1.a. Full Title: Metabolomic predictors of incident heart failure: a case-control study nested within the Atherosclerosis Risk in Communities (ARIC) study

b. Abbreviated Title (Length 26 characters): Metabolomics and heart failure

2. Writing Group:
Writing group members:
Jennifer A. Nettleton, Jack L. Follis, Alvaro Alonso, Laura Loehr, Eric Boerwinkle, others welcome

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

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ARIC author to be contacted if there are questions about the manuscript and the first author does not respond or cannot be located (this must be an ARIC investigator).
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3. Timeline: The full manuscript will be submitted for ARIC review before 12/1/2010; an abstract will be submitted to the spring 2011 American Heart Association Epidemiology Council meeting (submission deadline 10/1/2010)
4. Rationale:

Investigations that improve our ability to prevent, diagnose, and treat heart failure (HF) have become imperative due to increases in the rates of HF, shift in the age distribution of the population, and rising prevalences of important risk factors, such as obesity, diabetes, and hypertension\(^1\). Currently, most biomarkers of HF reflect metabolic changes subsequent to manifestation of overt HF but provide little information about the up-stream biological changes that drive progression from an at-risk state to one of overt disease.

Modern metabolomic technologies allow for global characterization of metabolic networks by quantifying all low molecular weight metabolites present in a given biological sample\(^2,3\). A metabolomic approach can not only quantify traditional biomarkers (e.g., plasma cholesterol), but can also quantify all the pathway metabolites\(^3\) (e.g., dietary sources and intermediates in the cholesterol biosynthesis pathway). The metabolome represents exogenous exposures and endogenous metabolism; thus, the human metabolome is the ultimate expression of the interplay between the environment and the genetic backbone that orchestrates biological function.

The majority of metabolomic studies conducted to date utilize samples from prevalent cases rather than incident cases. If metabolomic research is to inform prediction and/or prevention of disease, samples would ideally be collected prior to disease onset but within a short enough intervening time period as to show meaningful metabolic characteristics that distinguish those who remain healthy from those who do not. However, it is not known whether metabolomic data can be successfully applied to prospective data.

With this in mind, we compared the metabolomic profiles of 57 incident cases of HF with 57 age, race, sex, medication, and BMI-matched controls who were part of the Atherosclerosis Risk in Communities (ARIC) study. Baseline serum samples were used to quantify metabolites via GC-MS/LC-MS. Cases were individuals diagnosed with HF between exam 3 and exam 4 (≥5 years but <10 years after baseline); controls were free of HF through follow-up. Both cases and controls were hypertensive at baseline (i.e., at high risk for HF).

These ARIC participants were selected for metabolomic analysis in a pilot study, related to the larger ancillary study led by Drs. Nettleton and Boerwinkle (ARIC AS #2008.16).

5. Main Hypothesis/Study Questions:

- We hypothesize that metabolites and pathways reflecting a shift from fatty acid to carbohydrate metabolism will distinguish heart failure cases from controls (e.g., Kreb’s cycle intermediates)
We hypothesize that dietary factors, such as specific fatty acids, will distinguish heart failure cases from controls.

We hypothesize the traditional quantitative heart failure risk factors, such as dyslipidemia and hyperglycemia, will also distinguish heart failure cases from controls.

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).

As described in our original ancillary study proposal, we selected baseline serum samples from 57 incident heart failure cases (diagnosed between exam 3 and exam 4) and 57 controls matched to cases on age (within 1 year), race, sex, BMI (within 1.5 units), lipid-lowering medication use, and anti-hypertension medication use.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total pairs</td>
<td>57</td>
</tr>
<tr>
<td>Male pairs</td>
<td>28</td>
</tr>
<tr>
<td>Female pairs</td>
<td>29</td>
</tr>
<tr>
<td>Race: White pairs</td>
<td>42</td>
</tr>
<tr>
<td>Race: African American pairs</td>
<td>15</td>
</tr>
<tr>
<td>Pairs taking blood pressure-lowering medication</td>
<td>23</td>
</tr>
<tr>
<td>Pairs taking lipid-lowering medication</td>
<td>4</td>
</tr>
<tr>
<td>Age (mean)</td>
<td>56 years</td>
</tr>
<tr>
<td>BMI (mean)</td>
<td>29.0 kg/m²</td>
</tr>
</tbody>
</table>

Two sets of analyses are planned; in both analyses, metabolomic data will be log-transformed. In the first analysis, we will analyze only complete pairs of data, i.e., where a metabolite value (area under the peak) is present for both case and matched control. In the second analysis, we will use imputed minimum values, where values are imputed in instances when less than 50% of either the cases or controls were missing. Data will be analyzed using both paired t-tests and signed-rank tests. We will define statistical significance as $p < 1.9 \times 10^{-4}$ (0.05/268 named compounds in the metabolomics dataset), keeping in mind that this cut-point might be overly conservative given the exploratory nature of this work, our relatively small dataset, and the fact that many of the metabolites are not likely independent of one another.

7.a. Will the data be used for non-CVD analysis in this manuscript? 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? NA

(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? 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”?

NA

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

______ 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)?

None

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

11.b. If yes, is the proposal

___ A. primarily the result of an ancillary study (list number: 2008.16)

___ 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.

We understand and will comply

REFERENCES