1.a. Full Title: Physical Activity, Autonomic Function, and Incident Breast Cancer: ARIC

b. Abbreviated Title (Length 26 characters): PA, AF, and Breast Cancer

2. Writing Group:
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   I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. SGL [please confirm with your initials electronically or in writing]

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3. **Timeline:**

- Review IRB approval and obtain dataset: 6 months
- Data analysis: 6 months
- Manuscript preparation and submission: 6 months

4. **Rationale:**

There is substantive evidence that physical activity is inversely associated with breast cancer mortality.[1, 2] This relationship is graded (greater number of hours of activity, lower risk) and strongest in women with estrogen receptor positive tumors. Current Guidelines recommend at least 150 minutes a week of moderate–intensity activity in both healthy women and breast cancer survivors.[3, 4] Less well known is whether a change in physical activity patterns subsequently alters risk of incident breast cancer.

Physical activity can produce a beneficial effect on normal autonomic regulation of the cardiovascular system,[5] by reducing excessive sympathetic nervous system (SNS) activation in response to stress and improving the ability of the parasympathetic nervous system (PNS) to activate appropriately.[6] This results physiologically in a lower resting heart rate and a favorable effect on heart rate variability. Prior studies have demonstrated that a disruption in autonomic tone, or autonomic dysfunction (AD), is a strong predictor of cardiovascular disease mortality in women.[7] Resting heart rate, as the simplest measure of AD in healthy populations, has also been shown to predict future cancer mortality. [8] More recently, we have demonstrated that autonomic function is impaired in breast cancer patients.[9]

To date, no study has determined whether a relationship between physical activity and breast cancer mortality is mediated by autonomic function. However, such a relationship is biologically plausible. It is well accepted that lack of physical activity results in heightened sympathetic tone, resulting in excess catecholamine release. Catecholamines released into the bloodstream can modulate cell proliferation, cytokine production and migration.[10] B-adrenergic receptors, a key receptor for catecholamines, are present in mammary tissue[11] and B-adrenergic agonists stimulation mammary epithelial cell division.[12] In addition, beta-adrenergic receptor concentration is closely correlated with sex steroid hormones,[13] which is a key pathway involved in the development of mammary carcinogenesis.

We propose to study the interrelationship between physical activity, autonomic function, and breast cancer mortality in the ARIC Cohort. Specifically, we will assess the relationship between physical activity and breast cancer as well as determine whether autonomic function mediates a physical activity–breast cancer mortality relationship. We will also explore whether change in physical activity is associated with incident breast cancer and subsequent breast cancer mortality. ARIC has collected a comprehensive physical activity questionnaire that has been previously validated as well as resting, supine, 2-min beat-to-beat R-R interval data by standardized protocols for each participant.[14-16] Physical activity was obtained utilizing the same questionnaire at visit...
1-3, allowing for longitudinal assessment activity patterns. Incident breast cancer cases are currently being adjudicated by ARIC Cancer working group (PI: Elizabeth Platz). Breast cancer mortality data has been previously collected and adjudicated.

5. Main Hypothesis/Study Questions:

Objective 1: Determine an association between physical activity and breast cancer incidence and mortality.
   a. Assess differences in this relationship by race, menopausal status, and BMI category

Objective 2: Assess whether there is an association between physical activity and breast cancer and determine whether it is mediated by autonomic function.

Objective 3: Determine whether a change in physical activity is associated with breast cancer incidence and mortality.
   b. Assess differences in this relationship by race, menopausal status, and BMI category

6. Design and analysis (study design, inclusion/exclusion, outcome and other variables of interest with specific reference to the time of their collection, summary of data analysis, and any anticipated methodologic limitations or challenges if present).

Study design: prospective, observational cohort study of women without a diagnosis of breast cancer

Inclusion: participants with baseline variables, follow-up time, event status (mortality yes/no)
Exclusion: men; women who reported a cancer diagnosis other than nonmelanoma skin cancer at baseline
Outcome: incident breast cancer; breast cancer mortality (censor women at the time of death from other causes)
Baseline variables: *measures of autonomic function, **physical activity measures, age, sex, race, smoking, hypertension, use of blood pressure medication, diabetes history, body mass index, use of hormones, menopausal status, parity, age at first birth

*ARIC assessed autonomic function (AF) with a resting, supine, 2-minute beat-to-beat R-R interval. The following measures of autonomic function were collected: high frequency (HF), low frequency (LF), LF/HF ratio, very low frequency (VLF) standard deviation of all normal R-R intervals (SDNN), total power (TP), mean heart rate, and mean of the sum of squared differences (MSSD)

**Physical activity at baseline was derived from a slightly modified Baecke Physical activity questionnaire in which participants were asked to report in an open-ended format
the four sports or exercises in which they most frequently participated. They were then asked the hours per week and months per year spent in each activity. Each of the 150 reported sports and exercises was assigned a specific metabolic equivalent (MET) value according to the Compendium of Physical Activities, where 1.0 MET is considered a resting metabolic rate obtained during quiet sitting. MET values were then multiplied by the time and proportion of the year spent for a final value in units of MET-minutes per week. For comparison we also calculated the MET-minutes per week spent in moderate (3.0–6.0 METs) to vigorous (>6.0 METs) activity, as well as in vigorous activity alone.

Aim 1:

We will construct Kaplan–Meier curves to illustrate the unadjusted association between physical activity and breast cancer incidence and mortality, treating deaths as censored events. Physical activity will be treated as a continuous (1-MET change) and categorical variable (< 3 METs, 3–6 METs, >6 METs). We will use Cox proportional hazards regression analysis to estimate breast cancer mortality according physical activity, while adjusting for age (as a time-dependent covariate) as well as baseline measures of race, BMI, smoking status, hypertension, diabetes, alcohol use, hormone use in women, parity, age of first child, family history of cancer. Separate models will be explored after stratifying by race, menopausal status and BMI category.

Aim 2:

The mediation hypothesis will be tested using the Shrout et al. 2002 method for assessing statistical significance in mediation models.[17] The following simultaneous regression will be used to estimate path coefficients, a and b, representing the arms of the indirect pathway. The first path coefficient is an effect size estimated using regression of autonomic function on physical activity. The path coefficient b is effect size obtained from the Cox proportional hazard regression of breast cancer mortality on autonomic function adjusting for the physical activity. The magnitude of the indirect path is taken to be the product of the a and b coefficients, ab, the statistical significance of which will be tested using bootstrap-based 95% confidence intervals. Many methods exist to generate confidence intervals of the ab term; we favor the use of the percentile confidence interval as recent work (Fritz et al. 2012) suggests it has the best balance of type-I and type-II error.[18] This procedure will be repeated for each candidate mediator variables (e.g. nicotine dependence, craving and urges), with HL and relapse as the independent and dependent variables, respectively.

A natural logarithmic transformation will be used to normalize the distribution of the HRV frequency domain HRV indexes (HF and LF) when used as continuous variables. Pearson correlation coefficients between AF indexes will be calculated. In our primary models, HRV indexes will be entered into the models as “low HRV” versus “normal HRV” status based on the 25th percentile cut point for AF indexes.
Aim 3:

Changes in physical activity will be calculated as the difference in METs between baseline and the last examination (e.g. Exam1 - Exam 4), divided by the duration (number of years) between the two examinations. Because the intervals between those two examinations may vary among individuals in this cohort (due lack of follow-up at each exam year), we will use change in physical activity per year as our main exposure.

In addition, we will examine the associations between breast cancer incidence and mortality and changes physical activity according to specific cut-points. Participants who maintain a MET level >=3 at both baseline and the last examination will be classified as “active”; those who achieve <3 METs at both examinations will be classified as “inactive”; those who are inactive at baseline but active at the last examination will be classified as “became active”; those who were active at baseline but inactive at the last examination will be classified as “became inactive”.

Cox proportional hazard models will be used to estimate the hazard ratios (HRs) and 95% confidence intervals (95% CIs) for breast cancer incidence and mortality across changes in physical activity. Adjustments will be made for age (as a time-dependent covariate) as well as baseline measures of race, BMI, smoking status, hypertension, diabetes, alcohol use, hormone use in women, parity, age of first child, family history of cancer. Separate models will be explored after stratifying by race as well as baseline menopausal status and BMI category.

7.a. Will the data be used for non-CVD analysis in this manuscript? __x__ Yes ___ No

b. If Yes, is the author aware that the file ICTDER03 must be used to exclude persons with a value RES_OTH = “CVD Research” for non-DNA analysis, and for DNA analysis RES_DNA = “CVD Research” would be used? __x__ Yes ___ No

(This file ICTDER has been distributed to ARIC PIs, and contains the responses to consent updates related to stored sample use for research.)

8.a. Will the DNA data be used in this manuscript? ___ Yes ___x__ No

8.b. If yes, is the author aware that either DNA data distributed by the Coordinating Center must be used, or the file ICTDER03 must be used to exclude those with value RES_DNA = “No use/storage DNA”? ___ Yes ___x__ No

9. The lead author of this manuscript proposal has reviewed the list of existing ARIC Study manuscript proposals and has found no overlap between this
proposal and previously approved manuscript proposals either published or still in active status. ARIC Investigators have access to the publications lists under the Study Members Area of the web site at: http://www.cscc.unc.edu/ARIC/search.php

_____x____ Yes    _______ No

10. What are the most related manuscript proposals in ARIC (authors are encouraged to contact lead authors of these proposals for comments on the new proposal or collaboration)?

11a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data? __x__ Yes    ____ No

11b. If yes, is the proposal
    _x_   A. primarily the result of an ancillary study (list number* 2011.07)
    ____   B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list number(s)* _________ _________ _________)

*ancillary studies are listed by number at http://www.cscc.unc.edu/aric/forms/

12a. Manuscript preparation is expected to be completed in one to three years. If a manuscript is not submitted for ARIC review at the end of the 3-years from the date of the approval, the manuscript proposal will expire.

12b. The NIH instituted a Public Access Policy in April, 2008 which ensures that the public has access to the published results of NIH funded research. It is your responsibility to upload manuscripts to PUBMED Central whenever the journal does not and be in compliance with this policy. Four files about the public access policy from http://publicaccess.nih.gov/ are posted in http://www.cscc.unc.edu/aric/index.php, under Publications, Policies & Forms. http://publicaccess.nih.gov/submit_process_journals.htm shows you which journals automatically upload articles to PubMed central.