Panadol)					XX
Ibuprofen (Advil, Motrin)					XX
Other NSAIDS (Naproxen, Aleve, Naprosyn, Daypro, Celebrex, Vioxx, Indocin, Clinoril)					XX
Antidepressives (*Cymbalta, Zoloft, Paxil, Prozac)* Anti-anxiogenic (Ativan, Xanax)					XX
Statins: Drugs that lower cholesterol like atorvastatin (lipitor), fluvastin (lescol), rosuvastitin (crestor), pravastatin (pravachol), lovastatin (mevacor), simvastatin (zocor))					XX
Others:					

11c**. Antibiotics**: Have you taken antibiotics for **six consecutive months or more for a condition**? ☐
(0)No; (1) Yes; (9) Do not remember

If you have taken them, when was the last time? ___ ___ ___ ___ (Year)

For how long? _____months _____years

For what reason did you take antibiotics? _____

Which one? (if you took more than 1 antibiotic, please indicate the one you used the longest time) _____

Part II. Menstrual, reproductive and weight history

Read to participant

Now I will be asking you some questions about your menstruation and if you have been pregnant.

12. How old were you when you had your first menstruation (period)? ☐☐
(*If you don't remember, please tell me the best you can recall.*)
___ ____age __ ___ do not remember (99)

13. When was your last menstrual period? (Please mention the first day, month and year)
Day _____ Month _____ Year ___ ___ ___ ___

14. Has your menstruation stopped for **at least one (1) year**? ☐
(0) No (jump to question 16); (1) Yes **(jump to question 14a)**; (9) doesn't know (jump to question 16)

14a. what was the reason? ☐☐
1. Surgery (hysterectomy) – **(jump to question 15)**.
2. Naturally, age (menopause or change of life) – **(jump to question16)**.
3. Radiation or chemotherapy – **(jump to question16)**.
4. Other (specify) – **(jump to question16)**.
9. Doesn't know – **(jump to question16)**.
10. Doesn't want to answer – **(jump to question16)**.

15. When the surgery or hysterectomy was performed, how many of your ovaries were removed? ☐☐
(If the participant doesn't remember or doesn't understand the question you can say. *Generally when both ovaries are removed, the doctor prescribes hormones*.)
1. No ovaries were removed; just the uterus was removed
2. Yes, 2 ovaries and the uterus were removed
3. Yes, 1 ovary and the uterus were removed
9. Doesn't know
10. Does not want to answer

{Interviewer: Show the illustrations of the different types of medications. Interviewer Manual page --}

16. Have you ever used estrogen, progesterone, or other feminine replacement hormones during menopause (during or after your change of life) or for menopause symptoms? ☐
(0) No **(jump to question 17);** (1) Yes **(go to question 16a)**; (9) Don't know **(jump to question 17)**

16a. Since when did you start taking hormones?

Month ☐☐ Year ☐☐☐☐ (9) Don't remember ☐

[**If the participant cannot remember the exact date, *ask her how old she was.***] Age ☐☐

16b. What types of hormones have you used? ☐
(1) Only estrogen (Specify the dose _____)
(2) Only progesterone (Specify the dose _____)
(3) Estrogen and progesterone (Specify the dose _____)
(9) Don't know

{Interviewer: Show the illustrations of the different types of hormones. Interviewer Manual page --}

16c. Are you taking hormones at this moment? ☐

(0) No **(go to question 16d);** (1) Yes **(jump to question 17)**; (9) Don't know **(jump to question 17)**

16d. For how long did you take hormones? ☐

(1) Months (Specify how many months _____)
(2) Years (Specify how many years_____)
(9) Don't remember

17. Have you ever been pregnant? (Include any pregnancy, spontaneous abortion (miscarriage), ectopic pregnancy, abortions, etc.) ☐

(0) No **(jump to question 19);** (1) Yes **(go to question 17a)**

17a. How many times have you been pregnant? Number of pregnancies ☐☐

18. Please answer the following questions about each pregnancy (a to j), then go to the next pregnancy.

Items	Pregnancy							
	1	2	3	4	5	6	7	8
18a. How old were you when your pregnancy ended? __ __ age ; (99) Don't know ***If participant states a date -instead of the age- for the end of the pregnancy, enter date*** (__day/___month/___year)								
18b. What was the outcome of the pregnancy? __ 1=Baby was born alive 2= Multiple babies were born alive 3= Baby was born dead (still-born) 4=Spontaneous abortion (miscarriage)**, go to next pregnancy** 5=Abortion**, go to next pregnancy** 6=Ectopic pregnancy**, go to next pregnancy** 7 = Other _____ 9=Don't know, **go to next pregnancy**								
18c. How many months did this pregnancy last? __ __months or __ __ ***weeks*** **If she doesn't remember ask***:* 77=Premature (less than 9 months) 88=Completed term (9 months) 99=Don't know								
18d. How much weight did you gain during this pregnancy? __ __ # pounds she gained **;** 99=Don't know								
18e. Did you have nausea during this pregnancy?_ 0=No 1=Yes, during the first trimester 2= Yes, during the first 2 trimesters 3= Yes, during the all of the pregnancy 9=Don't know								
18f. Did you have gestational diabetes during this pregnancy? __ 0=No ;1=Yes ; 9=Don't know								
18g. Did you have high blood pressure or preeclampsia during your pregnancy?__ 0=No ;1=Yes ; 9=Don't know								
18h. What was the sex of this baby? __ 1=Male; 2=Female; 9=Don't know **(If the baby was still-born, GO TO next pregnancy or to 19**.)								
18i. Did you breastfeed the baby?___ 0=No, **go to next pregnancy or to question 19** 1=Yes 9=Don't know								
18j. For how many months did you breastfeed the baby? __ _ months or ___weeks; 99=Don't know								

18. (Cont.) Please answer the following questions about each pregnancy (a to j), then go to next pregnancy.

Items	Pregnancy							
	9	10	11	12	13	14	15	16
18a. How old were you when your pregnancy ended? _ __age ; (99) Don't know ***If participant states date – instead of the age- for the end of the pregnancy, enter***(_ day ___month ____yr)								
18b. What was the outcome of the pregnancy? __ 1=Baby was born alive 2= Multiple babies were born alive 3= Baby was born dead (still-born) 4=Spontaneous abortion (miscarriage)**, go to next pregnancy** 5=Abortion**, go to next pregnancy** 6=Ectopic pregnancy**, go to next pregnancy** 7 = Other _____ 9=Don't know, **go to next pregnancy**								
18c. How many months did this pregnancy last? __ __ months __ __ weeks **If she doesn't remember ask***:* 77=Premature (less than 9 months) 88=Completed term (9 months) 99=Don't know								
18d. How much weight did you gain during this pregnancy? __ __ number of pounds she gained **;** 99=Don't know								
18e Did you have nausea during this pregnancy?__ 0=No 1=Yes, during the first trimester 2= Yes, during the first 2 trimesters 3= Yes, during the all of the pregnancy 9=Don't know								
18f. Did you have gestational diabetes during this pregnancy? __ 0=No ;1=Yes ; 9=Don't know								
18g Did you have high blood pressure or preeclampsia during your pregnancy?__ 0=No ;1=Yes ; 9=Don't know								
18h What was the sex of this baby? __ 1=Male; 2=Female; 9=Don't know **(If the baby was still-born, GO TO next pregnancy or to 19**.)								
18i Did you breastfeed that baby? __ 0=No, **go to next pregnancy or to question 19** 1=Yes 9=Don't know								
18j For how many months did you breastfeed the baby? _ _ months; _ _ weeks; 99=Don't know								

19. Have you ever had a mammography?

___No (0) **(Jump to question 21**) ___Yes (1) ☐

20. a. What was the date of your last mammography?
_____/_____/_____
(day) / (month) / (year)

b. Do you have (keep) the mammography you had within **the last year?**
___No (0) ___Yes (1) _____Don't remember (9)

Interviewer: Write the age of the participant here (____) and use it to know (flag) when to finish question 21, that is, after getting to the age group of the participant

21. How much did you weigh when you were (____/Age)? If you were pregnant or breast feeding at that age, tell us how much you weighed a year before. For example, if you were pregnant or breast feeding a baby at age 20, then tell us how much you weighed when you were 19 years old.

21a. 20 years old Weight: _____lbs. or ___Kg. ___ Don't know

21b. 30 years old Weight: _____lbs. or ___Kg. ___ Don't know

21c. 40 years old Weight: _____lbs. or ___Kg. ___ Don't know

21d. 50 years old Weight: _____lbs. or ___Kg. ___ Don't know

21e. 60 years old Weight: _____lbs. or___Kg. ___ Don't know

21f. 70 years old Weight: _____lbs. or ___Kg. ___ Don't know

22. How much did you weigh a year ago? ***(*If the participant doesn't remember you can say *12 months ago; or during _____ a year ago on the same month of this interview***) If during this month you were pregnant or breastfeeding your baby, how much did you weigh a year before being pregnant?

Weight: _____lbs. or _____Kg. __999_ Don't know

Part III. Eating habits. Write in the box, the number that best indicates the frequency of food consumption. **Use the illustrations to estimate portion size.**
Read to participant
In this section we're going to ask about your eating habits. PLEASE try to remember and answer as accurately as possible.

23. Please tell us about the food and the portions that you consumed during the last 12-24 months. **(The interviewer can help by providing exact date from this month, back to a year ago: for example, during 1 year ago, from (write month _____of last year]**. Indicate how many times per month, week, or day you consumed the foods that we are going to read and if the portions were **S** (small), **M** (medium) or **L** (large) in comparison with the example illustrated. These questions do not include what you eat in fast food places. ***(Relocate portion after column for frequency).**

Cereals/Grains	**Portion**			**How many times did you consume it 12 MONTHS AGO? (Write the number of times in the box)**			
	S	**M**	**L**	**Never/ Rarely**	**Each Month**	**Each Week**	**Each day**
White bread							
Whole grain bread							
Soda crackers							
Hot Cereals: oatmeal, cream of wheat, corn meal, corn starch							
Cold boxed cereals							
White rice							
Yellow tubers (sweet potato, celery root, ripe plantain							
White tubers (potato, tannier, cassava, yam, taro root, ñame); breadfruit, green plantain							
Pasta: spaghetti, lasagna, elbow macaroni							

Vegetables/Legumes	**Portion***			**How many times did you consume it 12 MONTHS AGO? (Write the number of times in the box**			
	S	**M**	**L**	**Never/ Rarely**	**Each Month**	**Each Week**	**Each day**
Beans (red=1, pink=2, pinto=3, black=4, white=5; no=0) ***Indicate type of bean you consumed most frequently: __ code bean.***							
Legumes/peas (chick peas=1, pigeon peas=2, lentils=3, no=0) ***Indicate type of legume you consumed most frequently: __ code bean.***							

Vegetables/Legumes	**Portion***			**How many times did you consume it 12 MONTHS AGO? (Write the number of times in the box**			
	S	**M**	**L**	**Never/ Rarely**	**Each Month**	**Each Week**	**Each day**
Squash (yellow, winter),							
Dark leafy greens (Romaine lettuce, spinach, watercress)							
Other lettuce (iceberg, local PR lettuce)							
Cabbage							
Tomato							
Avocado – In season							
Cucumber (not pickles)							
Bell peppers, sweet peppers (green, red, yellow)							
Carrots							
Green beans							
Mixed vegetables							
Corn							
Sweet green peas							
Beets							
Eggplant							
Broccoli							
Cauliflower							
Mushrooms							
Christophine / prickly pear (“chayote”)							
Other: Okra=1, Asparagus=2, celery=3, radishes=4. *Indicate type of legumes you consume most frequently__*							

Meats and substitutes	**Portion***			**How many times did you consume it 12 MONTHS AGO? (Write the number of times in the box**			
	S	**M**	**L**	**Never/ Rarely**	**Each Month**	**Each Week**	**Each day**
Chicken							
Turkey							
Pork (pork roast, pork chops, pork loin)							
Beef (beefsteak, ground beef, etc.)							
White fish (haddock, snapper, etc.)							
Fatty fish (salmon, tuna, mackerel)							
Salted-cured codfish; salted, smoked herring							
Shrimp, lobster							
Tripe							
Lunchmeat (salami, bologna, ham, turkey, roast beef)							

Meats and substitutes	Portion*			How many times did you consume it 12 MONTHS AGO? (Write the number of times in the box			
	S	M	L	Never/ Rarely	Each Month	Each Week	Each day
Bacon							
Corned beef , deviled ham							
Hot dogs, Vienna sausage							
Other meats: Lamb=1, rabbit=2, goat=3. *Indicate type of meat you consume most frequently __.*							
Other seafood: Sardines=1, oysters=2, clams=3, scallops=4, crab=5 *Indicate seafood you consume most frequently __.*							
Eggs							
Sunflower seeds, Peanut butter, peanuts, almonds, other nuts, seeds *Indicate type of seed you consume most frequently __.*							
Tofu							

Fruits	Portion*			How many times did you consume it 12 MONTHS AGO? (Write the number of times in the box)			
	S	M	L	Never/ Rarely	Each Month	Each Week	Each day
Bananas, “fig-banana”, “Apple-banana”							
Citrus: Orange, grapefruit, “chironja”, sweet lime							
Pineapple							
Papaya, mamey							
Mango – In season							
Grapes (red, green)							
Raisins, dried prunes							
Apple, apple sauce, pear							
Watermelon							
Cantaloupe							
Peach, nectarine							
Apricots							
Fruit cocktail							
Plums							
Guava							
Passion fruit							
Strawberries							
Blueberries, blackberries, raspberries cranberries							

Fruits	**Portion***			**How many times did you consume it 12 MONTHS AGO? (Write the number of times in the box)**			
	S	**M**	**L**	**Never/ Rarely**	**Each Month**	**Each Week**	**Each day**
Other: Cherries, sour-sop, níspero, kiwi, "custard-apple", genip ("quenepas"), tamarind, "cherimoya/corazón" West Indian cherry. *Indicate type of fruit you consume most frequently __.*							

Dairy	**Portion***			**How many times did you consume it 12 MONTHS AGO? (Write the number of times in the box)**			
	S	**M**	**L**	**Never/ Rarely**	**Each Month**	**Each Week**	**Each day**
Milk (Whole milk=1, 2% milk=2, 1% milk=3, skimmed=4. *Indicate type of milk you consume most frequently __.* UHT (box)=1, powdered milk=1, evaporated milk=3. *Indicate type of milk you consume most frequently __.*							
Processed cheese (American, Velveeta, CheeseWhiz, etc.)							
Block or grated cheese (Edam, Swiss, Gouda, Cheddar, Mozzarella, etc.)							
Yogurt							
Cottage cheese							

Desserts	**Portion***			**How many times did you consume it 12 MONTHS AGO? (Write the number of times in the box)**			
	S	**M**	**L**	**Never/ Rarely**	**Each Month**	**Each Week**	**Each day**
Caramel custard							
Bread pudding							
Cake							
Ice cream							
Cookies							
Donuts							
Coconut cornstarch pudding, rice pudding							
Other desserts: Pie, "Tres-leches"cake, Cheesecake							
Candy bars with chocolate							
Other candy (non-chocolate): hard candy (bon-bons)							

Beverages / Drinks	Portion*			How many times did you consume it 12 MONTHS AGO? (Write the number of times in the box)			
	S	M	L	Never/ Rarely	Each Month	Each Week	Each day
Black coffee							
Coffee with milk							
Hot chocolate							
Hot Tea (herbal)							
Brewed tea (black or green)							
Natural juices (100% juices, fresh or canned)							
Fruit drinks (Kool-aid, Suiza fruit, Sunny Delight, powdered Iced-tea)							
Soda (Coke, Pepsi, 7-up etc.)							
Diet soda (Zero, Diet Coke, Diet Pepsi, etc.)							
"Malta " (non-alcoholic malt beverage)							
"Maví" (local root beer)							
Do you drink alcoholic beverages such as beer, wine, or mixed drinks? No ___ Yes____ Which one you prefer? __ Cuba libre __ Sangría __Torongin __Piña Colada __Martini ___Other: _____ _____							

Seasonings/Condiments	Portion*			How many times did you cook or consume food that were prepared with these condiments 12 MONTHS AGO? (Write the number of times in the box)			
	S	M	L	Never/ Rarely	Each Month	Each Week	Each day
Commercial and homemade "Sofrito" (pepper, onion, garlic, sweet pepper, tomatoes, condiment/salt, etc.)							
Garlic, onion (not in "sofrito")							
Cilantro, wide leaf cilantro, Chinese parsley (not in "sofrito")							
Orégano, bay leaves, cumin							
Tomato sauce (not in "sofrito")							
Lime / lemon/ lemon juice							
Salad dressing							
Olives							
Dried seasonings							
Mayonnaise							
Ketchup							
Other: Hot sauce=1, Mustard=2, Ají =3 (hot peppers). *Indicate type you consume most frequently* __.							

24. What type of oil or fat do you use **for frying** food?

____ Corn oil/Vegetable oil ____ Olive oil

____ Canola oil ____ Lard

____ Butter ____ Margarine

____ PAM (Non-stick cooking spray)

25. What type of oil or fat do you use **for cooking** (not frying) food? (Ex. Cooking rice)

____ Corn oil/Vegetable oil ____ Olive oil

____ Canola oil ____ Lard/Fat-back ("tocino")

____ Butter ____ Margarine

____ PAM (Non-stick cooking spray)

26. In the last 12 months, with what frequency did you go out to a **fast food** establishment (not a full service restaurant) to consume the following foods?

Type of fast food	**Portion***			**How many times did you consume it 12 MONTHS AGO? (Write the number of times in the box)**			
	S	**M**	**L**	**Never/ Rarely**	**Each Month**	**Each Week**	**Each day**
Fried chicken (Church's, KFC)							
Chicken roast / grilled							
Hamburger (Burger King, McDonald's)							
Pizza (Pizza Hut, Sbarro, Domino's, Papa John's, Little Caesar's)							
Chinese food (Panda Express)							
Mexican food (Taco Bell, Taco Maker)							
Fried fish (Long John Silvers', fish sandwich MacDonald's)							
Other							

27. In the last 12-24 months, with what frequency did you consume the following foods?

Traditional foods/ mixed dishes	**Portion***			**How many times did you consume it 12 MONTHS AGO? (Write the number of times in the box)**			
	S	**M**	**L**	**Never/ Rarely**	**Each Month**	**Each Week**	**Each day**
Salted codfish salad with tubers							
Heavy soup (rice-based)/ "asopao"							
Meat and vegetable stew							
"Pasteles"							
Tannier fritters/ "alcapurrias"							
Salted codfish fritters							
Ripe plantain and meat casserole ("pastelón")							
Potato & meat casserole ("pastelón de papa" ~similar to Shepherd's Pie)							
Other: Fried meat pie ("empanadilla" _____ _____							

Part IV. Exposure to Tobacco: <u>Read to participant</u>
I would like to ask you some QUESTIONS about YOUR SMOKING HABITS AND EXPOSURE TO TOBACCO or TO CIGARETTE SMOKE

28. Have you ever smoked cigarettes, pipe or cigar in your life? ☐
No (0) **(Jump to question 30)**; Yes (1); Don't know/Doesn't want to (9)
(Jump to question 30)

29. Have you smoked at least 100 cigarettes or 5 packs of cigarettes in your life? ☐
No (0) **(go to question 30)**; Yes (1); Don't know/Doesn't want to answer (9)
(go to question 30)

29a. At what age did you start smoking? _____ (age in years) ☐

29b. Do you actually smoke ? ☐
No (0); Yes (1) **(go to question 30;** Don't know/Doesn't want to answer (9)

29c. Since when did you quit smoking? _____ (month/year) ☐

Part V. Physical Activity: Before beginning these questions on physical activity you must write down the current age of the participant in the table where you will stop and move to the next question.

<u>Read to participant</u>

In this section we will be asking you questions about your physical activity during some moments in your life. We can remember if we were very active or less active when we were children, adolescents or young adults because we can relate this to where we used to live, or the school we went to, or the tasks we did at home or at work during those years.

We would like to ask you to help us understand the type of physical activities that women in Puerto Rico do. For this reason we will ask you questions about the physical activity that you used to do at six years old and at other moments of your life. PLEASE answer as accurately as you can remember. Think if these activities were done during the week, during the weekend, only during some days in particular o if they were daily activities. Also remember if you did these activities for a short time or for many hours during the week.

You can tell us about exercise or sports that you practiced such as lifting or moving heavy objects, aerobic exercise, swimming, volleyball, etc.; or recreational activities such as dancing, playing ball games, riding bicycle, etc. You can also tell us about physical activities related to daily living at home or workplace such as sweeping, cleaning windows, mopping/wiping, cutting the grass, picking up leaves, etc. We will also ask you about the hours or minutes per week that you spent brisk walking or that you walked briskly to school, to work, or to go shopping or visiting friends or how much time you spent walking for exercise.

Age	Interviewer: please describe in detail the physical activities reported	**Duration / Frequency of physical activities** **0 = never** **1** = <3 hrs./week **2 = 3 a 6 hrs.//week** **3** = ≥7 hrs./ week
30a. 6-11 years old (childhood)		
30b. 12-17 years old (adolescence)		
30c. 18 - 25 years old (young adult)		
30d. 26 - 40 years old (adult)		
30e. 41-55 years old		
30f. 56-64 years old		

30g. ≥65 years old		

31. Currently, on average, how many hours or minutes per week do you walk briskly? _____hours (If participant answers in minutes, record here:___minutes) (Access program should convert hours to minutes for final data output in minutes)

32. Please indicate which physical activity scenario on this list you have done mostly during your life (Select all that apply) _____:

1) Sports
2) Activity related to work
3) Activity inside the house
4) Activity in the yard
5) Recreational

Part VI: Read to participant (Write down age of participant where you should finish the questions). In this section we will be asking questions on your exposure to the SUN.

33. On average, how much time do you spend outside, during the daylight hours, during the ages listed below. Give us your best estimate. (*Interviewer, write down the amount given by interviewee*)

Age	**Less than 30 minutes (1)**	**30 minutes to 2 hrs (2)**	**More than 2 hours (3)**
33a. 6-11 years old			
33b. 12-17 years old			
33c. 18-25 years old			
33d. 26-40 years old			
33e. 41-55 years old			
33f. 56-64 years old			
33g. 65 years old or more			

34. Do you have freckles on your back? ___No (0) ___Yes (1) Don't remember (9)____

35. Do you burn easily when exposed to the sun in the afternoon?
___No (0) (jump to 34) ___Yes (1), my skin turns red Don't remember (9) (**jump to 34**) ____

36. If you are repeatedly exposed to the sun, does your skin bronze or get dark? (Instructions)
No (0) ____, or Don't know (9)_____
Yes (1) ____, my skin gets **very dark**, dark bronze.
Yes (2) ____, my skin gets **somewhat dark**.
Yes (3) ____, my skin gets **light bronze, light brown**

Part VII. Personal and family history of chronic diseases

Read to participant:

Now I would like to ask you to tell me about the illnesses that you have had or currently have. PLEASE ANSWER AS ACCURATELY AS POSSIBLE.

37. Have **you been diagnosed** by a doctor with any of the following illnesses?

Diagnosed illnesses	**No (0)**	**Don't know (9)**	**Yes (1) →**	**Age when diagnosed**
37a. Diabetes (not during pregnancy)			Did you use insulin? ___yes ___no	
37b. High or elevated blood sugar or urine				
37c. Childhood asthma (before age 14)				
37d. Adult asthma (after age 14)				
37e. Eye or nasal allergies				
37f. Skin allergies				
37g. Benign breast illness (non cancerous): tumors, fiber-tumors, or mastitis				
37h. Skin cancer				
37i. **Any other type of cancer**, (not skin cancer), please describe: Breast CA (1) (Flag: inclusion criteria =case) Other cancer (2) (Flag: exclusion criteria =control)				
37j. Polycystic Ovarian disease				
37k. Thyroid illness (for example: hyperthyroidism, hypothyroidism).			__hyperactive __hypoactive	
37l. At any time have you received treatment for parasites?			___yes ___# of times	

38 Has anybody **in your family** been diagnosed by a doctor with any of the following illnesses? (include your parents, and if you have brothers, sisters or children)

Diagnosed Illness	**No (0)**	**Don't know (7)**	**Yes (1)**	
38a. Skin cancer			**Relationship**	**Age when diagnosed**
38b. any other type of cancer, that is not skin cancer:_____			**Relationship**	**Age when diagnosed**

Part VIII. Other demographic information

Read to participant:

Sometimes we need to contact participants to ask additional questions. We would like to know if you would give us information that may allow us to contact you.

39. What is your Social Security number? [***Interviewer: If the participant shows concern, let them know that these questions will be kept confidential. If they don't want to answer skip this question***.]

__ __ __ __ __ ___ ___ ___ ___

40. Please give us the name of a family member or friend, that doesn't live with you, that could help us contact you in the event we were unable to:

Name: _____

Address: _____

_____

Telephone number: Area Code (__) _____

Relationship: _____

Mother's last name: _____

Father's last name: _____

41a. It is helpful to have information about where your parents and grandparents were born. Can you tell us where your mother was born?_____

On your mother's side of the family:

Place of birth of your grandmother: _____

Place of birth of your grandfather: _____

42b. Can you tell us where your father was born?_____

On your father's side of the family:

Place of birth of your grandmother: _____

Place of birth of your grandfather: _____

Part IX. Read only to participant that has been diagnosed with breast cancer.

We are almost finished but you mentioned (in question 35i) that you had been diagnosed with a breast tumor or a mass. We will like to know about this and possible treatments or illness.

43. Have you made any changes in your regular diet as reported earlier, in the past two years?

No (0) _____ Yes and the changes are the following (1) _____

_____

_____

_____

_____

44. Have you made any changes in your consumption of supplements (vitamins, minerals) as you reported earlier, in the past two years?

No (0) _____ Yes and the changes are the following (1) _____

_____

_____

_____

_____

45. Have you had any of the following treatments? (Mark as many as applicable)

____ Breast biopsy (No=0; Yes=1)
____ Mastectomy (Removal of all of the breast) (No=0; Yes=1)
____ Conservative breast surgery (Surgery without complete removal of breast, lumpectomy, or partial mastectomy (No=0; Yes=1)
____ Breast implant (No=0; Yes=1)
____ Breast remission surgery (No=0; Yes=1)
____ Surgery in the lymph nodes under the arm (No=0; Yes=1)
____ Radiation treatment for breast or chest (No=0; Yes=1)
____ Chemotherapy (No=0; Yes=1)

____ Don't know/Not sure (7)
____ doesn't want to answer (9)

46. Are you taking or have taken tamoxifen? (Hormonal preventative therapy)

___ No (0) ___ Yes (1)
___ Did take for some time, not taking now (3)
___ Considering the option but hasn't started yet (4)
___ Don't know/Not sure (7)
___ doesn't want to answer (9)

47. Are you taking or have taken aromatase inhibitors (i.e., Nolvadex)? [*Aromatase inhibitor is an enzyme that modifies the hormones and the inhibitors of the enzymes reduce the risk of cancer*] [Verify generic names and have pictures available].

___ No (0)
___ Yes (1)
___ Did take for some time, not taking now (3)
___ Thinking of taking; hasn't started yet (4)
___ Don't know/Not sure (7)
___ doesn't want to answer (9)

Read to participant

THESE ARE ALL OF THE QUESTIONS FOR THIS SURVEY. THANK YOU FOR LETTING US INTERVIEW YOU. YOUR PARTICIPATION IN THIS STUDY IS OF GREAT HELP. THANKS AGAIN. (*Now cordially say goodbye and demonstrate your satisfaction with the cooperation of the participant*).

ONLY FOR INTERVIEWER

IN THIS SECTION DESCRIBE THE CONDITIONS OF THE INTERVIEW

1. Interview ended at: _____ a.m. _____ p.m.

2. Answer each one:

Question	YES 1	No 0
2a. Participant appeared to be alert through the interview		
2b. Participant appeared to understand the questions		
2c. Participant appeared to be tired during some point in the interview		
2d. There were interruptions during the interview		
2e. The length of the interview was 30 minutes, as expected		

3. Code (internal code)/ 1=Case 2=Control

UNIVERSIDAD DE PUERTO RICO, RECINTO DE CIENCIAS MÉDICAS
UNIVERSITY OF PUERTO RICO, MEDICAL SCIENCES CAMPUS

OFICINA DEL RECTOR
OFFICE OF THE CHANCELLOR

COMITÉ DE DERECHOS HUMANOS (IRB)
INSTITUTIONAL REVIEW BOARD

Date: May 08, 2009

Protocol Number: 0750108

Principal Investigator: Dr. Cruz M. Nazario Delgado

Department / Division: School of Public Health - Epidemiology

Sponsor:

Title: ***Breast Cancer Epidemiology in Puerto Rico***

This is to certify that the above referenced research proposal/protocol was evaluated on **May 08,2009**and meets **expedite** IRB review category. The research proposal was **approved.**

This action involves:

- ☐ New proposal/project
- ☐ Waiver of Consents
- ☐ Continuing Review of Previously Approved Protocol
- ☒ Protocol Amendment #1
- ☐ Amendment
- ☐ Adverse Events
- ☐ Serious Adverse Events

The following documents were reviewed under this submission:

- ☒ Protocol
- ☐ Assent Document
- ☒ Informed Consent Document English and Spanish Version Main 4/6/09 and donors 3/27/09
- ☐ Letter of Amendment
- ☐ Survey Instrument
- ☐ Package Insert
- ☐ Advertisement
- ☐ Human Subject Certified
- ☐ Investigator Brochure
- ☐ Authorization Letter
- ☐ Informative Sheet
- ☐ Curriculum Vitae
- ☐ HIPAA Certified
- ☐ FDA #1572
- ☐ Others:

In compliance with federal regulations, the approval for this study is valid through: **March 04, 2010**
For additional information please contact Human Research Subjects Protection Office at 787-282-0010 or 787-282-0018; e-mail opphi@rcm.upr.edu

Cordially,

Alan Preston, PhD
Chairperson IRB 1

bcg

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1. Research must be conducted according to the proposal that was approved by the IRB.
2. Changes to the protocol or its related consent document must be approved by the IRB prior to implementation.
3. All serious or unexpected adverse events/drug reactions should be reported.
4. Each subject should receive a copy of the consent document, if appropriate.
5. Records must be retained for at least three years.
6. Any future correspondence should include the IRB identification number provided and the study title.

---------- Forwarded message ----------
From: Wilberding, Julie A Dr CTR USA MEDCOM USAMRMC <
Julie.Wilberding@amedd.army.mil>
Date: Mon, Jun 22, 2009 at 11:58 AM
Subject: **FW: A-14268.a, HRPO Approval Memorandum (Proposal Log Number BC060131, Award Number W81XWH-07-1-0329) (UNCLASSIFIED)**
To: Cruz M Nazario <cruz.nazario@upr.edu>

Classification: UNCLASSIFIED
Caveats: NONE
Please let me know if you receive this. See below:
-----Original Message-----
From: Duchesneau, Caryn L Ms CIV USA MEDCOM USAMRMC
Sent: Monday, June 15, 2009 1:32 PM
To: 'Cruz M Nazario'
Cc: Miller, Theresa J Dr DoD Af USA MEDCOM CDMRP; Bennett, Jodi H Ms CIV USA MEDCOM USAMRMC; Duchesneau, Caryn L Ms CIV USA MEDCOM USAMRMC; Brosch, Laura R Dr CIV USA MEDCOM USAMRMC; Kline, Andrea J Ms CIV USA MEDCOM USAMRMC; Wilberding, Julie A Dr CTR USA MEDCOM USAMRMC; Baker, Wendy A Ms CIV USA MEDCOM USAMRAA; 'nperez@rcm.upr.edu'; Drake, Carrie EMs CTR USA MEDCOM USAMRMC
Subject: A-14268.a, HRPO Approval Memorandum (Proposal Log Number BC060131, Award Number W81XWH-07-1-0329)
SUBJECT: Initial Approval for the Protocol, "Breast Cancer Epidemiology in Puerto Rico," Submitted by Cruz M. Nazario, PhD, University of Puerto Rico, San Juan, Puerto Rico, Proposal Log Number BC060131, Award Number W81XWH-07-1-0329, HRPO Log Number A-14268.a

1. The subject protocol (dated 27 March 2009) was approved by the University of Puerto Rico Medical Sciences Campus Institutional Review Board (IRB) on 8 May 2009. This protocol was reviewed by the U.S. Army Medical Research and Materiel Command (USAMRMC), Office of Research Protections (ORP), Human Research Protection Office (HRPO) and found to comply with applicable Federal, DOD, U.S. Army, and USAMRMC human subjects protection requirements.
2. This no greater than minimal risk study is approved for the enrollment of 1000 subjects.
3. Please note the following reporting obligations:
   a. Major modifications to the research protocol and any modifications that could potentially increase risk to subjects must be submitted to the USAMRMC ORP HRPO for approval prior to implementation. All other amendments must be submitted with the continuing review report to the HRPO for acceptance.
   b. All unanticipated problems involving risks to subjects or others, serious adverse events related to study participation, and deaths related to study participation must be reported promptly to the HRPO.
   c. Any deviation to the subject protocol that affects the safety or rights of the subject and/or integrity of the study data must be reported promptly to the HRPO.
   d. All modifications, deviations, unanticipated problems, adverse events, and deaths must also be reported at the time of continuing review of the protocol.
   e. A copy of the continuing review report approved by the University of Puerto Rico Medical Sciences Campus IRB must be submitted to the HRPO as soon as possible after receipt of approval. It appears the next continuing review by the University of Puerto Rico Medical

Sciences Campus IRB is due no later than 4 March 2010.

f. In addition, the current version of the protocol and consent form (if applicable) must be submitted along with the continuing review report and the University of Puerto Rico Medical Science Campus IRB approval notice for continuation of the protocol.

g. The final study report submitted to the University of Puerto Rico Medical Science Campus IRB, including a copy of any acknowledgement
documentation and any supporting documents, must be submitted to the HRPO as soon as all documents become available.

4. Do not construe this correspondence as approval for any contract funding. Only the Contracting Officer or Grants Officer can authorize expenditure of funds. It is recommended that you contact the appropriate contract specialist or contracting officer regarding the expenditure of funds for your project.

5. The HRPO point of contact for this study is Julie Wilberding, PhD, Human Subjects Protection Scientist, at 301-619-6766/julie.wilberding@us.army.mil.

CARYN L. DUCHESNEAU, CIP
Chief, Human Subjects Protection Review
Human Research Protection Office
Office of Research Protections
U.S. Army Medical Research and Materiel Command

Note: The official copy of this approval memo is housed with the protocol file at the Office of Research Protections, Human Research Protections Office, 504 Scott Street, Fort Detrick, MD 21702. Signed copies will be provided upon request.

Classification: UNCLASSIFIED
Caveats: NONE

PROTOCOL

I. Title: Breast Cancer Epidemiology in Puerto Rico

II. Personnel

A. Minority Institution

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III. Study Locations

University of Puerto Rico, **-FWA00005561 (10/23/2010)**
School of Public Health, Office A463 & A462
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P O Box 365067, San Juan PR 00936-5067

Core Laboratory:
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University at Buffalo- **FWA00008824**
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270 Farber Hall
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Roswell Park Cancer Institute
Elm and Carlton Streets
Buffalo, NY 14263
(716) 859-2092

IV. Background

Puerto Rico is a particularly interesting place for breast cancer research, because the incidence rate of breast cancer in Puerto Rico is about half that in the United States, but the risk of this disease among women in Puerto Rico is increasing at a faster rate than it is in the United States (1). The rapidly increasing rates are likely indicative of changes in the environment with resulting variability that can be exploited to better understand risk factors. In particular, there is likely to be more variation in early life exposures which will allow for less misclassification of exposures that are inherently difficult to measure. Further, historically low breast cancer rates in Puerto Rico are interesting in that a number of adverse risk factors are more common for women in Puerto Rico than in the United States. Among the 52 US states and territories, Puerto Rico has one of the highest prevalence of overweight and obesity, and lowest physical activity profiles (2). We want to understand how risk factors operate for this understudied Hispanic population of the United States. In addition, the racial mixture in the Puerto Rican population may provide an opportunity to better understand the observed differences in disease incidence and prognosis by race in the United States. Puerto Ricans have European, African, and Native American ancestry; the variation in racial admixture can generate greater variation, again allowing for the development of hypotheses that could lead to the elucidation of genetic risk factors and gene-environment interactions in future studies. As noted above, study of

genetic factors is beyond the scope of the current study given its size. However, this study could serve as the basis for larger studies that would include the examination of ancestry informative markers in relation to risk as well as the examination of genetic susceptibility in other pathways. Finally, there are aspects to the diet of Puerto Ricans that are of interest. These include different consumption patterns of legumes as well as other foods that are high in antioxidants. There are hypotheses that these foods are protective but to study them in most other US populations is difficult because consumption is low. Nevertheless, there have been few studies of breast cancer epidemiology in low risk populations

There are indications that exposures in early life, as early as pre-natal exposures, have significance in the etiology of breast cancer (3). It has been hypothesized that the ductal tissue in the breast is more sensitive to environmental insults such as endocrine disruptors while *in utero* when there is terminal differentiation of the ductal end buds (4). Research has focused on indicators such as birth weight, birth in a pregnancy with pre-eclampsia or eclampsia as indicators of hormonal exposure *in utero* since it appears that increased exposure to estrogens increases risk (4). Further, there is some evidence, although inconsistent, that having been breast fed is related to a decrease in risk (5). In animal studies, there is evidence that the timing of intake of a nutritional factor may affect subsequent response to a carcinogenic insult (6, 7). Genistein, a phytoestrogen found in soy, if given before puberty, has been shown to decrease mammary carcinogenesis in animal models, up-regulating EGF with increased cell differentiation (8).

Breast cancer risk is modified by food intake and body weight. But there are only sparse data on diet in childhood and breast cancer risk; there are studies that indicate that childhood diet, particularly fat intake (9) and soybeans (10, 11) may impact subsequent breast cancer risk. There is no evidence regarding the effect of intake of other, non-soy legumes. There is considerable evidence that adult height is determined, at least in part by childhood diet; and there is relatively consistent evidence that height is related to increased risk of breast cancer (12-14). There is also evidence that low weight in adolescence and high growth velocity may be related to increase subsequent breast cancer risk (13, 14). There is a small number of case control studies of childhood diet and breast cancer risk, most of them were conducted in the United States. The foods examined and the findings from these studies are inconsistent (15-18). There have been several studies of breast cancer among women who were adolescent during the WWII time of famine in Europe. These studies provide some indication that adult breast cancer risk was affected by dietary restrictions during their childhood (19-23). It has been hypothesized that increased exposure to estrogens during adolescence may enhance breast tissue differentiation and may be protective (7); the observations of decreased risk associated with high adolescent BMI and with soy phytoestrogens are consistent with this hypothesis. Better understanding of the role of infant, childhood and adolescent diet may be important in understanding nutrition in the etiology of breast cancer. Examination of a population with greater variability in dietary intake can provide insight into the role of early dietary exposures.

In this study we will address the relationship between dietary intake during different time periods of female participants as well as early exposures (*in utero*). While it is difficult for women to recall their past intakes, there is evidence of fairly good relative validity and reliability for recalled food intake from adult participants reporting intake during childhood (24-27). Further, we expect that with greater variability in intake of a population in dietary transition, it might be possible to recall broad categories of intake with less misclassification. An important study objective is to develop an instrument that is sensitive, culturally appropriate, and reliable as well as capable to motivate recall from participants.

Endocrine disruptors are environmental substances that appear to have the ability to interfere with endogenous hormonal processes. Some of the compounds that are suspected to be endocrine disruptors include: insecticides, herbicides, plasticizers such as the phthalates, and other industrial compounds such as cadmium, lead, mercury, styrene, dioxin and polychlorinated biphenyls (28). Exposure to these substances has been suggested to play a role in the etiology of breast cancer. Recently, research efforts have focused on considering exposures to a mixture of these compounds, since low levels of single estrogen-like pollutants can act together contributing to the internal estrogenic load (29, 30). Exposure to such substances may be relevant even at low levels, especially when occurring at vulnerable stages of development (i.e. pre-natal exposure, pre-adolescence, etc). For instance, a study conducted in PR found phthalate esters levels in serum of young Puerto Rican girls diagnosed with thelarche (prepubertal breast development in girls less than eight years of age) to be significantly higher than in the control group, suggesting a possible association between these estrogenic compounds and premature breast development (31).

We have also examined other environmental factors in relation to breast cancer risk in western New York and find that exposures in early life may relate to risk (32, 33). Using residence location as a proxy for environmental exposure and using publicly available data on environmental exposures (particulates in air and traffic exhaust as proxy measures for polycyclic aromatic hydrocarbons (PAHs), we found that women who lived in areas with greater exposure to higher levels of PAHs were at higher risk of breast cancer. These associations were limited to exposures in childhood: at the time of birth and at menarche (32). Physical activity has been associated with risk reduction for various cancers, including breast cancer.

Physical activity is defined as movement produced by the activation of skeletal muscle that increases energy expenditure above resting levels (34). It can be classified by three major domains: occupational, household, and leisure. For this study we propose to use a modified version of the lifetime total physical activity questionnaire (35).
We propose to conduct a population-based case-control study of breast cancer in PR that would examine in-depth: diet (recent and in childhood), physical activity (lifetime including childhood), lifetime energy balance (including adult weight gain and BMI at different periods of life), other measures related to early life exposures (height, birth weight, residential history) and environmental exposures (PAHs, endocrine disruptors) and reproductive history.

V. Objectives / Specific Aims and Research Questions:

The objectives are:

To design a reliable, sensitive, and culturally appropriate questionnaire.

To identify and interview 500 women aged 30-79, with incident, primary, histologically confirmed breast cancer cases, who are residents of San Juan, Bayamón, and Guaynabo municipalities around the Metropolitan area (Annex A: Map) and to collect biological samples (blood or saliva sample preferably before treatment) for analysis and long-term storage, pathological information from the Puerto Rico Central Cancer Registry as well as interview data from the participants to test study hypothesis.

To identify and interview 500 women aged 30-79, as population controls, who live in the same geographic area as the breast cancer cases and are free of cancer (except non-melanoma skin cancer) and to collect biological samples (blood or saliva samples) for analysis and long-term storage, as well as interview data from the participants to test study hypothesis.

To design and implement a training plan that will provide hands on experience for the University of Puerto Rico (UPR) minority institution scientists in the design and conduct of breast cancer research. In addition, to facilitate their participation in lectures, conferences and courses that will extend their research skills.

To design future studies, with larger sample size, that will allow researchers from the UPR to further explore and test hypotheses that arise from this pilot study. Such studies could examine genetic factors, ancestry informative markers in relation to breast cancer risk; elucidate gene-environment interactions as well as examining genetic susceptibility in other pathways.

We hypothesize that factors associated with more traditional lifestyles will be less common in breast cancer cases than in controls. We propose to conduct a case control study of breast cancer among Hispanic women in Puerto Rico to examine the hypotheses that the frequency of those factors differs between cases and controls. Because this study will be small, it will only provide pilot data for some of the questions of interest.

Research questions:

1) Identify dietary risk factors in Puerto Rican women that differ between breast cancer cases and controls. We hypothesize that factors associated with more traditional diet (e.g., phytoestrogens and antioxidants, fruits and vegetables, folate, carotenoids, as well as other nutrients and bioactive food components) will be less common in cases than in controls. We will examine adult and childhood dietary factors, both at the macro and micronutrient level. We will examine risk factors in relation to breast cancer and also in relation to tumor characteristics (e.g., estrogen and progesterone receptor status). We will examine these dietary risk factors in relation to genetic variability for genes related to the metabolism of the dietary factors of interest.

2) Examine factors related to breast cancer risk and early life exposure, including *in utero*, such as birth weight, having been breastfed, birth in a pregnancy with pre-eclampsia or eclampsia, and residential history since birth. We hypothesize that early life exposure patterns will be different among cases and controls. Women who during early childhood and adolescence lived in areas with greater exposure

to PAHs (such as air and traffic exhaust exposures), endocrine disruptors and these factors related to *in utero* environment will differ in relation to risk of breast cancer. We will do a preliminary examination of these risk factors in relation to genetic variability for genes related to their metabolism.

3) Evaluate lifetime energy balance (e.g., BMI, adult weight gain, weight history, height, sitting height, etc.) and lifetime physical activity, and usual adult and childhood physical activity (e.g., type of activity, time spent on vigorous and moderate physical activities, etc.) among breast cancer cases and controls. We hypothesize that lifetime exposure patterns to physical activity and energy balance will be associated with breast cancer risk. We will do a preliminary examination of energy balance and physical activity in relation to genetic variability for genes related to their metabolism.

4) Examine other established risk factors for breast cancer (e.g., reproductive history, age at menarche and menopause, age at first full-term pregnancy, parity, personal and family history of diseases, and use patterns of vitamin, minerals, hormones, and medication, etc.) in this low risk population. We hypothesize that in spite of the ecological differences in population characteristics and disease trends that the associations of these factors will be the same or similar to what has been observed in other, better studied populations. We will do a preliminary examination of these risk factors in relation to genetic variability for genes related to the pathways of interest. We hypothesize that the differences in distribution of genetic variants in this population will provide insight into the mechanism for the observed associations.

VI. Research Design

Type of study

This is a population-based case-control study of breast cancer in Puerto Rico. Breast cancer cases will be compared to populations controls to estimate the magnitude of the associations (OR), and to test exploratory hypotheses.

Study Variables

1) Diet: usual frequency and quantity data for fruits and vegetables, cereals, dairy, meat, desserts and sweets, fats and oils use for cooking, as well as condiments use for seasoning, beverages including alcohol and traditional food items will be collected. Participants will also be asked frequency and type of traditional food items that study participants consumed during pubertal time period (12-13 years of age). Past and usual dietary intake will be assessed with a food frequency questionnaire (FFQ). In particular we will identify food items which contribute to the total intake of and to variance in the intake of the nutrients and bioactive food components that are the focus of the planned study.

2) Lifetime energy balance (anthropometry, BMI, height, adult weight gain). We will measure actual body weight and height using a balance beam scale and stadiometer, waist circumference using an anthropometric tape. We will also measure sitting

height. Skin fold measurements will be also collected. We will collect history of body weight (participants' body weight since birth) with a group of standardized questions in the personal interview.

3) Lifetime physical activity, both usual adult and childhood. Physical Activity (PA) is defined as movement produced by the activation of skeletal muscle that increases energy expenditure above resting levels. It can be classified by three major domains: occupational, household, and leisure. For this study we will use a modified version of the lifetime total physical activity questionnaire. The questionnaire will include information regarding the type, frequency, duration, and intensity of recent usual and lifetime household, occupational and leisure time physical activity.

4) Residential history: We will ask each woman about her address at the time she was born, at the time of her menarche and the address where she lived for the longest time as an adult. We will geocode residence information and combine it with publicly available information on other environmental exposures to estimate exposure to environmental factors such as polycyclic aromatic hydrocarbons and endocrine disruptors.

5) Reproductive history, hormonal, early life exposures: We will collect information on age at menarche and menopause (natural or due to surgery), history of pregnancies and lactation, as well as hormone use. We will ask the participant to recall if she was breast fed and if her mother had suffered eclampsia during the pregnancy from which she was born.

6) Personal and family history of diseases, vitamins, mineral, and medication use: We will ask about personal history of diseases such as allergies, infections, diabetes, asthma, cancer, fibrocystic and polycystic disease, and thyroid problems. We will also ask the participant to report on intake of vitamins, mineral, antibiotics, NSAIDs, aspirins, drugs for blood cholesterol and antidepressants consumption. Sun exposure patterns, as source of pre-vitamin D will also be collected in the questionnaire. Two skin pigmentation measurements will be obtained using a colorimeter to estimate facultative skin pigmentation –exposed to solar radiation (dorsal hand) and constitutive pigmentation –not exposed to solar radiation (underarm) (36). Family history of cancer will also be asked.

7) Socio demographic information: We will collect age, marital status, last completed school grade, place of birth of parents and grandparents. Since we hope to extend the study to allow for addressing additional questions regarding participant morbidity and mortality, participants will be asked to provide social security number, her address and a relative's address for assistance in contacting her in the future, if necessary. Participants will be informed that they can refuse to answer particular questions that they consider sensitive without any negative consequence.
8) Biological information: We will collect a fasting (wherever possible) blood sample. Blood samples (three tubes) will be aliquot to tubes of 0.5 ml for serum plasma, red blood cells and buffy coat at the Clinical Research Center at the Medical

Sciences Campus of the UPR (CRC-MSC) and sent to Roswell Park Cancer Institute for DNA extraction and long term storage. If the participant can not provide a blood sample, a saliva sample could be collected. The biological sample will be stored for future analysis for only those participants that provide consent. Samples will be ID coded with scannable bar codes. Using this DNA, we will examine genetic variation in relation to study variables (diet, physical activity, reproductive history, etc.). We will examine genetic variation in relation to metabolic pathways of interest. In addition, we will examine ethnic ancestry variation in relation to breast cancer risk. Further, because the field of genetics and cancer is changing so rapidly, we will examine other variants that are identified as of interest in breast carcinogenesis. We will not be studying highly penetrant variants such as BRCA1 or BRCA2. Rather, the focus will be on more common variants that may interact with other exogenous factors in breast carcinogenesis.

At the end of the interview, the interviewer asks the participant about cancer diagnosis. If she indicates that she has been diagnosed with breast cancer cases, she will be asked to sign a separate consent to allow us to collect a sample of her tumor block, if possible. We will not collect tumor samples at this time; however we want to leave access to tumor tissue as an option for future research. Neither incidental findings nor DNA test results will be given to individual participants. Pathological information will be abstracted from the Puerto Rico Central Cancer Registry database.

9) Treatment information: Breast cancer cases will be asked if they had treatments such as breast biopsies, surgery, radiotherapy and chemotherapy. She will also be asked about breast implants. Specific treatments such as tamoxiphen and aromatase inhibitors will be recorded.

Methods to obtain a sample of volunteers (N/A)

Group assignment (N/A)

Reliability of psychometric measures (N/A)

VII. Study Population:

The target population is women in Puerto Rico. Study Base: The study population will be women aged 30-79 who are current residents of San Juan, Bayamón, and Guaynabo, municipalities around the Metropolitan area. Included as breast cancer cases will be 500 women with incident, primary, histologically confirmed breast cancer diagnosed within one year of the time of interview. Controls will be 500 women of the same age, living in the same geographic area with no previous history of cancer other than non-melanoma skin cancer.

VIII. Inclusion/Exclusion Criteria:

Inclusion criteria: Participants will be female aged between 30 and 79 years. This age group is appropriate in that it is the group of women at the highest risk of breast cancer and accounts for 85% of all breast cancer cases in Puerto Rico.

Cases - women aged 30-79 with incident, primary, histologically-confirmed breast cancer who are residents of the municipalities described in Section VII of the Protocol, with no previous history of cancer other than non-melanoma skin cancer. DCIS cases will also be included; we will conduct separate analysis to determine whether they should be included in analyses with the invasive tumors.

Controls - women aged 30-79 with no previous history of cancer other than non-melanoma skin cancer who are residents of the municipalities described in Section VII of this Protocol.

Exclusion Criteria:

Women who have had cancer other than non-melanoma skin cancer will be excluded. We excluded women older than 79 because in our experience it is difficult for many older female cases to participate in epidemiological studies. The study validity can be reduced if the response rate of older participants is low. Breast cancer risk is extremely low among women less than 30 years of age. There are no ethnicity exclusions in this study, but those that live outside of the municipalities described in Section VII of this Protocol will be excluded to comply with the population-based design of this study. Women (cases or controls) who can not consent due to mental illness or physical limitations will be also excluded.

IX. Description of the Recruitment Process

The Project Coordinator (JH) and the Clinic Patient Coordinator from the Clinical Research Center at the Medical Sciences Campus (CRC-MSC) will synchronize the most appropriate appointment time for the participant's visit to the CRC-MSC. If the participant is willing to participate but refuses to travel to the CRC-MSC clinic, study personnel (interviewer and nurse) will conduct the interview and collect specimens in the participant's home.

Recruitment Process for Breast Cancer Cases

Cases will be ascertained from the Puerto Rico Central Cancer Registry (PRCCR), a population-based cancer registry that has been collecting data (diagnosis and treatment reports, demographic data, etc.) from all cancer cases diagnosed and treated in Puerto Rico since 1950. In 1951, it was mandated by law, that all cancer data collected by pathologists, therapists, surgeons, physicians and other health professionals be reported to the PRCCR. This Cancer Law also stated that collected cancer data should be used for surveillance and research initiates (Annex B: PRCC letter). The PRCCR is supported by funds from the government of Puerto Rico and the Center for Disease Prevention and Control (the National Program of Cancer Registries (NPCR)). The process for the identification of cancer cases from the cancer registry is detailed in the letter of support from Dr. Nayda Figueroa-Vallés, Director of the PRCCR. Her recommendations are as follows:

PRCCR staff will meet on a regular basis (weekly) with study staff to evaluate pathology reports of newly diagnosed (primary and not a recurrence or a metastasis) breast cancer cases. The pathological information will be obtained from the PRCCR, and if required from the medical record. The PRCCR designated personnel will verify that the case is a new breast cancer case and our study personnel will evaluate other inclusion criteria requirements (age and residential address). The breast cancer patients' name and primary physician (or referring physician) will be collected from the PRCCR databank. The primary physician will be contacted (first by letter from Project's PIs) and followed by a telephone call) to request authorization to contact the breast cancer patient. If the primary physician presents no objection, the patient will be contacted by study personnel to invite her to participate, verify eligibility and to schedule an appointment for the study. A brief description of the research project (study objectives, procedures, etc) and pertinent information (study location and name, telephone, and address of personnel, etc.) will be provided.

Recruitment Process for Controls

Controls will be women aged 30-79 with no previous history of cancer other than non-melanoma skin cancer who are also residents of the municipalities described in Section VII of this Protocol. A population sample of women living in these municipalities will be obtained from the *Estudio Continuo de Salud* (ECS), a National Health Survey in Puerto Rico, conducted by the School of Public Health, University of Puerto Rico for the Puerto Rico Department of Health. The ECS is based on a multistage sampling probabilistic procedure of geo-political conglomerates (cities & municipalities) as defined by the US Bureau of the Census. The primary sampling unit is the census block and the secondary sampling units are household segments. The database of the ECS contains the address and the survey information of all members of the household selected as part of a population sample. ECS will provide a list of households with potential controls from the municipalities described in Section VII: Study Population. This strategy will provide the population-based design of this study with a representative sample of the population from where the cases are obtained.

The Project Coordinator (JH) and the Project PI (CMN) will evaluate the list of households provided by ECS. The list of potential controls will be given to a trained study staff who will contact the potential control. If more than one female lives in the house, one will be selected based on age of the potential participant and the needs of the study for frequency matching of controls to the cases that we are ascertaining. The study staff will determine if they meet the study inclusion and exclusion criteria and invite them to participate in the study. If the woman is interested, study personnel will schedule an appointment for the study with her.

X. Description of the Informed Consent Process

Trained study personnel [Project Coordinator (JH) or the project Research Assistant (MB)] will explain that during the visit to the CRC-MSC she will get detailed information on the research project. If she agrees to participate and consents, she will be interviewed and biological samples will be taken along with other bio-measurements.

A trained interviewer will receive the potential participant at the CRC-MSC facility. The study interviewer (SN) will be the person responsible to explain the research project in detail, review the potential participant's eligibility for the study, answer questions and obtain the informed consent. This process will be conducted in one of the CRC-MSC private examination rooms. Because of rapid changes in cancer epidemiology we want to have the opportunity to test new hypothesis and examine other variants in breast cancer carcinogenesis in future studies. Therefore, since biological samples could be used in future studies, consent to store and use samples for future studies will also be requested by the interviewer. Participants may choose not to provide a biological sample but she can still participate by completing the questionnaire. Additionally, a participant may elect not to have their sample stored for future use. In that case, a sample will be collected only for short term storage and analyzed in this study. The participant will have time to read the informed consent and ask questions before deciding whether or not to participate. If the potential participant chooses to discuss the study with relatives or other study personnel she will be provided with a copy of the informed consent document and a new appointment will be scheduled.

In addition, only for breast cancer cases, at the end of the general interview, a separate consent form will be explained and offered to allow us to collect tumor block tissue. We will not access those blocks at this time, but we want to leave this as an option for future research.

The participation in this study is completely voluntary. The entire group of participants for this study must have full capacity to consent. If the participant is illiterate, the interviewer will read and explain the consent to her in the presence of a witness. If she decides to participate in the study, she will sign the consent with her signature or will mark with an X. The witness will also sign.

Because the study will be of women in Puerto Rico, consent forms and all study documents will be written in Spanish, the vernacular of the target population. All study documents, including the consent form and the study questionnaire will be translated and back-translated by bilingual study personnel to verify the consistency of Spanish and English versions. If the participant prefers, the English version of the informed consent and questionnaire will be used.

XI. Volunteer Screening Procedures (N/A)

XII. Study Procedures/Research Interventions

This study will not include experimental interventions. The study will include collection of biological samples (blood or saliva samples), anthropometric measurements and a face-to-face interview.

To answer research questions regarding breast cancer risk in Puerto Rico we plan to conduct a population-based case-control pilot study that will explore differences between breast cancer cases and controls regarding the following factors: diet (past year and during childhood), physical activity (during different periods of life since childhood),

lifetime energy balance (including adult weight, skin folds measures, adult weight gain, birth weight, and BMI during different periods of life), early life and lifetime exposures (birth weight, being breastfed, height, residential history), personal and family history of disease, reproductive factors (age at menarche, menopause, use of hormones), lifestyles (sun exposure history and differences in skin color measured with a colorimeter, use of vitamins and medications, tobacco and alcohol consumption) and environmental exposures (PAHs and endocrine disruptors). We will also examine genetic variation in relation to these risk factors.

Potential breast cancer cases will be identified from the Puerto Rico Central Cancer Registry and potential controls will be identified from a list provided by Estudio Continuo de Salud. Potential participants will be approached by study personnel and they will invite them to participate in the study and ascertain their eligibility as described in Section IX.

The study interviewer (SN) will receive the potential participant at the Clinical Research Center- Medical Sciences Campus (CRC-MSC) and will obtain the informed consent. This process will be conducted in one of the CRC-MSC private examination room, as described in Section X. If the participant signs the consent form, a trained research nurse from the CRC-MSC will collect the blood sample. If study participant is not willing to donate blood, but is willing, a saliva sample can also be obtained to collect DNA information. The nurse will measure the participant's height, weight, sitting height, waist circumference, skin folds and skin color. Once this process is completed, the participant will receive a small snack. The interviewer will then conduct the general interview with a structured questionnaire.

The biological samples (blood or saliva) will be processed and stored for a short time at the CRC-MSC and then shipped to Roswell Park Cancer Institute, in Buffalo NY (RPCI) for DNA extraction and other analyses. For those participants that consent, the samples will be stored for future studies. All samples will be coded with a unique identifier.

All identifying information regarding study participants will be kept confidential. Hard copy information of study participants (identifying information) will be kept in locked file cabinets which only the principal investigator and project coordinator will have access to. The electronic data linking participant name with the study identifier will be password protected and accessible only to the principal investigator and project coordinator. All other data files will have only the participant unique identifying code to link to different data sets. Study results may be reported in professional journals or at scientific meetings. Results will be reported for the aggregate of appropriate study groups and individuals in the study will not be identified in any way. Analysis of genetic variation will be limited to common variants and will not include rare, highly penetrant mutations.

XIII. Description of Protocol Drugs or Devices. (N/A)

XIV. Laboratory Evaluations.

Specimens to be collected:

All blood draws will be done on participants in the fasting state, between 7 AM and 9AM, whenever possible. If the participant has eaten, that information will be recorded. Blood samples (3 tubes) will be processed according to the protocols developed previously for another case control study of breast cancer. For participants who are unwilling or unable to provide blood specimens, saliva samples can be obtained to allow for genotyping. Samples will be aliquoted to tubes of 0.5 ml for serum, plasma, red blood cells and buffy coat will be stored in -80ºC freezers. Computerized storage maps will be developed based on our existing programs. The blood draw and processing of samples will be conducted by a trained research medical technician at the CRC-MSC. So that there is minimal variability in time between blood draw and blood processing, all specimens will be aliquoted into 0.5ml tubes and frozen as soon as possible for banking. Aliquots will be shipped in batches to RPCI for DNA extraction and long term storage. Shipment of samples will be on dry ice using a 24-hour delivery service. Each sample will be assigned a unique identifier for banking. This ID number will be on all tube labels, with scannable bar codes.

Evaluations to be made:

Blood specimens will be used to examine case control differences in genotype. Using this DNA, we will examine genetic variation in relation to other study variables (diet, physical activity, reproductive history, etc.). We will examine genetic variation in relation to metabolic pathways of interest. Further, because the field of genetics and cancer is changing so rapidly, we will examine other variants that are identified as of interest in breast carcinogenesis. We will focus on more common variants that may interact with other exogenous factors in breast carcinogenesis. We will not be studying highly penetrant variants such as BRCA. In addition, other assays such as blood vitamins or other metabolites may be on interest for future research. Incidental findings or DNA test results will no be given to individual participants.

Storage:

Biological specimens (blood and saliva) will be stored in -80º C freezers at Roswell Park Cancer Institute. All samples will be identified with bar codes that provide information on the unique study identifier for that participant as well as indicating the sample type (e.g., serum, plasma). Samples will be analyzed for this study and stored for future studies. Included in the consent will be the information as to the future us of these samples. Participants will indicate on the consent whether they are willing to allow for this further use. If they do not consent to participate in future studies, the collected biological samples will be for this study only and they will not be stored for future studies. We will maintain a list of such participants so that their biological samples will be destroyed at the end of the study.

XV. Sample Size Justification.

The proposed pilot study has the power to examine a limited number of hypotheses that are of considerable interest in breast cancer epidemiology. The unique aspects of this understudied population make this a particularly strong research plan. While there are

biases inherent in case control studies which cannot be avoided, primarily recall bias, there is evidence that the amount of bias may not be great. Every effort will be made to make the interview situation the same for cases and controls. The expected high rates of response in this population are important; selection bias is not likely. There is a trend in epidemiology at this time to discard the case control design, a trend that we find disturbing. The case control study is an elegant and efficient means to explore exposure-disease associations. In particular, when response rates are high and the study is carefully designed, this study design allows for investigations in less-studied populations. Further the variability in exposure will improve our ability to examine risk factors in spite of misclassification. We will be examining both well-established risk factors in a population where they have not been well studied. Particularly important is the study of some novel hypotheses about diet and early life exposures in relation to breast cancer risk and the likelihood that this study can provide some insight into these hypotheses.

Minimum detectable risks (MDR) are shown in table 1, for 500 cases and 500 controls, with a two-sided p-value set at 0.05 and power of 0.80. In addition, MDRs are shown for data stratified on menopausal status, assuming about one third of women will be premenopausal and the remainder postmenopausal. Odds ratios on the order of 1.5 to 2.0 are detectable. Only for premenopausal women with an exposure present in 80% of controls was the MDR 2.64.

This is a pilot study and will have limited power to examine interactions, particularly gene-environment interactions. As indicated above, our intention is to eventually expand this study so that we can look in more detail at the study hypotheses and to examine the interactions that are of interest in this population.

Table 1. Minimum Detectable Risks (MDR) for Puerto Rican Breast Cancer Study, total sample and stratified on menopausal status.

Exposure among controls	MDR, total sample	MDR, premenopausal women	MDR, postmenopausal women
0.2	1.54	2.08	1.69
0.4	1.44	1.90	1.57
0.6	1.46	1.99	1.60
0.8	1.64	2.64	1.87

XVI. Data Analysis.
In this case-control study of breast cancer, the primary outcome will be breast cancer. Cases will be limited to incident, primary, pathologically-confirmed cases. Cases with ductal carcinoma in situ (DCIS) will be included; we will also run analyses excluding those participants with DCIS to determine whether results differ.

The exposures of interest are the dietary intake variables, physical activity at different points in the lifetime, weight change during adulthood and body mass index during life at critical time periods (e.g., decades of life, at menopause), birth weight, height, and environmental exposures such as PAHs. All models will be examined adjusting for age and education. In addition, the other known risk factors for breast cancer will also be considered as possible confounders. These would include age at menarche (primarily for premenopausal breast cancer), age at first birth, parity, body mass index, history of lactation, history of breast cancer in a first degree relative, history of benign breast disease, and for the postmenopausal women, age at menopause and history of use of postmenopausal hormone replacement therapy. For analyses of dietary variables, models including adjustment for energy will be examined. For all analyses, effect modification by menopausal status will be considered. If there is effect modification, analyses will be stratified.

Distributions of all exposure variables and other covariates will be compared between cases and controls. Summary statistics will be evaluated; means will be compared with the Student's t-test and categorical variables compared with the chi-squared test. Correlations among the variables will be calculated. Unconditional logistic regression will be the primary modeling tool for this case-control study with frequency-matched controls.

XVII. Data Management.

Data collection:

The information for this study will be collected through a face to face interview (structured questionnaire) and the pathological information for breast cancer cases will be obtain from the Puerto Rico Central Cancer Registry database. Biological (blood or saliva) sample, and anthropometric measurements will be collected by a trained nurse. Since this is not a follow-up study, participants will not be followed further.

Each blood sample and all interview data will be assigned a unique numeric code identifier (scannable bar codes). Hard copies of information connecting participant identifying information with the ID codes will be kept in locked file cabinets. Electronic files with identifying information will be password protected. Only the study personnel who require this information will have access to these files. All results will be published or discussed in conferences for aggregated data, no information will be included that would reveal the participant identity.

Researchers from Roswell Park Cancer Institute, and the University at Buffalo will work with the University of Puerto Rico investigators on data collected for this study. In general, they will not have access to participant identifiers unless there is a scientific need for such access in which case there will be access only as required with care to protect participant confidentiality. Also authorized representatives of the U.S. Army Medical Research and Material Command can check the files as part of their responsibilities to protect human subjects in research. Data will be stored indefinitely. There are no plans to dispose of the data. Included in the consent will be information as to the future use of the biological samples. Participants will indicate on the consent whether they are willing to

allow for this further use. If they do not agree to participate in future studies the biological samples will be collected and used in this study but they will be disposed according to lab procedures for such specimens and they will not be stored for future studies.

XVIII. Risks/Benefits Assessment.

This study presents no personal risk since it does not involve any drug or clinical treatment. The procedure to collect the blood sample is simple and the risk to participant health is minimal. The blood draw will be done by a trained phlebotomist. Blood drawing is not very risky, but a bruise at the site of the blood draw, inflammation of the vein, or infection may occur. Extreme care will be taken to prevent these risks. Most questions in the questionnaire are not sensitive and there are just a few questions that could be considered as moderately sensitive (i.e., abortions). A trained interviewer (female) will conduct the interview of study participants. Particular emphasis has been placed by training and supervision of the interviewer in order to minimize the potential risk during the administration of the questionnaire. Participants will be assured that they can refuse to answer any question that makes them uncomfortable without any negative consequence. The female interviewer will be trained to minimize discomfort during the administration of the questionnaire.

In the event of physical and/or mental injury resulting from this research study, the participant will receive medical treatment free of change at the University Hospital or any other hospital designated by the Chancellor or the Medical Sciences Campus of the University of Puerto Rico. The University of Puerto Rico has no plans to provide any form of compensation directly to the participant.

The only other possible risk is the loss of confidentiality of information provided in the interview. As indicated above, we will make every effort to ensure that there is no breach of the confidential information provided. We have considerable combined experience in the conduct of epidemiologic studies and will put all needed mechanisms into effect to protect confidentiality.

The participant does not receive any personal benefit from this study. Nevertheless, the participation in this study is very important because it will help us expand the knowledge on the factors that affects the risk of developing breast cancer among women living in Puerto Rico. The results of this study will increase our knowledge about how diet, physical activity, and hormone metabolism are related to breast cancer. Breast cancer is the most frequently diagnosed cancer among women in Puerto Rico and the largest cause of cancer death among Puerto Rican women.

XIX. Study Personnel.

Minority Institution

Cruz M. Nazario, PhD, Principal Investigator and Project Director:
The minority institution will identify study participants, conduct the interviews, collect data and draw blood for consenting females. Dr Nazario will be responsible for the organization, direction and management of the project, and all phases of the design and conduct of the research in the selected sites with the collaboration of Dr J Freudenheim (Primary Collaborating Mentor). Dr Nazario will have a primary role in the design of the survey questionnaire, questionnaire pre-testing and in the analysis and interpretation of the qualitative and quantitative data. Along with Dr. Freudenheim, she will be responsible for the development and conduct of the training of the interviewers and the development of all necessary documents pertaining to the training and data collection. Dr. Nazario will collaborate with the co-PIs in sample design, data management, statistical analysis of data, and implementation of the analytical process. As PI from the HBCU/MI institution, she will conduct regular meetings with Dr. Freudenheim and the co-PIs. Dr. Nazario will be responsible for meetings with the consultants and will have a major role in report writing, publications, and presenting results at scientific meetings and health care agencies.

Farah A. Ramirez-Marrero, PhD, Co-PI: will take charge of the research area of physical activity and breast cancer. She will participate in the design and validation of questions to collect data on physical activity that will be incorporated in the questionnaire.

Michelle Schelske-Santos, PhD, Co-PI: will take leadership on the part of the study related to diet and cancer, working closely with Drs Nazario and Freudenheim. She has collaborated with epidemiologists as part of her training in nutrition and most of her work has been laboratory-based.

Imar Mansilla-Rivera, PhD, Co-PI: will take leadership on the part of the study related to environmental exposures and breast cancer risk. She will also participate in all the planning and implementation of this large population-based epidemiologic study.

Johan Hernández, M.P.H., Project Coordinator: will assist the PI and co-PIs, overseeing all aspects of day-to-day operations. Ms. Hernandez will oversee and keep track of the data gathering process, assist with interviewer training, supervision and quality control. She will collaborate with the PI in the planning, development and pre-testing of the questionnaire. Ms. Hernández will assist Dr. Nazario in the development of the administrative and interview procedures' manuals for the study. Prior to the survey, she will collaborate with the biostatistician in obtaining information for updating maps of selected sites and in the sample design, analysis and review of data results.

Mentoring Institution

Jo L. Freudenheim, Ph.D., Primary Collaborating Mentor:
The mentoring institution will provide scientific advice and support to the research activities conducted at the minority institution. Expert scientists at UB will be available

as consultants as particular issues arise during the research. Dr Freudenheim will facilitate and coordinate consultations between the two institutions.

Dr Freudenheim will work with the PI and the co-PIs on training, career planning and all aspects of the planned research project. She will also work with the Puerto Rican investigators on all issues related to day to day management of the study, design, data analysis and interpretation and publication of oral presentations and reports. She will meet by conference call weekly or more often to discuss issues regarding the study. She will also be in regular e-mail contact with the Puerto Rican investigators regarding all aspects of the study.

Jing Nie, Ph.D., Programmer: He will work with the PI and co-PIs in Puerto Rico in Year 1 to design a database. In the subsequent years, he will work with them in the maintenance of that database, in data cleaning and in analysis of the final data set.

Christine Ambrosone, PhD –Consultant: She will be available as needed to consult in data analysis regarding gene-environment interaction. Dr Ambrosone has extensive field experience in molecular epidemiology studies in relation to cancer risk.

Susan McCann, PhD –Consultant: Dr. McCann has broad experience in nutritional epidemiology. She will be available as needed to consult her in relation to breast cancer risk and nutrition.

Kirsten Moysich, PhD –Consultant: Dr Moysich has extensive experience in large population based-studies. She will be consulted as needed in population-based issues.

Peter Shields, MD –Consultant: Dr Shields will be consulted as needed in cancer genetics issues.

Conflict of Interest.
All investigator and key study staff certify that they have no real or apparent conflicts of interest with a research sponsor or that can affect or be affected by the research.

XX. Roles and Responsibilities of Medical Monitor. (**N/A**)

XXI. Withdrawal from the Protocol.
The participation in this study is completely voluntary. If the potential participant chooses not to participate, that choice will not affect their relationship with the University of Puerto Rico, Medical Sciences Campus or their right to health care or other services to which they are otherwise entitled. If the potential participant decides to participate, she will be free to withdraw her consent and discontinue participation at any time without prejudice.

The investigator may withdraw the participant from the study if it is necessary. This decision may be made either to protect the participant health and safety.

XXII. Modifications to the Protocol.

XIII. Protocol Deviations.

XXIV. Reporting of Serious Adverse Events and Unanticipated Problems.
This study presents no personal risk since it does not involve any drug or clinical treatment. The procedure to collect the blood sample is simple and the risk to participant health is minimal. The blood draw will be done by a trained phlebotomist. Blood drawing is not very risky, but a bruise at the site of the blood draw, inflammation of the vein, or infection may occur. Extreme care will be taken to minimize these risks. Should any adverse event occurs, they would be reported following the adverse reporting protocol of the CRC-MSC and to the UPR-MSC IRB officials

"The protocol will be conducted in accordance with the protocol submitted to and approved by the USAMRMC ORP HRPO and will not be initiated until written notification of approval of the research project is issued by the USAMRMC ORP HRPO."

"Accurate and complete study records will be maintained and made available to representatives of the U.S. Army Medical Research and Materiel Command as a part of their responsibility to protect human subjects in research. Research records will be stored in a confidential manner so as to protect the confidentiality of subject information."

"All unanticipated problems involving risk to subjects or others, serious adverse events related to participation in the study and subject deaths related to participation in the study should be promptly reported by phone (301-619-2165), by email (hsrrb@det.amedd.army.mil), or by facsimile (301-619-7803) to the USAMRMC, Office of Research Protections, Human Research Protection Office. A complete written report will follow the initial notification. In addition to the methods above, the complete report will be sent to the U.S. Army Medical Research and Materiel Command, ATTN: MCMR-ZB-PH, 504 Scott Street, Fort Detrick, Maryland 21702-5012." ***Note: This language supersedes the language found on pg 18 of the protocol.***

"Any deviation to the protocol that may have an effect on the safety or rights of the subject or the integrity of the study must be reported to the USAMRMC ORP HRPO as soon as the deviation is identified."

"Major modifications to the research protocol and any modifications that could potentially increase risk to subjects must be submitted to the USAMRMC ORP HRPO for approval prior to implementation. All other amendments will be submitted with the continuing review report to the USAMRMC ORP HRPO for acceptance."

"A copy of the approved continuing review report and the local IRB approval notification will be submitted to the USAMRMC ORP HRPO as soon as these documents become available. A copy of the approved final study report and local IRB approval notification will be submitted to the USAMRMC ORP HRPO as soon as these documents become available."

"The knowledge of any pending compliance inspection/visit by the FDA, OHRP, or other government agency concerning clinical investigation or research, the issuance of Inspection Reports, FDA Form 483, warning letters or actions taken by any Regulatory Agencies including legal or medical actions and any instances of serious or continuing noncompliance with the regulations or requirements will be reported immediately to USAMRMC ORP HRPO."

XXV. Continuing Review and Final Report.
An annual review and approval is required by UPR-MSC IRB until the study period concluded. IRB approval notification will be submitted to the USAMRC ORP HRPO as soon as the UPR-MSC IRB office provides it.