ARIC Manuscript Proposal #4020

PC Reviewed: 3/8/22	**Status: _____**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: Adipokines as Markers of Metabolic Health Among Individuals with and without Obesity: The Atherosclerosis Risk in Communities (ARIC) Study

b. Abbreviated Title (Length 26 characters): Adipokines and Metabolic Risk

2. Writing Group:

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

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3. Timeline: We aim to complete the manuscript <6 months from the date of approval of this manuscript proposal.

4. Rationale:

Obesity is a major risk factor for cardiovascular diseases (CVD) by means of contributing to the development of metabolic risk factors, in addition to its direct effects on the cardiovascular system. It is frequently accompanied by other metabolic abnormalities, clustering of which is defined as metabolic syndrome. Metabolic syndrome predisposes to a 5-fold higher risk of type 2 diabetes and an up to 2-fold higher risk of CVD[1]. Individuals with obesity but without metabolic syndrome, described as having metabolically healthy obesity (MHO), have lower risk of adverse cardiovascular outcomes than individuals with metabolically unhealthy obesity (MUO). [2,3]. However, MHO is an inherently unstable phenotype, with approximately half developing MUO over a few years of follow-up[4].

Public health interventions to facilitate weight reduction have not yet mitigated the obesity epidemic, and individuals with obesity suffer from a high CVD burden. Thus, novel approaches are needed to better understand the mechanisms by which excess adiposity increases metabolic risk to mitigate the cardiovascular risk in the general population.

Adipokines are cytokine-like molecules secreted from adipose tissue, which have paracrine and endocrine effects on target tissues[5]. Excess adiposity, particularly visceral obesity, leads to adipose tissue dysfunction, which manifests as altered production and secretion of pro- and anti-inflammatory adipokines[5,6]. Two of the most studied adipokines are leptin, with mainly pro-inflammatory properties and adiponectin, an anti-inflammatory adipokine[7].

These adipokines mediate cardiovascular risk through effects on insulin resistance, hypertension[8,9], dyslipidemia[10], and other direct end-organ effects[11,12]. It is therefore plausible that among individuals with similar weight, adipokines might help explain the heterogeneity in metabolic risk status (metabolically healthy vs unhealthy), and in the development of metabolic syndrome and diabetes.

In this study, we aim to assess the associations of adipokines with prevalent metabolic risk status (metabolically healthy or unhealthy), and prevalent metabolic syndrome and diabetes, among middle-aged Atherosclerosis Risk in Communities (ARIC) study participants with and without obesity. Furthermore, we will assess how adipokine levels relate to incident metabolic syndrome and diabetes, and transitions in metabolic health, among individuals with similar weight status. Our study will provide valuable insights regarding the heterogeneity in metabolic risk among individuals with obesity and help guide therapeutic strategies targeting adipokines to address adverse health outcomes associated with obesity.

5. Main Hypothesis/Study Questions:

Aim 1: To investigate and compare the associations of adipokines with prevalent metabolic risk status (metabolically healthy vs metabolically unhealthy), and with prevalent metabolic syndrome and diabetes

Aim 2: To investigate how adipokines are related to the development of metabolic syndrome and diabetes and to transitions in metabolic risk status over time.

Aim 3: To examine the associations of adipokines with individual components of metabolic syndrome

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 evaluate the cross-sectional associations between adipokines (adiponectin, leptin, and leptin/adiponectin ratio) and prevalent metabolic risk status among ARIC participants who attended ARIC Visit 2 (1990-1992) and have variables of interest measured. We will further evaluate the prospective associations of adipokines with incident metabolic syndrome and diabetes at Visit 4 (1996-1998), among individuals with and without obesity. We will also evaluate the associations of adipokines with transitions in metabolic risk status from Visit 2 to Visit 4.

Exclusions: We will exclude individuals missing measurements of adipokine levels. We will also exclude a small number of individuals who are not of black or white race, and those who are missing covariates of interest. For the primary analyses, we will exclude the relatively small number of individuals with diabetes without metabolic syndrome (as most individuals developing type 2 diabetes progress from having metabolic syndrome), but these individuals will be included in secondary analyses. For prospective analyses examining the risk for incident MetS and diabetes, we will exclude participants with MetS and/or diabetes at Visit 2.

Exposures: Our main exposures will be adipokines: adiponectin, leptin, and leptin-adiponectin ratio. Adipokines are measured from stored samples from ARIC Visit 2 participants using Slow Off-rate Modified Aptamer (SOMAmer)-based capture array, that has been shown to correlate highly with ELISA-based targeted protein measurements of adiponectin and leptin. We will model adipokines as tertiles in primary analysis, but will also consider analyses with adipokines modeled continuously with log transformation as needed for skewed distributions. Obesity will be defined as having a body mass index (BMI; defined by weight in kg / height in meters squared) of $\geq$30 kg/m^2. Individuals with a BMI of $<$30 kg/m^2 will be classified as individuals without obesity.

Outcomes: We will use AHA/NHLBI criteria to define metabolic syndrome[13]. Presence of 3 or more of the following 5 risk factors will be classified as having metabolic syndrome: (1) waist circumference $\geq$102 cm in males and $\geq$88 cm in females; (2) HDL cholesterol $<$40 mg/dL in males and $<$50 mg/dL in females; (3) triglycerides $\geq$150 mg/dL; (4) elevated blood pressure (systolic blood pressure $\geq$ 130 mmHg and/or diastolic blood pressure $\geq$ 85 mmHg and/or use of anti-hypertensive medications); and (5) fasting hyperglycemia (fasting glucose $\geq$ 100 mg/dl without diabetes mellitus). To be consistent with prior ARIC studies, diabetes status will be defined based on self-reported physician diagnosis, anti-diabetic medication use, or elevated plasma glucose (fasting $\geq$126 mg/dL; non-fasting $\geq$200 mg/dL). Our three primary outcomes are as follows:

1) Metabolically healthy, defined as no metabolic syndrome or diabetes (reference)
2) Metabolically unhealthy, defined as the presence of metabolic syndrome
3) Metabolically unhealthy (metabolic syndrome present) with diabetes

We will also evaluate overall prevalent metabolic syndrome and prevalent diabetes at Visit 2.

In prospective analyses we will evaluate the associations of Visit 2 adipokines with transitions in metabolic risk status from Visit to Visit 4, as well as with incident metabolic syndrome and diabetes from Visit 2 to Visit 4. We will consider further analyses examining incident diabetes any time after Visit 2 as an additional outcome.

Covariates: We will adjust for Visit 2 measures of age, sex, race-center, smoking status, socioeconomic status, C-reactive protein. We will conduct analyses stratified by obesity status.

Main analyses:

1) We will present baseline characteristics of the participants as means for continuous variables and proportions for categorical variables, stratified by metabolic risk category and baseline obesity
2) In cross-sectional analyses, we will use multinomial logistic regression to evaluate the associations of adipokines with prevalent metabolic risk status at Visit 2 using the following categories:
    a. Metabolically healthy, defined as no metabolic syndrome or diabetes (reference)
    b. Metabolically unhealthy, defined as the presence of metabolic syndrome
    c. Metabolically unhealthy (metabolic syndrome present) with diabetes
        - Analyses will be performed in the overall study population, and also stratified by obesity status
        - We will conduct sensitivity analyses including a category for persons with diabetes but without metabolic syndrome.
        - We will also perform logistic regression analyses examining the association of adipokines with prevalent metabolic syndrome and prevalent diabetes at Visit 2. Adipokines will be modeled continuously in cubic spline models in secondary analyses.
3) We will demonstrate the cross-sectional univariate associations between measures of adiposity (BMI and waist circumference) and adipokine levels comparing adipokine distributions by obesity status and using restricted cubic splines.
4) In prospective analyses, we will use relative risk regression (or logistic regression) to estimate the relative risks and confidence intervals for the association of tertiles of adipokines and incident MetS and diabetes at Visit 4.
5) For each of the metabolic risk categories at baseline (Visit 2), we will investigate the associations between adipokines and transitions between metabolic risk categories (from Visit 2 to Visit 4). Analyses will be performed in the overall population and stratified by obesity status. Metabolic risk transitions of particular interest are:
    a. Odds of progression in metabolic risk from metabolically healthy to unhealthy
    b. Odds of any increase in metabolic risk category: from metabolically healthy to unhealthy or from metabolically unhealthy to metabolically unhealthy with diabetes
    c. Among those with metabolically unhealthy status at Visit 2, odds of progression (to metabolically unhealthy with diabetes) vs regression (to metabolically healthy) in metabolic risk

6) We will examine associations of adipokines with individual components of metabolic syndrome, and with overall metabolic syndrome severity as defined by an established metabolic syndrome Z-score[14].
7) We will test for effect measure modification by obesity status, sex (male, female) and race (White, Black). We will report stratified results if statistically significant effect measure modification is present.

Limitations:

- We may not be able to fully eliminate residual confounding due to the observational nature of the study.
- We cannot assess the directionality of a potential causal association in cross-sectional analyses.
- We may have limited statistical power to evaluate further interactions by different covariates after stratification by metabolic risk and obesity status.

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 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 ____ 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)?

ARIC Manuscripts:

Zhu, Na et al. "High-molecular-weight adiponectin and the risk of type 2 diabetes in the ARIC study." *The Journal of clinical endocrinology and metabolism* vol. 95,11 (2010): 5097-104. doi:10.1210/jc.2010-0716

Schmidt, M I et al. "Leptin and incident type 2 diabetes: risk or protection?." *Diabetologia* vol. 49,9 (2006): 2086-96. doi:10.1007/s00125-006-0351-z

Duncan, Bruce B et al. "Adiponectin and the development of type 2 diabetes: the atherosclerosis risk in communities study." *Diabetes* vol. 53,9 (2004): 2473-8. doi:10.2337/diabetes.53.9.2473

Ballantyne, C M et al. "Metabolic syndrome risk for cardiovascular disease and diabetes in the ARIC study." *International journal of obesity (2005)* vol. 32 Suppl 2,Suppl 2 (2008): S21-4. doi:10.1038/ijo.2008.31

Luft, Vivian C et al. "Chronic inflammation role in the obesity-diabetes association: a case-cohort study." *Diabetology & metabolic syndrome* vol. 5,1 31. 27 Jun. 2013, doi:10.1186/1758-5996-5-31

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* _AS2017.27,__)

___ 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 https://sites.cscc.unc.edu/aric/approved-ancillary-studies

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.

References:

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2 Kramer, C. K., Zinman, B. & Retnakaran, R. Are metabolically healthy overweight and obesity benign conditions?: A systematic review and meta-analysis. *Ann Intern Med* **159**, 758-769, doi:10.7326/0003-4819-159-11-201312030-00008 (2013).

3 Commodore-Mensah, Y. *et al.* High Burden of Subclinical and Cardiovascular Disease Risk in Adults with Metabolically Healthy Obesity: The Atherosclerosis Risk in Communities (ARIC) Study. *Diabetes Care*, doi:10.2337/dc20-2227 (2021).

4 Eckel, N. *et al.* Transition from metabolic healthy to unhealthy phenotypes and association with cardiovascular disease risk across BMI categories in 90 257 women (the Nurses' Health Study): 30 year follow-up from a prospective cohort study. *Lancet Diabetes Endocrinol* **6**, 714-724, doi:10.1016/S2213-8587(18)30137-2 (2018).

5 Longo, M. *et al.* Adipose Tissue Dysfunction as Determinant of Obesity-Associated Metabolic Complications. *Int J Mol Sci* **20**, doi:10.3390/ijms20092358 (2019).

6 Feijoo-Bandin, S. *et al.* Adipokines and Inflammation: Focus on Cardiovascular Diseases. *Int J Mol Sci* **21**, doi:10.3390/ijms21207711 (2020).

7 Fantuzzi, G. Adipose tissue, adipokines, and inflammation. *J Allergy Clin Immunol* **115**, 911-919; quiz 920, doi:10.1016/j.jaci.2005.02.023 (2005).

8 Ding, W. *et al.* Adipokines are Associated With Hypertension in Metabolically Healthy Obese (MHO) Children and Adolescents: A Prospective Population-Based Cohort Study. *J Epidemiol* **28**, 19-26, doi:10.2188/jea.JE20160141 (2018).

9 Bell, B. B. & Rahmouni, K. Leptin as a Mediator of Obesity-Induced Hypertension. *Curr Obes Rep* **5**, 397-404, doi:10.1007/s13679-016-0231-x (2016).

10 Marso, S. P. *et al.* Low adiponectin levels are associated with atherogenic dyslipidemia and lipid-rich plaque in nondiabetic coronary arteries. *Diabetes Care* **31**, 989-994, doi:10.2337/dc07-2024 (2008).

11 Nakamura, K., Fuster, J. J. & Walsh, K. Adipokines: a link between obesity and cardiovascular disease. *J Cardiol* **63**, 250-259, doi:10.1016/j.jjcc.2013.11.006 (2014).

12 Zhao, S., Kusminski, C. M. & Scherer, P. E. Adiponectin, Leptin and Cardiovascular Disorders. *Circ Res* **128**, 136-149, doi:10.1161/CIRCRESAHA.120.314458 (2021).

13 Alberti, K. G. *et al.* Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation* **120**, 1640-1645, doi:10.1161/CIRCULATIONAHA.109.192644 (2009).

14 Gurka, M. J., Lilly, C. L., Oliver, M. N. & DeBoer, M. D. An examination of sex and racial/ethnic differences in the metabolic syndrome among adults: a confirmatory factor analysis and a resulting continuous severity score. *Metabolism* **63**, 218-225, doi:10.1016/j.metabol.2013.10.006 (2014).