ARIC Manuscript Proposal #3427

PC Reviewed: 7/9/19 **Status: _____** **Priority: 2**
SC Reviewed: _____ **Status: _____** **Priority: ____**

1.a. Full Title:

Identification of plasma sphingolipids as biomarkers of physical function decline in older adults

b. Abbreviated Title (Length 26 characters):

Plasma sphingolipids and physical function decline

2. Writing Group:
Writing group members:

Fang Yu, B. Gwen Windham, Alvaro Alonso, Danni Li, Aniqa Alam, Anna Newton and others are welcome (*The order in which the writing group members are currently listed in the manuscript proposal does not reflect the order they will appear in the final manuscript. The final authorship order will be discussed at a later time and will be based on authors' contributions*).

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

First author: **Danni Li**
Address: 420 Delaware St SE, Minneapolis, MN 55406

Phone: 6126260299 Fax:
E-mail: dannili@umn.edu

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).
Name: **Alvaro Alonso**
Address: Emory University
1518 Clifton Rd NE
Atlanta, GA 30307
Phone: 404 727 8714
E-mail: alvaro.alonso@emory.edu

3. Timeline:

A manuscript will be submitted to a journal for publication within one year from the date the proposal is approved.

4. Rationale:

Physical function decline affects nearly 40% of Americans older than 65 years of age,[1] and leads to many adverse health outcomes.[2-4] To date, the molecular mechanisms underlying physical function decline are unknown. Studies have linked demyelinating diseases to mobility impairments (e.g., multiple sclerosis).[5-7] Our previous work in ARIC was the first study that identified a positive cross-sectional association of three plasma odd numbered fatty acid sphingomyelins (SMs): SM (41:1), SM (41:2) and SM (43:1) with physical function.[8] These odd number fatty acid SMs are alpha oxidation products of hydroxy SM,[9] which play important role in long-term stability of myelin sheath.[10] Therefore, these odd chain fatty acid SMs have considerable potential as plasma biomarkers of myelination stability underlying physical function.

The objective of this study is to validate our previous findings in an independent sample (a different subset of the same Visit 5 ARIC cohort data which were used in the initial analyses referenced above) and to evaluate the longitudinal association of these odd chain fatty acid SMs with physical function decline in older persons (mean age ± SD: 77.5 years ± 5.5). In addition to physical function, we will also include self-reported function status as secondary outcomes as they are not included in our previous study. Furthermore, because SMs and ceramides belong to the same sphingolipids (SLs) family and Cer (41:1) and Cer (43:1) are precursors to synthesis of SM (41:1) and SM (43:1), respectively, we will explore two ceramide equivalents of SM (41:1) and SM (43:1) [Cer (41:1) and Cer (43:1)] as biomarkers of physical function decline. Blood biomarkers of physical function decline will inform studies of age-related mechanisms underlying physical function decline. They may be used as minimally invasive biomarkers to predict older persons at risk of physical function decline for enrollment into intervention programs aimed at boosting physical function.

5. Main Hypothesis/Study Questions:

The **central hypothesis** is that lower plasma levels of odd number fatty acid sphingolipids (SLs) [SM (41:1), SM (41:2), SM (43:1), Cer (41:1) and Cer (43:1)] are associated with poorer physical function and steeper decline of physical function in older adults.

Aim 1. Determine the cross-sectional association of plasma odd number fatty acid SLs with physical function in older adults. Hypothesis 1: Low plasma SM (41:1), SM (41:2), SM (43:1), Cer (41:1) and Cer (43:1) are associated with poor physical function.

Aim 2. Evaluate the longitudinal relationship of plasma odd number fatty acid SLs with physical functional decline in older adults over time. Hypothesis 2: Low plasma SM (41:1), SM (41:2) SM (43:1), Cer (41:1) and Cer (43:1) are associated with steep physical function declines over 5 years.

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 use the cross-sectional study design to determine the association between 5 plasma SLs [SM (41:1), SM (41:2), SM (43:1), Cer (41:1) and Cer (43:1)] and 3 physical function measures and self-reported FS (Aim 1). We will use the cohort study design to determine the association of baseline 5 plasma SLs with physical function decline and self-reported FS decline in these same participants in Aim 2. We have randomly selected 400 participants based on the following eligibility criteria from the ARIC Visit 5/ARIC-NCS. We will compare the demographic of these 400 participants to the entire Visit 5 population to determine how representative they are of the entire Visit 5 population.

Eligibility criteria:

We have established the following inclusion criteria: (i) "frozen never-thawed" plasma samples from ARIC Visit 5 are available; (ii) brain MRI data from Visit 5/ARIC-NCS are available; and (iii) physical function data (grip strength, SPPB score, 4-m walking speed) are available from both Visits 5 and 6; (iv). self-reported functional status derived from standardized questionnaires which ascertain difficulty performing instrumental and basic activities of daily living and functional measures at two time points since V5 (not including V5, the study baseline) (i.e., GEN [2011] and GNB [2012]); (v). cognition data are available from both Visits 5 and 6. We will exclude participants included in the previous study.[11] Brain MRI and cognition data will be included as covariates in statistical analyses, because lacunar infarct detected by brain MRI and poor cognition are associated with physical function decline.[12, 13]

Study cohort selection:

R21 (R21_AG059068) selected 400 participants meeting the inclusion criteria described above. The cohort includes a similar number of men and women and at least 25% African Americans. The 400 participants do not include 383 participants included in our previous study.[8] In addition, 40 blind duplicates were included. Therefore, the total number of plasma samples for biochemical analysis will be 440.

Biochemical Analysis:

We have applied flow injection method of the Biocrates p400 kits to measure 5 plasma SLs [SM (41:1), SM (41:2), SM (43:1), Cer (41:1) and Cer (43:1)]. The method overall measures 55 acylcarnitines, 196 glycerophospholipids, 14 cholesteryl esters, 60 glycerides, and 40 sphingolipids [SM (n=31) and Ceramides (n=9)] including the 5 plasma SLs.

These 440 samples will be analyzed across 6 batches (each p400 kit can measure up to 80 samples). MedIDQ software used by the p400 kits normalize data based on quality control samples (e.g., plate- and analyte- specific normalization factors will be applied). The normalization may correct for batch difference between QC samples (i.e., QC2) across plate. However, the normalization may not correct for batch effects existed for samples. We will use quality control samples that are included in the p400 kits to monitor batch effects and overall variability of the biochemical measurement. Furthermore, the 40 blind duplicates will be analyzed by the ARIC Coordinating Center to evaluate variability of the biochemical analysis. Lastly, our statistical analysis will adjust for batch effects in regression models.

Primary and Secondary Outcomes:

Physical function as primary outcome. This study will consider 3 physical function outcomes measured at the ARIC Visits 5 and 6: grip strength, SPPB score and 4-meter walking speed. Even though 4-meter walking speed is included in the SPPB score, it will also be examined as a separate outcome, because it is the most clinically feasible of these measures, is considered

the sixth vital sign of health in older adults, and is a sensitive predictor of mortality and frailty.[14-16]

Grip strength (kg) was assessed in the participant's preferred hand using an adjustable, hydraulic grip strength dynamometer, with the better score from 2 trials used in the analysis.[17, 18] Lower extremity physical function will be assessed using the SPPB,[2] which is composed of 3 tasks: (i) repeated chair stands; (ii) balance (standing, semi-tandem, tandem); and (iii) a 4-meter usual-paced walk (m/s).[18] Walking aids will be allowed for the walking task only. Scores of 0–4 will be assigned for each task, based on timed performance of the 3 tasks using established age-based thresholds, then summed for a final score of 0–12. Higher scores will represent better function.

We will determine physical function decline using changes for each function measure over 5 years: grip strength (kg); 4-meter walking speed (m/s); and SPPB (score).

Self-reported FS as secondary outcome:

Self-reported functional status (FS) are assessed using a modified Rosow-Breslau questionnaire which was administrated during routine semi-annual telephone interviews. This study will use the FS questionnaire conducted during 2011-2017 administration of the GEN (2011), GNB (2012), GNE (2015), and GNF (2017) semi-annual questionnaires. Questions included in the FS questionnaire were based on the Rosow-Breslau Guttman scale of functional healthy for older people.[19] We will construct an FS score by summing responses (yes=1, no=0) across the following four questions, comprising a modified version of the Rosow-Breslau questionnaire.[20]

1. Are you able to do your usual activities, such as work around the house or recreation?
2. Are you able to walk up and down stairs without help?
3. Are you able to do heavy work around the house, such as shoveling snow or washing windows,
4. Are you able to walk half a mile without help?

Other variables of interests:

From our previous study,[8] we know that female gender, low prevalence of diabetes, low use of hypertension medication, low use of lipid lowering medications, low prevalence coronary heart disease, high concentration of total cholesterol or HDL cholesterol, high executive function z score, and lowprevalence of stroke are associated with high concentrations of SMs. Addditionally, female sex,[21] diabetes,[22] hypertension,[23] use of lipid lowering medications,[24] plasma total cholesterol (or HDL cholesterol),[25] executive function z score,[26] and stroke[20] are also associated with the presence of physical function impairment in older adults. Our previous study did not find a significant association between 3 odd chain fatty acid SMs and age or BMI,[8] likely due to the narrow age and BMI range in our previous study. Unlike our previous study, Mielke MM et al. has shown significant positive or biphasic association between SMs and age (as age increases, some SMs increase first and then decrease) and positive association between SMs and BMI.[27] Therefore, we will adjust analyses for age and BMI. Lastly, we will adjust all analyses for race-study center. The final analytical model will include the following ten factors (age, sex, BMI, race-site, diabetes,[22] hypertension,[23] use of lipid lowering medications, plasma total cholesterol [or HDL cholesterol], executive function z score, and stroke) as

covariates to evaluate whether plasma SMs are independently associated with physical function or its change.

All covariates will be ascertained at baseline (ARIC Visit 5), except sex, which was ascertained at ARIC study baseline (Visit 1).

Statistical analysis

Hypothesis driven data analysis

We will explore the distribution of each SLs (3 SMs and 2 Cers) and perform variable transformations if required. We will also assess correlations of each SLs. We will correct for multiple testing using the Bonferroni adjusted p value 0.01 (0.05/5).[28]

In Aim 1, we will test the ***association*** between plasma SLs and each primary outcome (i.e., grip strength, SPPB score and walking speed) and secondary outcomes (i.e., self-reported FS) using multivariable linear or logistic regression models as appropriate. In addition, we will examine the association of these 5 plasma SLs with self-reported functional status (FS) assessed using a modified Rosow-Breslau questionnaire which was administrated during routine semi-annual telephone interviews conducted in 2011-2013 (GEN [2011] or GNB [2012] whichever is closer [e.g., within +/- 6 months] to the ARIC Visit 5 exam for individual participants).

In Aim 2, we will estimate the association of plasma SLs measured at Visit 5 with physical function decline [measured at two time points, five years apart: ARIC Visit 5 (2011–2013) and ARIC Visit 6 (2016–2018)] or self-reported physical function decline between 2011-2017 [(GEN [2011], GNB [2012], GNE [2015], and GNF [2017]) using multivariate linear or logistic regressions, with the difference in function as the dependent variable. We will consider using Generalized Additive Models to evaluate dose response relations between plasma SLs and physical function decline. We will adjust for batch effects in the regression models.

Our study is a complete case study therefore we do not expect missing data. However, in case of missing data (e.g., to account for losses to follow-up between Visits 5 and 6 for the Aim 2 analysis), we will use guidelines established by the ARIC NCS Working Group and apply inverse probability of attrition weighting or multiple imputation with chained equations to account for the impact of attrition on our estimates of association.[29, 30] Lastly, for Aim 2 we will conduct additional sensitivity analyses that consider the influence of coexisting cognitive decline (i.e., change in the executive function z score) that will occur between Visits 5 and 6.

Hypothesis-generating data analysis

Because cholesterol esters and SLs measured by the p400 kits were not included in our previous study, we will also explore cholesterol esters (14) and other SLs (35) regarding their associations with physical function and decline using multivariable linear or logistic regression models. We will explore the distribution of each SLs and perform variable transformations if required. We will also assess correlations of each SLs. We will correct for multiple testing using the Bonferroni method or false discovery rate.[28]

Other anticipated methodological limitations or challenges

This study has a few limitations. First, this study itself does not have a replication cohort, although the study is an independent validation study of our previous study. Second, it has a moderate sample size of 400 participants. Third, our study is a complete case analysis since all these 400 selected participants should have outcome data available (i.e., physical function,

cognitive function, and self-reported functional status). Due to these eligibility criteria, there may be selection bias in that our study sample may not be representative of the entire ARIC Visit 5 population. Therefore, we will compare the demographics of this study to those of the entire ARIC Visit 5 study population. Lastly, despite our consideration of an extensive list of covariates, it is possible that there are unmeasured confounding factors. [31]

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? __X__ 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 ICTDER03 must be used to exclude those with value RES_DNA = "No use/storage DNA"? __X__ 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/mantrack/maintain/search/dtSearch.html

__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)?

MS1697 Functional status and cardiovascular disease

MS3350 Association of arterial stiffness with frailty among older adults

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

11.b. If yes, is the proposal
___ 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 https://www2.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

1. Tsai Y. Education and disability trends of older Americans, 2000-2014. *Journal of Public Health (Oxf).* 2016;10.1093/pubmed/fdw082:1-8

2. Guralnik JM, Simonsick EM, Ferrucci L, Glynn RJ, Berkman LF, Blazer DG, Scherr PA, Wallace RB. A short physical performance battery assessing lower extremity function: association with self-reported disability and prediction of mortality and nursing home admission. *Journal of Gerontology.* 1994;**49**(2):M85-94

3. Guralnik JM, Ferrucci L, Pieper CF, Leveille SG, Markides KS, Ostir GV, Studenski S, Berkman LF, Wallace RB. Lower extremity function and subsequent disability: consistency across studies, predictive models, and value of gait speed alone compared with the short physical performance battery. *Journals of Gerontology. Series A: Biological Sciences and Medical Sciences.* 2000;**55**(4):M221-31

4. Groessl EJ, Kaplan RM, Rejeski WJ, Katula JA, King AC, Frierson G, Glynn NW, Hsu FC, Walkup M, Pahor M. Health-related quality of life in older adults at risk for disability. *American Journal of Preventive Medicine.* 2007;**33**(3):214-8

5. Lucchinetti C, Bruck W, Parisi J, Scheithauer B, Rodriguez M, Lassmann H. Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. *Annals of Neurology.* 2000;**47**(6):707-17

6. Martin CL, Phillips BA, Kilpatrick TJ, Butzkueven H, Tubridy N, McDonald E, Galea MP. Gait and balance impairment in early multiple sclerosis in the absence of clinical disability. *Multiple Sclerosis.* 2006;**12**(5):620-8

7. Trapp BD, Nave KA. Multiple sclerosis: an immune or neurodegenerative disorder? *Annual Review of Neuroscience.* 2008;**31**:247-69

8. Li D, Misialek JR, Huang F, Windham BG, Yu F, Alonso A. Independent association of plasma hydroxysphingomyelins with physical function in the Atherosclerosis Risk in Communities (ARIC) study. *Journals of Gerontology. Series A: Biological Sciences and Medical Sciences.* 2017;10.1093/gerona/glx201

9. Foulon V, Sniekers M, Huysmans E, Asselberghs S, Mahieu V, Mannaerts GP, Van Veldhoven PP, Casteels M. Breakdown of 2-hydroxylated straight chain fatty acids via peroxisomal 2-hydroxyphytanoyl-CoA lyase: a revised pathway for the alpha-oxidation of straight chain fatty acids. *Journal of Biological Chemistry.* 2005;**280**(11):9802-12

10. Zöller I, Meixner M, Hartmann D, Büssow H, Meyer R, Gieselmann V, Eckhardt M. Absence of 2-hydroxylated sphingolipids is compatible with normal neural development but causes late-onset axon and myelin sheath degeneration. *Journal of Neuroscience.* 2008;**28**(39):9741-54
11. Li D, Misialek JR, Huang F, Windham GB, Yu F, Alonso A. Independent association of plasma hydroxysphingomyelins with physical function in the Atherosclerosis Risk in Communities (ARIC) study. *The Journals of Gerontology: Series A.* 2017;**73**(8):1103-10
12. Appelros P, Samuelsson M, Lindell D. Lacunar infarcts: functional and cognitive outcomes at five years in relation to MRI findings. *Cerebrovascular Diseases.* 2005;**20**(1):34-40
13. Black SA, Rush RD. Cognitive and functional decline in adults aged 75 and older. *Journal of the American Geriatrics Society.* 2002;**50**(12):1978-86
14. Cummings SR, Studenski S, Ferrucci L. A diagnosis of dismobility--giving mobility clinical visibility: a Mobility Working Group recommendation. *Journal of the American Medical Association.* 2014;**311**(20):2061-2.PMC5012417
15. Fritz S, Lusardi M. White paper: "walking speed: the sixth vital sign". *Journal of Geriatric Physical Therapy.* 2009;**32**(2):46-9
16. Studenski S, Perera S, Patel K, Rosano C, Faulkner K, Inzitari M, Brach J, Chandler J, Cawthon P, Connor EB, Nevitt M, Visser M, Kritchevsky S, Badinelli S, Harris T, Newman AB, Cauley J, Ferrucci L, Guralnik J. Gait speed and survival in older adults. *Journal of the American Medical Association.* 2011;**305**(1):50-8.PMC3080184
17. Kucharska-Newton AM, Palta P, Burgard S, Griswold ME, Lund JL, Capistrant BD, Kritchevsky SB, Bandeen-Roche K, Windham BG. Operationalizing Frailty in the Atherosclerosis Risk in Communities Study Cohort. *Journals of Gerontology. Series A: Biological Sciences and Medical Sciences.* 2017;**72**(3):382-8
18. Burke BT, Kottgen A, Law A, Windham BG, Segev D, Baer AN, Coresh J, McAdams-DeMarco MA. Physical Function, Hyperuricemia, and Gout in Older Adults. *Arthritis Care & Research.* 2015;**67**(12):1730-8.PMC4698232
19. Rosow I, Breslau N. A Guttman health scale for the aged. *Journal of Gerontology.* 1966
20. Kucharska-Newton A, Griswold M, Yao ZH, Foraker R, Rose K, Rosamond W, Wagenknecht L, Koton S, Pompeii L, Windham BG. Cardiovascular Disease and Patterns of Change in Functional Status Over 15 Years: Findings From the Atherosclerosis Risk in Communities (ARIC) Study. *J Am Heart Assoc.* 2017;**6**(3):e004144.PMC5523991
21. Valentine RJ, Misic MM, Rosengren KS, Woods JA, Evans EM. Sex impacts the relation between body composition and physical function in older adults. *Menopause (New York, NY).* 2009;**16**(3):518
22. Sinclair AJ, Conroy SP, Bayer AJ. Impact of diabetes on physical function in older people. *Diabetes Care.* 2008;**31**(2):233-5
23. Mitchell GF. Arterial stiffness and hypertension. *Hypertension.* 2014;**64**(1):13-8
24. Gray SL, Aragaki AK, LaMonte MJ, Cochrane BB, Kooperberg C, Robinson JG, Woods NF, LaCroix AZ. Statins, angiotensin-converting enzyme inhi bitors, and physical performance in older women. *Journal of the American Geriatrics Society.* 2012;**60**(12):2206-14
25. Schalk BW, Visser M, Deeg DJ, Bouter LM. Lower levels of serum albumin and total cholesterol and future decline in functional performance in older persons: the Longitudinal Aging Study Amsterdam. *Age and Ageing.* 2004;**33**(3):266-72
26. Carlson MC, Fried LP, Xue Q-L, Bandeen-Roche K, Zeger SL, Brandt J. Association between executive attention and physical functional performance in community-dwelling older women. *The Journals of Gerontology Series B: Psychological Sciences and Social Sciences.* 1999;**54**(5):S262-S70
27. Mielke MM, Bandaru VV, Han D, An Y, Resnick SM, Ferrucci L, Haughey NJ. Factors affecting longitudinal trajectories of plasma sphingomyelins: the Baltimore Longitudinal Study of Aging. *Aging Cell.* 2015;**14**(1):112-21

28. Hochberg Y. A Sharper Bonferroni Procedure for Multiple Tests of Significance. *Biometrika.* 1988;**75**(4):800-2
29. Rawlings AM, Sang Y, Sharrett AR, Coresh J, Griswold M, Kucharska-Newton AM, Palta P, Wruck LM, Gross AL, Deal JA. Multiple imputation of cognitive performance as a repeatedly measured outcome. *European Journal of Epidemiology.* 2016:1-12
30. Weuve J, Tchetgen EJT, Glymour MM, Beck TL, Aggarwal NT, Wilson RS, Evans DA, de Leon CFM. Accounting for bias due to selective attrition the example of smoking and cognitive decline. *Epidemiology.* 2012;**23**(1):119-28
31. Living cell microarray