

## ARIC Manuscript Proposal # 1322

PC Reviewed: 12/\_11/07  
SC Reviewed: \_\_\_\_\_

Status: A  
Status: \_\_\_\_\_

Priority: 2  
Priority: \_\_\_\_\_

**1.a. Full Title:** Test of a Biomarker for Consumption of Sweets in the ARIC-MRI study

**b. Abbreviated Title (Length 26 characters):** Biomarker for Sweets

**2. Writing Group:**

Writing group members:

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

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**3. Timeline:** We will conduct measurement and analysis of C13/C12 in December 2007. We plan to submit an abstract to American Diabetes Association Annual Scientific Sessions due beginning of January.

**4. Rationale:**

Current methods for gathering nutrition information on consumption of sweets (including high fructose corn syrup (HFCS) and sugar cane derived foods) is limited by problems with recall and misreporting.<sup>1</sup> Therefore, even though there has been ecological evidence<sup>2</sup> showing a strong relationship between the trends of obesity and HFCS consumption, epidemiologic studies have been inconclusive in showing associations. A biomarker that would reflect either the long or short term intake of HFCS and sugar-cane based foods would be helpful in investigations of the dietary effects of sweets consumption with the development of obesity and diabetes.

The scientific basis for the proposed biomarker comes from studies related to paleontology/paleobotany research. C4 plants have the distinct feature of utilizing an additional enzyme (phosphoenolpyruvate carboxylase) in their photosynthetic process leading to the concentration of C13, a stable isotope of carbon which has one more neutron than its more predominant form, C12. Corn and sugar cane are the two major C4 plants that are consumed in our diet. Other C4 plants include crabgrass, sorghum, and other non-edible grasses. We have found in previous investigation of over 100 grocery products that the C13/C12 signature of products such as sweetened beverages and solid sweets such as candy, produce a unique C13/C12 ratio that distinguishes them from other products. Those products that consisted of corn or sugar derived sweeteners had high values of C13/C12 (towards -11 permil) whereas fruits and vegetables and other products without these sweeteners were measured at -26 permil C13/C12 (which indicates a low concentration).<sup>3</sup>

We hypothesized that food items consumed would translate into differences in C13/C12 signature in blood samples. Previous studies in cattle have found that such tissue turnover occurs with corn feeding.<sup>4</sup> Furthermore, we have tested over 200 anonymous blood samples and found that serum samples from humans had variability that could be related to dietary C13/C12 sources. Thus, we proposed to test whether measurement of the stable isotope ratio of C13 to C12 could serve as a biomarker for sweetened beverage consumption in a group of participants with diets characterized by food frequency questionnaire (FFQ). Our ancillary study is titled "Test of a Unique Biomarker for Studies of Obesity and Diabetes" (PI: Cheryl Anderson, AS# 2007.05). This manuscript proposal reflects the analysis of our primary outcome of C13/C12 ratio.

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

This manuscript will address three aims:

- 1) To determine the association between serum C13/C12 levels and self-reported sweetened beverage consumption. We hypothesize that individuals with low consumption, as recorded by FFQ, will have low serum C13/C12 concentrations while individuals with high consumption will register a C13 signal closer to that of corn/sugar cane (i.e. towards -17 permil).

- 2) To determine the effects of corn consumption on C13/C12 levels. We hypothesize that corn consumption will influence serum C13 concentrations but that it can be adjusted for in analysis with sweetened beverage consumption.
- 3) To describe associations between C13/C12 levels with other dietary variables and with anthropometric values (i.e. BMI, WHR). We hypothesize that high C13 concentrations will also be associated with obesity by anthropometry.

## 6. Design and analysis

### Study population:

Washington County participants from the ARIC-MRI study cohort who had FFQ data gathered during their 2005-2006 visit. The selection for analysis was based on having either very high sweetened beverage consumption (one drink a day or more of sugary drinks) or very low consumption (less than one drink a month). We also ensured that these individuals had low or high intake of total sugar, as calculated by quintiles of total sugar intake derived from the FFQ.

### Exclusion:

We excluded participants who had very low stored serum samples to reserve samples for other investigations

### Laboratory Methods:

C13/C12 values were measured from stored serum samples by mass spectroscopy (in units permil) using methods previously described.<sup>5</sup> The analysis of the serum samples was conducted by Dr. Jahren lab group at their laboratory in Johns Hopkins University, Department of Earth and Planetary Sciences, Baltimore, MD.

### Statistical Methods:

We will conduct data checking for outliers and assess distributions for normality, transforming variables as necessary.

For aim #1, we will conduct a t-test of difference in C13/C12 ratio between those with high versus those with low consumption of sweetened beverages as indicated by their responses to FFQ items (i.e. for sugary carbonated and non-carbonated drinks such as coke, caffeine free coke, 7-Up, Hawaiian punch, etc).

For aim #2, we will conduct t-test of difference in C13/C12 ratio between the high vs. low sugary drinks consumers stratified by consumption of corn and corn products including whole corn, popcorn, corn oil, corn cereal, etc. We will use multiple linear regression to examine whether the association between sugary drinks and C13 will be accounted for by corn consumption.

For aim #3, we will conduct Spearman correlations and multiple linear regression adjusting for corn intake between different dietary factors including the consumption of protein, fiber, etc to characterize the C13/C12 ratios. We will conduct linear regression to determine the relationship between C13/C12 ratio and body mass index and waist-circumference first stratified by sweetened beverage intake (to account for sample selection). We will look both cross-

sectionally and retrospectively (i.e. BMI from visit 1). If the association is similar in both groups, we will pool the groups together and adjust for sweetened beverage and corn intake to see if the relationship changes. We are limited by sample size in the number of variables we can include in our multivariate models. We are also limited by the strong association between sweetened beverage consumption and gender. However, previous investigations have found no association between gender and C13 values in a random sample of 200 anonymous samples.

**7.a. Will the data be used for non-CVD analysis in this manuscript?** ☒ Yes ☐ No

**b. If Yes, is the author aware that the file ICTDER02 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 ICTDER02 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 ☒ No

**8.b. If yes, is the author aware that either DNA data distributed by the Coordinating Center must be used, or the file ICTDER02 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.csc.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)?**

- Manuscript #737 (Stevens et al., Diabetes Care 2002; 25(10):1715-21) which found an association between dietary cereal fiber intake with the development of diabetes although no association was found with glycemic index.
- Manuscript #930 (Paynter et al., Am. J. Epi 2006; 164(11):1075-84) where investigators looked at sweetened beverage consumption by FFQ but found no relationship to the development of type 2 diabetes.

**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\* 2007.05)

\_\_\_\_ **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/>

**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.**

#### Reference List

1. Winkler JT. The fundamental flaw in obesity research. *Obes Rev.* 2005;6:199-202.
2. Bray GA, Nielsen SJ, Popkin BM. Consumption of high-fructose corn syrup in beverages may play a role in the epidemic of obesity. *Am J Clin Nutr.* 2004;79:537-543.
3. Jahren AH, Saudek C, Yeung EH et al. An isotopic method for quantifying sweeteners derived from corn and sugar cane. *Am J Clin Nutr.* 2006;84:1380-1384.
4. Bahar B, Monahan FJ, Moloney AP et al. Alteration of the carbon and nitrogen stable isotope composition of beef by substitution of grass silage with maize silage. *Rapid Commun Mass Spectrom.* 2005;19:1937-1942.
5. Jahren AH, Saudek C, Yeung EH et al. An isotopic method for quantifying sweeteners derived from corn and sugar cane. *Am J Clin Nutr.* 2006;84:1380-1384.