ARIC Manuscript Proposal # 3164

PC Reviewed: 5/8/2018   **Status: _____**   **Priority: 2**
SC Reviewed: _____   **Status: _____**   **Priority: ____**

1.a. Full Title: Independent and Joint Associations of Obesity, Physical Activity, and Galectin-3: The Atherosclerosis Risk in Communities Study (ARIC)

  b. Abbreviated Title (Length 26 characters): Obesity, Physical Activity, and Galectin-3

2.   Writing Group: Roberta Florido, Vijay Nambi, Lucia Kwak, Ron C. Hoogeveen, Mariana Lazo-Elizondo, Gary Gerstenblith, Roger S. Blumenthal, Josef Coresh, Aaron R. Folsom, Elizabeth Selvin, Christie M. Ballantyne, Chiadi E. Ndumele; others welcome

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

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3.   Timeline: We aim to submit this manuscript to the ARIC publications committee <6 months from the date of approval of this manuscript proposal.

4.   Rationale:

Galectin 3 (gal-3) is a β-galactosidase-binding lectin that orchestrates several physiological processes and is a key molecule in the pathogenesis of various diseases [1]. Gal-3 is expressed at low levels in healthy cardiac tissue and at much higher levels during cardiac injury [1,2]. Gal-3 plays an important role in tissue repair, however sustained over-expression can lead to cardiac inflammation and fibrosis [1,3]. Pre-clinical studies have suggested a critical role of gal-3 in the development of adverse cardiac remodeling and dysfunction that can be suppressed via genetic modification of pharmacologic blockage of gal-3 [4]. Several epidemiologic studies have confirmed an association between elevated levels of circulating gal-3 and left ventricular hypertrophy, left ventricular dysfunction, and incident heart failure (HF) [5,6]. Among patient with HF higher gal-3 has been associated with increased risk of re-hospitalization and mortality [7,8]. Despite the well-recognized associations of gal-3 with cardiovascular disease, its determinants are incompletely understood.

Obesity and physical inactivity are established and inter-related risk factors for incident HF, with the highest HF risk seen among those who are both obese and inactive. These risk associations are independent of traditional cardiovascular risk factors and the underlying mechanisms that have not been fully elucidated [9]. Obesity is independently associated with adverse cardiac remodeling, marked by evidence of fibrosis, which predisposes to HF [10,11]. Additionally, animal studies have shown that obesity may upregulate gal-3 expression in the cardiovascular system. Laboratory studies also suggest that physical activity prevents age-associated cardiac inflammation and fibrosis, but studies of endurance athletes have conversely demonstrated a higher prevalence of myocardial fibrosis compared to age-matched controls [15-18]. Prior work has demonstrated that physical activity may attenuate the likelihood of subclinical myocardial damage, as reflected by high-sensitivity troponin-T, particularly among those with obesity. However, there is limited clinical data regarding the associations of obesity and physical activity with gal-3. It is also unclear whether the implications of gal-3 for incident HF differ among subgroups defined by obesity and activity level; for example, whether gal-3 levels have similar HF risk associations among non-obese individuals with high levels of activity and obese individuals who are sedentary.

Therefore, we propose to examine the independent and joint associations of obesity and physical activity with myocardial fibrosis, as reflected by gal-3 levels, among participants in the community-based Atherosclerosis Risk in Communities Study (ARIC) study. We anticipate that this work will further our understanding of the pathways linking obesity and physical inactivity to HF.

5. **Main Hypothesis/Study Questions**:

Aims:
1. To evaluate the association between obesity and fibrosis, as reflected by gal-3 levels
2. To evaluate the association between physical activity and gal-3 levels

3. To evaluate the combined associations of obesity and physical activity with gal-3 levels
4. To assess the implications of gal-3 for incident HF among subgroups defined by obesity status and physical activity levels

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: We will perform cross-sectional and non-concurrent cross-sectional analyses to assess the independent and combined associations of obesity (measured at Visit 4) and physical activity (measured at Visit 3) with gal-3 (measured at Visit 4). Additionally, we will perform prospective analyses to assess the association of gal-3 with incident HF among subgroups defined by obesity status and physical activity levels, with Visit 4 serving as the baseline for prospective analyses.

Exposures: Body-mass index (BMI) and physical activity measured at Visits 4 and 3 respectively, will be the main exposures of interest for the cross-sectional analyses. Additionally, gal-3 measured at Visit 4 will serve as the exposure of interest for prospective analyses.
1. BMI (kg/m^2): Calculated from height and weight measured at Visit 4. Will be categorized as: normal weight (18.5-25 kg/m^2), overweight (25-29.9 kg/m^2), obese (30-34.9 kg/m^2) and severely obese ($\geq$35 kg/m^2). When considering obesity status in concert with physical activity levels, BMI will be dichotomized as: non-obese (18.5-29.9 kg/m^2) and obese ($\geq$30 kg/m^2). We will also model BMI as a continuous variable (in restricted cubic spline models and scaled per 5 kg/m^2 higher BMI).
2. Physical activity: We will consider moderate (3-6 METs) and vigorous (>6 METs) sports physical activity measured by a modified Baecke questionnaire at Visit 3. As done in prior analyses, we will categorize physical activity according to AHA guidelines as: poor (no moderate or vigorous activity), intermediate (less than recommended moderate or vigorous physical activity), and recommended ($\geq$75 min/week of vigorous intensity or $\geq$150 min/week of any combination of moderate and vigorous intensity). Physical activity will also be modeled as a continuous variable in METs*min/week (in restricted cubic spline models and scaled per 1-SD).
3. Gal-3: Gal-3 was measured in stored blood samples (plasma) collected at Visit 4. In prospective analyses, gal-3 will be the main exposure of interest, with categorization by obesity and physical activity levels evaluated as an effect modifier. For these analyses, gal-3 will be categorized into quartiles and also modeled continuously (per 1-SD, with log transformation as needed).

Outcomes: Gal-3 will be the main outcome for cross-sectional analyses and incident HF for prospective analyses.

1. Gal-3: As described above, gal-3 measured from stored blood samples collected at Visit 4 will be used for the current analysis. When considered as an outcome, gal-3 will be classified as elevated vs non-elevated. Elevated gal-3 will be defined as: gal-3 level within the highest quartile, which has been shown to be associated with CVD events including HF; we will additionally consider gal-3 level above the 90th percentile as the definition of elevated gal-3.
2. HF: Incident HF will be defined as HF hospitalization or death due to HF occurring after Visit 4 (baseline for prospective analyses), through 2016 or most recently available data.

Exclusions: Participants with coronary heart disease (CHD) or HF (self-reported HF or CHD at Visit 1; or HF events, adjudicated CHD events, or silent MI at or prior to Visit 4). prior to Visit 4 will be excluded from the current analyses. We will also exclude those of non-black or non-white race due to small numbers, those with BMI <18.5 kg/m^2 due to the confounding associated with underweight, and those missing data on the exposure variables.

Covariates: Age, sex, race*center, smoking status, alcohol use, systolic blood pressure, anti-hypertensive medications use, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, diabetes, estimated glomerular filtration rate (eGFR).

Main Analyses:
1. We will perform bivariate analyses of participant baseline (Visit 4) characteristics according to obesity status (normal weight, overweight, obese, severely obese); we will perform similar analyses according to physical activity category (poor, intermediate, recommended). The chi-square test will used for comparison of categorical variables and analysis of variance (ANOVA) for continuous variables.
2. We will construct regression models with two levels of adjustment:
   a. Model 1: adjusted for age, sex, race*center, smoking status, and alcohol use.
   b. Model 2: Adjusted for the variables in Model 1 + systolic blood pressure, anti-hypertensive medications use, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, diabetes, and eGFR.
   c. We will also consider including rs4644 in our adjustment models. rs4644 is a common single-nucleotide polymorphism (SNP) in the galectin-3–encoding gene (LGALS3) that influences serum gal-3 levels by changing the epitope of the antibody-binding region of the gal-3 assay, leading to falsely low levels of gal-3.
3. We will use adjusted linear regression to assess the difference in average gal-3 (using log transformation if appropriate) with higher BMI category; we will also use adjusted logistic regression to study the association of each BMI category with elevated gal-3 at Visit 4, using those with normal weight as the reference. We will evaluate the continuous association of BMI with the odds

of elevated gal-3, estimating the associations per each 5 kg/m^2 higher BMI and of continuous BMI in restricted cubic spline models.

4. We will use adjusted linear regression to assess the difference in average gal-3 with higher physical activity category; we will also use adjusted logistic regression to study the association of higher physical activity category and elevated gal-3 at Visit 4. We will repeat these analyses stratified by obesity status (non-obese and obese), and use the likelihood ratio test to test for interaction between physical activity and obesity. We will scale physical activity per 1-SD and use restricted cubic spline models to study the association of continuous physical activity with odds of elevated gal-3.

5. We will create cross-categories of obesity (non-obese and obese) and physical activity (recommended, intermediate and poor). We will then use adjusted linear and logistic regression models to evaluate the association of cross-categories of obesity and physical activity with gal-3, as described previously. The subgroup of non-obese individuals with recommended physical activity will be used as reference.

6. In prospective analyses, we will use adjusted Cox proportional hazards models to assess the association of quartiles of gal-3 and of elevated galectin 3 with incident HF after Visit 4. We will perform this analysis in the overall population, stratified by BMI category, stratified by physical activity category, and within subgroups defined by cross-categories of obesity and physical activity. If significant differences across groups are identified, we will perform tests for statistical interaction.

7. We will evaluate the associations of obesity and physical activity with gal-3 in analysis stratified by sex, race and age (>= or <65).

Secondary Analyses:

- We will perform secondary analyses using waist circumference and waist-to-hip ratio as alternative measures of adiposity.
- We will also consider sensitivity analyses examining the associations of obesity and physical activity with change in gal-3 levels from Visit 4 to Visit 6.

Limitations:
1. Residual confounding due to the observational nature of the study.
2. Limitations in measurement of physical activity due to self-report via a questionnaire.
3. Physical activity and gal-3 were not measured at the same study Visit, therefore non-concurrent cross-sectional analyses will have to be performed.

7.a. Will the data be used for non-CVD analysis in this manuscript?   ____ Yes   __X__ 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? _____
Yes _____ No
(This file ICTDER03 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** _____ **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)?

Florido R, Ndumele CE, Kwak L, Pang Y, Matsushita K, Schrack JA, Lazo M, Nambi V,
Blumenthal RS, Folsom AR, Coresh J. Physical activity, obesity, and subclinical
myocardial damage. *JACC: Heart Failure*. 2017 May 31;5(5):377-84

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Ballantyne CM, Nambi V. Obesity, Subclinical Myocardial Injury and Incident Heart
Failure. *JACC: Heart Failure*. 2014 Dec;2(6):600-7.

Fashanu OE, Norby FL, Aguilar D, Ballantyne CM, Hoogeveen RC, Chen LY, Soliman
EZ, Alonso A and Folsom AR. Galectin-3 and incidence of atrial fibrillation: The
Atherosclerosis Risk in Communities (ARIC) study. Am Heart J. 2017;192:19-25.

McEvoy JW, Chen Y, Halushka MK, Christenson E, Ballantyne CM, Blumenthal RS,
Christenson RH and Selvin E. Galectin-3 and Risk of Heart Failure and Death in Blacks
and Whites. J Am Heart Assoc. 2016;5.

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

11.b. If yes, is the proposal
_x_ **A. primarily the result of an ancillary study (list number* __)**

___   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/

12. 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.

References:

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