1.a. **Full Title**: Lifestyle-related health behaviors and six-year change in high-sensitivity cardiac troponin T

b. **Abbreviated Title (Length 26 characters)**: Health Behaviors and Troponin T

2. **Writing Group**:
   Writing group members:

   Anna Fretz; John McEvoy; Casey Rebholz; Chiadi Ndumele; Christie Ballantyne; Liz Selvin... others welcome

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

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3. **Timeline**: Data is currently available. Analysis is planned to start as soon as approval is obtained and will take between 3 and 6 months. Manuscript will be prepared during the 3 months following the completion of the analysis.

4. **Rationale**:

   Cardiac troponin T is a widely-used biomarker in clinical practice for diagnosing myocardial infarction in persons with chest pain. A novel highly sensitive assay for cardiac troponin T can detect concentrations of troponin 10-times lower than assays currently used in clinical practice, extending the potential utility of this biomarker to asymptomatic populations. Previous studies have demonstrated that roughly 70% of a healthy middle-aged population have detectable troponin levels using this assay (1-3). Having detectable levels of high-sensitivity cardiac troponin T (hs-cTnT) has been shown to strongly and independently predict cardiovascular morbidity and mortality (2-6). Moreover, in studies using serial measures of hs-cTnT, individuals with increases in hs-cTnT over time demonstrate greater risk of cardiovascular events relative to individuals with no significant change (5-7). Additionally, individuals with decreases in hs-cTnT over time demonstrate lower risk of cardiovascular events than those with no significant change (6,7). Given the prognostic utility of serial measures of hs-cTnT, it is important to understand determinants that may be associated with increased or decreased subclinical myocardial damage over time.

Only one previous study has explored clinical factors associated with temporal changes in hs-cTnT (8). This study demonstrated that hypertension, obesity and diabetes were most strongly associated with progression of subclinical myocardial damage. It also demonstrated that among modifiable clinical risk factors, individuals with obesity and diabetes were less likely to have temporal decreases in hs-cTnT. Because hypertension, obesity and diabetes are strongly influenced by lifestyle and environmental factors, the impact of health behaviors on temporal changes in hs-cTnT is of substantial clinical interest. To our knowledge, only two previous studies have addressed a component of this question, by examining the association between physical activity and temporal increases in hs-cTnT (9,10). In these studies, low levels of physical activity were indeed associated with progression of subclinical myocardial damage.

However, it is important to better understand other modifiable health behaviors that are associated with temporal reductions in hs-cTnT, as this has potential utility for
monitoring the beneficial impacts of health behaviors on CVD risk through regression of myocardial damage during the subclinical period.

There are five key health behaviors, highlighted by the American Heart Association, recommended for the prevention of cardiovascular disease: physical activity, diet, weight management, smoking cessation and emotional wellbeing (11). There is exhaustive literature on the cardiovascular benefits of these health behaviors. With the exception of physical activity that has already been studied (9,10), our study will focus on diet, adiposity, smoking, alcohol consumption and emotional well-being as potential lifestyle risk factors for subclinical myocardial damage. We will also assess health behaviors modeled in aggregate, using the American Heart Association Life’s Simple 7 score, which has shown that a higher health score is associated with reduced risk of cardiovascular disease (12).

**Diet** is a well-studied risk factor for cardiovascular disease. Previous studies show that having a healthy diet or improving diet over time is associated with decreased risk of cardiovascular events (13-18) perhaps through improved endothelial function and reduced levels of inflammatory markers and oxidized lipids, which contribute to vascular disease (19-21).

**Adiposity** is strongly influenced by diet, but has been independently studied as a risk factor for cardiovascular disease and mortality, both in terms of excess weight (22-24) and underweight (25). Previous studies also demonstrate that weight gain is associated with increased CVD risk, compared to stable weight (26-30). However, data are conflicted on the association of weight loss and cardiovascular risk, with some studies showing increased CVD risk (29,30), some showing no association (26-28) and some showing decreased mortality, only when weight loss is intentional (31,32). It is hypothesized that the data are conflicted due to confounding factors such as comorbidities and illness that could cause weight loss, but also lead to increased risk of cardiovascular events and mortality. However from a pathophysiologic standpoint, weight loss is associated with improved cardiometabolic markers (33-36), reduced blood pressure (37,38) and reduced cardiac wall stress (39,40).

**Smoking** cessation is associated with rapid reduction in risk of cardiac events (41-43), but its mechanism is less well-studied than the risks associated with active smoking. The literature suggests that there are improvements in endothelial function after just one year (44). It is also hypothesized that many of the negative impacts of cigarette smoking, such as increased sympathetic activity of the heart (45), increased inflammatory markers (46,47) and inhibition of thrombolytic processes (47), are simply reversed with cessation (48).

Poor **emotional wellbeing** and psychological stress have been assessed in population-based studies as vital exhaustion using the well-established Maastricht Vital Exhaustion Questionnaire (49,50). High levels of baseline vital exhaustion are independently associated with increased coronary events (51-54) as well as microvascular disease
The literature suggests that this risk could be mediated by reduced cardiac parasympathetic activity (56) as well as impaired thrombolysis (57,58).

Lastly, moderate alcohol consumption, although not part of the core AHA prevention guidelines, is a known health behavior that demonstrates cardiovascular protective effects (59-64). The mechanisms by which there is decreased risk are hypothesized to be: improved endothelial function (65,66), decreased inflammation of the endothelium and myocardium (67,68), reduced fibrinogen levels and platelet aggregation leading to improved hemodynamics (69-73), as well as inhibition of oxidation of lipids (74,75).

Given the associations between these health behaviors and cardiovascular pathology, as well as clinical cardiovascular outcomes, it could be of clinical utility to understand their association with changes in hs-cTnT, specifically temporal reductions, to assess for regression in myocardial damage during the subclinical stage.

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

**Aim 1.** To evaluate the association between baseline health behaviors (healthy diet score, moderate alcohol consumption, smoking status, body mass index (BMI) and vital exhaustion) and six-year categorical changes in hs-cTnT. These associations will be evaluated before and after adjustment for demographic and traditional cardiovascular risk factors.

**Hypothesis:** Healthier behaviors will be positively associated with incident undetectable hs-cTnT (<5 ng/L) among persons with detectable hs-cTnT (≥5ng/L) at baseline, compared to those who had sustained detectable hs-cTnT levels. We will also conduct analyses assessing risk of incident detectable hs-cTnT (≥5ng/L) among those with undetectable levels (<5ng/L) at visit 2, which we hypothesize to have a negative association.

**Aim 2.** To evaluate the association between health behaviors (healthy diet score, alcohol consumption, smoking status, body mass index (BMI) and vital exhaustion), treated as time-varying exposure variables, and six-year categorical change in hs-cTnT. These associations will be evaluated before and after adjustment for demographic and traditional cardiovascular risk factors.

**Hypothesis:** Increased duration of exposure to better health behaviors will be positively associated with incident undetectable hs-cTnT among those with detectable hs-cTnT at baseline compared to those who had sustained detectable hs-cTnT levels. We will also conduct analyses assessing risk of incident detectable hs-cTnT among those with undetectable hs-cTnT at baseline, which we hypothesize to be a negative association.
Aim 3. To evaluate the association between Life’s Simple 7 score and six-year categorical change in hs-cTnT.

Hypothesis: A higher score (indicative of healthier behaviors) will be positively associated with incident undetectable hs-cTnT (<5ng/L) among persons with detectable hs-cTnT (≥5ng/L) at baseline, compared to individuals with sustained detectable hs-cTnT 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: Prospective analysis to measure the association of health behaviors and Life’s Simple 7 score with changes in hs-cTnT, measured at two time points, six years apart (visit 2: 1990-1992 and visit 4: 1996-1998). Visit 2 will serve as baseline for the study. We will also assess health behaviors as time-varying exposures, using information from visit 3 and visit 4.

Study Population (Inclusion/Exclusion Criteria):
All ARIC participants who attended visits 2 and 4 and who did not meet any of the following exclusion criteria:
- MI, angina, stroke, or revascularization before visit 2
- Missing hs-cTnT value at visits 2 or 4
- Missing drinking status, smoking status, diet information, BMI, or vital exhaustion score at visit 2
- Missing any component of the Life’s Simple 7 score (BMI, physical activity score, diet information, smoking status, total cholesterol, fasting blood glucose, blood pressure)
- Identify as non-white race in Minnesota or Maryland
- Identify as non-black race in Mississippi

Exposure:
There are five health behaviors that will be assessed: physical activity, diet, smoking status, alcohol consumption, weight loss, and emotional well-being. We will also assess Life’s Simple 7 score, which is composed of: total cholesterol, fasting blood glucose, blood pressure, smoking, body mass index, physical activity, and diet.

Diet will be measured using a healthy food score, adapted from a study by Rebholz et al (76). The score will be a sum of 5 individual food groups obtained from the Food Frequency Questionnaire (FFQ): fruits and vegetables, fish, fiber-rich whole grains, sodium, and sugar-sweetened beverages. A point will be given for each group where recommended intake levels were met: (1) ≥4.5 servings of fruits and vegetables per day; (2) ≥7 ounces of fish per week; (3) ≥3 ounces of fiber-rich whole grains per day.
(≥1.1 g of dietary fiber/10 g of carbohydrate per day); (4) <1500 mg of sodium per day; and (5) ≤36 ounces of sugar-sweetened beverages per week. Thus, a higher score indicates a healthier diet. This score demonstrates consistency with AHA scientific statements (77, 78) as well as the US Dietary Guidelines (79).

**Smoking Status** will be measured by self-reported current, former or never user.

**Alcohol Consumption** will be measured by self-reported drinking status and number of drinks per week. Alcohol consumption will be divided as never drinker, former drinker, current drinker up to 7 drinks per week, current drinker 7-14 drinks per week, current drinker 14-21 drinks per week, and current drinker greater than 21 drinks per week.

**Body Mass Index** will be measured using height and weight, assessed by standard protocol.

**Emotional Well-being** will be assessed using Vital Exhaustion, measured by the Maastricht Questionnaire. This questionnaire consists of 21 questions with a higher score corresponding to greater level of vital exhaustion and poorer emotional well-being. A score of ≥14 is considered the threshold for clinically evident vital exhaustion and thus is the threshold for our analyses (80).

**Life’s Simple 7 score** will be determined by summing the number of the 7 individual health components that are achieved at the ideal level. Ideal levels of the health components were: healthy diet score ≥4, body mass index <25 kg/m², ≥ 150 minutes/week of physical activity, never smoker or quit >12 months ago, blood pressure <120/80 mmHg, fasting blood glucose <100 mg/dL, and total cholesterol <200 mg/dL.

**Outcome:**
Cardiac troponin T was measured using a highly-sensitive novel (pre-commercial) assay, Elecsys Troponin T (Roche Diagnostics, Indianapolis, Indiana). For the purposes of these analyses, we will define 5.0 ng/L as the lower limit of detection (81,82) although we will conduct sensitivity analyses using the lowest level of measurability (3.0 ng/L). The assay demonstrated between-assay coefficients of 2.6% for control materials with mean cTnT concentration of 2378 ng/L and 6.9% for control materials with mean cTnT concentration of 29 ng/L. Laboratory reliability was 0.99 in participants with HF and 0.94 in participants without HF (83); reliability coefficient was assessed to be r=0.98 with a coefficient of variation of 15% after excluding outliers of greater than SD from the mean. Serum samples were drawn at visit 2 and plasma samples were drawn at visit 4. These samples were stored at -80° until time of assay, which was 2011 for visit 4 plasma samples and 2013 for visit 2 serum samples. A formal calibration study demonstrated comparability between visit 2 serum and visit 4 plasma measurements (84). Detectable hs-cTnT will be defined as concentrations ≥5ng/L; undetectable hs-cTnT will be defined as concentrations<5ng/L.

**Covariates:**
Age (years, continuous), sex (male/female), race/field center (Maryland whites; Minnesota whites, North Carolina whites; North Carolina blacks; Mississippi blacks), education (years of education), diabetes (yes/no), total cholesterol (continuous), HDL cholesterol (continuous), systolic and diastolic blood pressure (continuous), blood pressure medication use (yes/no), eGFR (mL/min/1.73 m2), LV hypertrophy (yes/no). All measured at baseline visit 2, unless otherwise noted.

Statistical Analysis:
For the primary analysis, we will look at hs-cTnT as a binary outcome. The primary outcome will be incident undetectable hs-cTnT (<5ng/L) at visit 4 among persons with detectable hs-cTnT (≥5ng/L) at visit 2, using those who had sustained detectable hs-cTnT at visit 4 as the reference group. For these binary outcomes we will use Poisson regression to generate adjusted risk ratios.

We will perform two statistical models:
- Model 1: adjust for demographic factors (age, gender, race/field center [only if not a modifier], education level)
- Model 2: Model 1 + additional cardiovascular risk factors (total cholesterol, HDL cholesterol, systolic and diastolic blood pressure, blood pressure medication use, diabetes, eGFR, LVH)

Sensitivity Analyses:
We will perform 4 sensitivity analyses:
1) Sensitivity analysis excluding individuals with a cardiovascular event between visits 2 and 4 (MI, HF).
2) Sensitivity analysis for the prospective association of incident non-elevated hs-cTnT (<14 ng/L) among those with elevated hs-cTnT (≥14ng/L) at visit 2, using those with sustained elevation of hs-cTnT as the reference group.
3) Sensitivity analysis imputing visit 4 hs-cTnT as 60ng/L for those who died between visit 2 and 4.
4) Sensitivity analysis looking at continuous change in hs-cTnT among those with detectable hs-cTnT at visit 2.
5) Sensitivity analysis using data only from North Carolina to assess race-field center aliasing.

Potential effect modifiers:
We will test for interaction by age, race, and sex. A stratified analysis will be performed if statistically significant effect modification for any of these variables is observed.

Limitations:
We are limited by the two measurements of hs-cTnT at visit 2 and then visit 4, which are six years apart, as a means of characterizing the progression or regression of subclinical myocardial injury. There may be more detailed trends in changes of hs-cTnT that we are unable to assess. The use of Vital Exhaustion as a variable to measure emotional well-being is not ideal, but has been shown to be of use in predicting cardiovascular outcomes (51). Lastly, because this is an observational study only, we cannot rule out the potential of residual confounding.

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.cscce.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)?
MS #2128: Six-year change in high sensitivity cardiac troponin T and risk of cardiovascular events (Selvin)

MS #2441: Obesity, Physical Activity and Myocardial Injury: The Atherosclerosis Risk in Communities (ARIC) Study

MS #2269: Risk factors for Progression of Subclinical Myocardial Injury: Six-year change in highly-sensitive troponin T in a community-based population study (McEvoy)

MS #2025: Obesity and Subclinical Myocardial Injury: The Atherosclerosis Risk in Communities (ARIC) Study (Ndumele)

MS #2442: Alcohol consumption and myocardial biomarkers

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

ARIC Ancillary Study #2008.10: Measurement of NT-pro-BNP and troponin T at visit 4 for the full ARIC cohort (Ballantyne)

ARIC Ancillary Study #2009.16: Short-term Markers of Glycemia and Long-term Outcomes (Selvin)

11.b. If yes, is the proposal  _X_ A. primarily the result of an ancillary study (list number* #2008.10, #2009.16)  ____  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.csc.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.

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