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# Association of Cardiovascular Disease Risk Factors with Preclinical Biomarkers of Alzheimer's Disease in the Emory Healthy Brain Study Cohort

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By

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M.B.B.S, University of Balochistan, 2022
B.A., University of Balochistan, 2018
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# Abstract

## Association of Cardiovascular Disease Risk Factors with Preclinical Biomarkers of Alzheimer's Disease in the Emory Healthy Brain Study Cohort

By Rida Akbar

**Background:** Alzheimer's disease (AD), a leading cause of dementia and severe cognitive impairment, remains asymptomatic for decades before clinical symptoms emerge. Recent evidence suggests that cardiovascular health metrics may influence early neurodegenerative changes associated with AD. This study examines the relationship between cardiovascular disease (CVD) risk factors and AD biomarkers, aiming to uncover potential early intervention targets.

**Objective:** The primary aim of this research is to test the hypothesis that cardiovascular health risk factors, as defined by the American Heart Association's Life's Essential 8 metrics, are associated with AD-specific biomarkers, including Beta-amyloid 42 ($A\beta_{42}$), total Tau (tTau), and phosphorylated Tau (pTau), measured in cerebrospinal fluid (CSF) samples.

**Methods:** This cross-sectional study utilized comprehensive datasets from the Emory Healthy Aging Study (EHAS) and Emory Healthy Brain Study (EHBS) cohorts. Data from participants (N=826) aged 50-75 years, was gathered through self-reported online questionnaire and clinical assessments from in-person visits. These characteristics included demographic information, lifestyle and health behaviors, clinical health indicators, and biomarker profiles. These biomarker data were analyzed by immunoassays run on CSF samples. Multiple linear regression models were applied to assess the associations between CVD risk factors: Diet, Sleep, Physical activity, Smoking, BMI, and Hypertension with AD biomarkers: $A\beta_{42}$, Ttau and Ptau, while adjusting for potential confounders.

**Results:** The regression analyses showed a significant association between physical activity and increased $A\beta_{42}$ concentrations (Model 1: $\beta = 0.31$, $p=0.003$), indicating a protective effect. Unexpectedly, adherence to a healthy diet was also significantly associated with increased levels of both tTau and pTau biomarkers. While smoking and sleep duration influenced A $\beta$ 42 levels, these associations did not achieve statistical significance.

**Conclusion:** This study provided the basis of association between CVD risk factors and AD biomarkers in the EHBS cohort. Our findings emphasize the importance of modifiable lifestyle factors and health behaviors, in promoting cardiovascular health that could also benefit cognitive health. The study calls for further research to explore the underlying mechanisms through which lifestyle factors affect AD- biomarkers for early diagnosis. Public health policies should enhance the quality of life for the aging population by supporting broader efforts to mitigate the global burden of AD.

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## Background

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that significantly impairs cognitive functions such as memory, executive functioning, and the ability to perform daily activities. The global prevalence of AD presents a pressing public health challenge. Projections indicate that by 2050, the number of individuals living with dementia could exceed 139 million, predominantly in low- and middle-income countries [1]. Annually, AD is more lethal than the combined toll of breast and prostate cancer, highlighting its severity [2]. Additionally, the lifetime risk of developing AD for women is twice that of men, emphasizing the gender disparity in AD risk [2]. Most Americans express a preference for early diagnosis, hoping it might lead to more effective treatment options [2].

The multifaceted nature of AD is attributed to complex etiological mechanisms, including genetics, epigenetics, and comorbid medical conditions such as diabetes and hypertension. Recent research has underscored the association between vascular risk factors and biomarkers indicative of AD, such as changes in cerebrospinal fluid (CSF) tau markers, correlating with cognitive decline in preclinical stages [3]. Furthermore, plasma total tau levels have emerged as a promising predictive tool for the early detection of AD [4]. Notably, alterations in CSF biomarkers, including $A\beta_{42}$ and Tau levels, begin up to two decades before the clinical onset of AD symptoms [5], underscoring the potential of early biomarker identification in delaying or preventing the progression of late-onset AD. The American Heart Association's Life's Essential 8, an expansion of the original Life's Simple 7 to include sleep, outlines critical modifiable and non-modifiable lifestyle health factors for improved cardiovascular health [6]. Adherence to these factors has

been linked to a lower risk of cardiovascular disease (CVD) [7], diabetes, cognitive impairment, and even benefits to cancer patients [6]. Optimal cardiovascular health, as defined by these criteria, is associated with a significantly reduced incidence of cognitive impairment [6]. This supports Life's Essential 8 as a comprehensive strategy to mitigate the risk of cognitive impairment and various chronic diseases across different populations [6]. Despite this, studies characterizing the relationship between cardiometabolic risk factors and preclinical biomarkers in the progression of AD remain scarce.

This thesis investigates the complex relationship between CVD risk factors and preclinical CSF biomarkers of AD, using data from the Emory Healthy Brain Study cohort, as numerous studies have shown established links between hypertension, diabetes, dyslipidemia and risk of AD and dementia. [5] This research question is of high impact and significant to preventive clinical research. It aligns with broader public health aims to enhance quality of life and reduce healthcare costs through effective preventive measures. By offering novel insights into early intervention and prevention strategies, this study aims to transform the approach to managing AD, potentially altering the course of the disease through early detection and lifestyle modifications.

# Materials and Methods

## Emory Healthy Aging and Brain Studies

This research utilized subsets of data from the Emory Healthy Brain Study (EHBS) and the Emory Healthy Aging Study (EHAS), that has been going on since 2016 [5, 8]. These studies enrolled consenting, English-speaking adults from the U.S., representing diverse ethnicities [8]. These

individuals had no prior or present self-reported diagnosis of AD or any other cognitive impairment [5]. Designed as community-based prospective cohorts, these studies aimed to explore the relationships between health and aging, particularly how understanding these relationships could help delay morbidity and promote health and independence among older adults [8]. A significant aspect of this research was examining the link between cardiometabolic health risk factors and biomarkers for AD progression to understand the disease's pathogenesis and identify early manifestations of age-related illnesses [8].

## Study Population

EHAS was primarily an online longitudinal study, enrolling participants, both men and women over 18 years old, who consented to future contact for studies. The annually administered questionnaire collected extensive information on participant demographics, socioeconomic status, lifestyle, psychosocial factors, past or present medical conditions, among other areas [8]. Meanwhile, EHBS focused on a narrower demographic, enrolling participants aged 50–75 years who met a defined health criterion. The exclusion criteria from the study were having a Body Mass Index (BMI) $\geq 32$, anti-coagulation use, chronic medico-surgical conditions including Congestive Heart Failure, stroke, AD or other dementias, epilepsy, Multiple Sclerosis (MS), kidney disease on dialysis, cancer not in remission $\geq 5$ years, HIV (Human Immunodeficiency Virus), active TB and untreated Hepatitis B or C infection [8].

This cohort underwent thorough assessments at biennial visits to the Emory Brain Health Center, including neuropsychological testing, brain imaging, cardiovascular measures, and biospecimen (blood and CSF) collection [5]. All participants provided informed consent, and the study protocols were approved by the Emory University Institutional Review Board [5]. The datasets

for this thesis were accessed with approval from the EHBS's principal investigator at Emory University's School of Medicine.

## Study Design

A cross-sectional study design was used to explore the association between cardiovascular disease risk factors and biomarkers indicative of preclinical Alzheimer's Disease. Comprehensive datasets were used from the Emory Healthy Aging Study and Emory Healthy Brain Study, including those containing demographic information, biomarkers profile, cardiovascular health indicators, and relevant data from the questionnaire to use for this study.

### Exposure Variables Categorization

The exposure variables for this study were rigorously defined according to the metric for cardiovascular health by the American Heart Association, known as Life's Essential 8. This includes scoring an algorithm for health behaviors and factors such as diet, physical activity, nicotine exposure, sleep, BMI, blood lipid and glucose profile, and blood pressure, each contributing to a composite cardiovascular health score [6]. These recommendations are important in prescribing a healthy lifestyle to lower the risk of developing cardiometabolic or heart disease. These include the following for adults [6]:

1. Maintaining cholesterol/triglyceride levels below 200 mg/dL without medication.
2. Keeping fasting glucose below 100 mg/dL without medication.
3. Ensuring blood pressure remains under 120/80 mmHg without medication.
4. Being a non-smoker or having ceased smoking for more than a year.
5. Maintaining a BMI below 25 $kg/m^2$.
6. Engaging in at least 150 mins of moderate or 75 mins of vigorous exercise weekly.

7. Following the Dietary Approaches to Stop Hypertension (DASH) diet, which includes consuming at least 4.5 cups of fruits and vegetables daily, at least two weekly fish servings, and at least three daily servings of whole grains, while limiting sugar-sweetened beverages to 36 oz/week and sodium intake to 1500 mg/day.
8. Ensuring an adequate night-time sleep duration of 7-9 hours.

**Outcome Variables Assessment**

Different biomarkers associated with preclinical AD, specifically $A\beta_{42}$, tTau and pTau were measured from CSF samples of participants collected from standardized methods. Trained clinicians performed lumbar puncture (LP) procedures to collect CSF samples from participants who had fasted for at least 6 hours prior to using sterilized equipment [5]. After clearing any blood contamination, CSF samples were placed in dry ice and transferred to –80°C freezers. Assays were performed on them for the specific biomarkers ($A\beta_{42}$, tTau and pTau) using Elecsys CSF immunoassays on a cobas e 601 analyzer [5]. In biomarker studies, CSF biomarker and Total Tau concentrations below ($A\beta_{42}$) or above (tTau and pTau) a specific threshold are considered biomarker-positive. Previously established cut-off value for $A\beta_{42}$ is 1,100 pg/mL , while optimal tTau and pTau cut-offs are approximated at 300 pg/mL and 27 pg/mL [9]. One more study analyzed the EHBS cohort biospecimens, where a tTau/$A\beta_{42}$ ratio > 0.24 was considered biomarker-positive for asymptomatic AD [5]. These are considered as robust biomarkers for predicting risk of clinical decline and conversion to dementia in non-demented patients, and they are also helpful in AD diagnosis in clinical practice [9].

**Inclusion of Indicator Variables**

From the EHAS questionnaire and EHBS study data, indicators of socioeconomic status and demographic information obtained included age, gender, primary race, highest level of education

and income levels. Other covariates like the presence of comorbid conditions including hypertension, diabetes and high cholesterol were also included, as well as the use of medication for these medical conditions. Except age, all the data included was self-reported from the EHAS questionnaire.

## Statistical Analysis

The primary analytical framework for this study was based on multiple linear regression modeling to dissect the association between CVD risk factors and preclinical AD biomarkers, quantified through continuous measures of $A\beta_{42}$, tTau, and pTau. The regression models were structured into two distinct categories: Model 1 (Reduced Model) and Model 2 (Full Model), each tailored to the unique requirements of the study's hypotheses and data structure. Model 1 adjusted for basic sociodemographic factors including age, gender, race, income, and education level. This model facilitated initial insights into the direct relationships without the influence of extended covariates. Model 2 expanded on the first by incorporating a broader set of covariates, including all other exposure variables, alongside health-related variables like self-reported diagnosis and use of medication for diabetes, hypertension, and high cholesterol.

Extensive exploratory data analysis was conducted to prepare a refined and an integrated final dataset for comprehensive analysis. This involved standardizing and merging multiple data sources, variable transformations like encoding, creation or formatting necessary adjustment. Specific imputation methods based on the degree of missingness were also applied. Exposure variables were selected based on their relevance to cardiovascular health assessment according to the metric set by the AHA's Life's Essential 8. These included BMI, systolic BP (Blood Pressure), smoking status,

physical activity, dietary adherence, and sleep duration. Total physical activity was quantified by aggregating the durations of mild and moderate exercise and multiplying these by their respective frequencies per week, creating a continuous variable. Moreover, smoking status was categorized into never smokers, former smokers, and current smokers to capture tobacco exposure variations.

To assess dietary habits aligned with the AHA guidelines, we utilized the EHAS questionnaire data that contained detailed information about different dietary components with serving sizes. For example, the serving size for fruits and vegetables was measured in cups, and for fish, a 3.5-ounce portion size was used. Whole grains were measured in ounces or cups. For sugar-sweetened beverages, the total number of sugar-beverages consumed per week was measured. From this data, we made a composite diet score variable. For fruits and vegetables, a point was assigned if the combined daily consumption was more than 4.5 cups. For whole grains, a point was given for consuming over 21 ounces per week. Fish intake earned a point at two or more servings per week, and for sugar-sweetened beverages, a point was allocated if consumption was limited to 4.5 servings or less per week. These points were then summed to create an overall diet score for each participant, which ranged from 0 (no adherence) to 3 (full adherence), providing a graded metric of dietary compliance.

Hypertension was defined as a binary variable that identified individuals as hypertensive if they either had a SBP $\geq$ 140 mm Hg or they were on anti-hypertensive medication. This definition includes both controlled and uncontrolled hypertension.

The outcome variables for the analysis consisted of biomarkers associated with AD as identified in established literature, that include continuous measures of $A\beta_{42}$, tTau, and pTau in the CSF.

Additional covariates included in the analysis comprised age, gender, primary race, income, and education level. Data for age, gender, and education were collected during participant visits for the EHBS study, while data for other variables were obtained from the EHAS questionnaire that was self-reported. The income variable was ordinally categorized into “Low” , “Middle”, “Upper-middle” and “High”, each corresponding to income ranges of “Under $30,000”, “$30,000 to $59,999” , “$60,000” and “Over $100,000” per year. Racial categories included White, Black, and Other, which encompassed Asian, American Indian/Alaskan Native, and Native Hawaiian/Other Pacific Islander, to accommodate the low representation of these groups in the "Other" category. Education levels were converted into factored ordinal variables as specified in the codebook: "Less than high school (HS)," "HS graduate (GED)," "College graduate," "Master's degree," "Associate degree," and "Doctorate." We also incorporated binary variables for the presence of hypertension, diabetes, and high cholesterol, as well as for the use of medications for these conditions.

To ensure standardized data quality practices in our study, we handled missing data by utilizing imputation techniques depending on the nature and extent of missing data. For continuous and categorical variables with < 20% missingness, we used single imputation techniques to adjust for the missingness. Mean was used to replace the missing values for continuous variables (BMI, SBP, diastolic BP and heart rate) and mode for categorical variables (education and sleep hours). For diet variable, missing data was assumed to be in the non-adherence category (i.e. diet score = 0).

For more substantive missing data of > 45%, particularly cholesterol and triglycerides, multiple imputation method was used with Predictive Mean Matching (PMM). However, due to significant inaccuracies in cholesterol measurements, which averaged 246 mg/dL—well above the normal threshold (< 200 mg/dL) —and high levels of missing data, cholesterol and triglycerides were

excluded from the final modeling analysis. This exclusion was essential to mitigate potential biases and maintain the integrity of our regression analysis.

## Results

The initial EHAS study cohort included 17,831 participants. After merging with other demographic datasets and ensuring completeness of biomarker data from the EHBS study, the final analytic sample was reduced to 826 participants **(Figure 1).** A summary of the baseline characteristics stratified by gender shows that more females (N =571, Mean age=62) were present than men (N =255, Mean age=63) were present in the study cohort **(Table 1).** Other key characteristics included demographic information, clinical indices, biomarker profiles and lifestyle habits of the study population**.** In the regression analyses results exploring the association between CVD risk factors and preclinical AD biomarkers, several key findings were found **(Table 2, 3 and 4).**

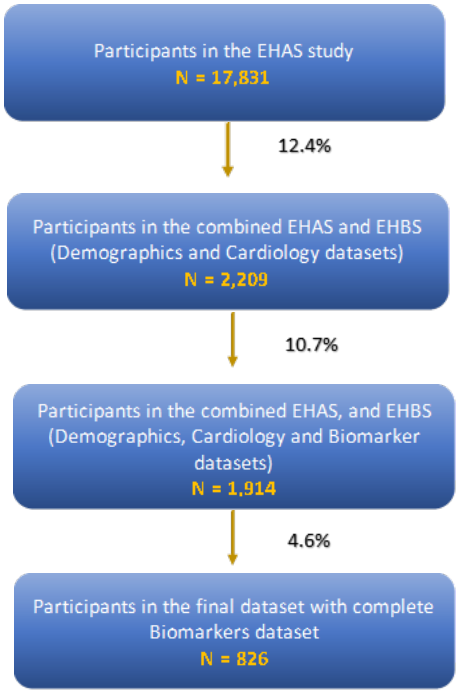
For $A\beta_{42}$ outcome **(Table 2)**, physical activity was significantly associated with an increase in $A\beta_{42}$ concentrations, showing a possible protective effect, in both Models 1 and 2 [Model 1: $\beta$ = 0.31 (95%CI: 0.11, 0.50), p=0.003; Model 2: $\beta$ = 0.30 (95%CI: 0.09, 0.50), p=0.005]. In contrast, BMI, hypertension, diet and sleep did not show any significant associations with $A\beta_{42}$ levels in either model.

In addition, current smokers (vs. never smokers) showed a larger effect size in $A\beta_{42}$ levels (Model 1: $\beta$ = 126.67, p=0.103; Model 2: $\beta$ = 129.37, p=0.097) than former smokers (vs. never smokers) (Model 1: $\beta$ = 36.73, p=0.167; Model 2: $\beta$ = 26.78, p=0.324), these associations did not reach

statistical significance. Similarly, compared to individuals who slept 7.5 hours per night, individuals reporting < 5 or 3.5 hours of sleep per night had the highest β-coefficient for $A\beta_{42}$ (Sleep 3.5 - Model 1: β = 183.02, p=0.098 ; Model 2: β =194.59 , p=0.078 ), with negligible effects for those sleeping 5.5 or 9.5 hours per night.

Moreover, in both models, the effect sizes for diet adherence categories (Diet scores 1,2 &3), relative to the non-adherent reference group (Diet score 0), showed negative β-coefficients for $A\beta_{42}$. These findings also were not statistically significant.

## Figure 1




For tTau outcome **(Table 3)**, hypertension showed a significant association with tTau in Model 1, suggesting a protective effect [β = -10.22 (95% CI: -20.26, -0.17), p = 0.046], although the

association in Model 2 was not statistically significant [β = -9.55 (95% CI: -19.97, 0.88), p = 0.073].

For BMI, the effect size across both models for Ttau levels, showed a decrease with an increase in BMI, which was statistically significant in Models 1 and 2 [Model 1: $\beta$ = -1.42, 95% CI: (-2.78, -0.05), p=0.042] [Model 2: $\beta$ = -1.78, 95% CI: (-3.24, -0.33), p=0.016], implying that as BMI increases, there is a small but statistically significant decrease in Ttau levels. Physical activity and sleep did not show a significant relationship with Ttau levels in either model.

Among former smokers (vs. non-smokers), there was a positive association with Ttau levels relative to non-smokers, in both models, reaching statistical significance in Model 2 [β = 14.30 (95% CI: 2.92, 25.68), p = 0.014]. The effect size among current smokers(vs. non-smokers) was inversely related to Ttau levels, but this did not reach significance level.

Higher diet adherence (Diet score 3) seemed to show a statistically significant positive association with Ttau levels in both the models [Model 1: $\beta$ = 22.77, 95% CI: (4.02, 41.53), p=0.017; Model 2: $\beta$ = 19.85, 95% CI: (1.00, 38.71), p=0.039]. Other diet scores did not show any significant association with Ttau.

For pTau outcome **(Table 4)**, hypertension showed a statistically significant inverse association with pTau levels in Model 1 [β = -1.09 (95% CI: -2.15, -0.03), p = 0.045], indicating that hypertension may be associated with lower Ptau concentrations, but this association did not approach significance in Model 2 [Model 2: β = -1.04 (95% CI: -2.14, 0.07), p = 0.065].

Former smokers showed a significant positive association with Ptau in both models [Model 1: $\beta$ = 1.28, p = 0.034; Model 2: $\beta$ = 1.62, p = 0.009], indicating higher Ptau levels relative to non-smokers. However, for current smokers, the effect size was negative with when compared to non-smokers and was not significant.

Additionally, a significant positive association was seen for Diet score 3 with pTau levels [Model 1: $\beta$ = 2.67, p = 0.009; Model 2: $\beta$ = 2.40, p = 0.019], suggesting that a diet with high adherence to the AHA dietary guidelines may be associated with higher Ptau levels.

There was no significant association observed for other exposure variables including BMI, physical activity, and sleep with pTau levels.

## Discussion

Our study provides useful insights in understanding the complex and intricate relationships of cardiometabolic health, in particular how CVD risk factors and preclinical AD is associated in the EHAB study cohort. As highlighted by AHA's Life's Essential 8, understanding the impact of modifiable lifestyle factors such as BMI, diet, physical activity, sleep, smoking status and to an extent hypertension, on our health, particularly its link with neurodegenerative disorders like AD remains a high priority [6]. This exploration is particularly pertinent as AD and CVD share several common risk factors, and optimizing cardiovascular health may have a positive impact on cognitive decline and AD progression.

The significant association found between physical activity and increased concentrations of $A\beta_{42}$, warrants caution in interpretation. Previous research suggests that exercise may have a neuroprotective effect, potentially delaying the onset of AD [13]. Physical activity is also known to promote neurogenesis, enhancing brain vascularization, and even influencing the clearance of amyloid plaques [14], which might temporarily increase Aβ42 concentrations in the CSF.

The observed protective but non-significant association between current smoking status and Ttau was counterintuitive. Former smokers showing higher Ttau and pTau levels may reflect a cumulative impact of past smoking behavior, potentially indicating long-term effects on neuronal integrity. Cigarette smoking has been shown to impact neuroinflammation and other pathophysiological processes related to AD [15]. Incorporating objective measures of smoking exposure, such as nicotine levels, could provide a clearer picture of how smoking and its cessation influence AD pathophysiology.

Interestingly, the statistically significant association between high adherence to healthy dietary habits (Diet score 3) and increased tTau and pTau levels raises questions about potential confounders or the extent to which these dietary guidelines are beneficial to cognitive health outcomes, since the AHA dietary guidelines are primarily targeted to improve cardiovascular health [6]. To further explore these associations, future studies could implement stratified or sensitivity analyses using robust statistical models and potentially more comprehensive dietary assessment tools.

The relationship between BMI and Ttau also provides a compelling insight, with a higher BMI associated with a decrease in Ttau levels in Model 2, suggesting a nuanced protective effect of

BMI on tau levels. The exact mechanism behind this association requires further research, considering the typically adverse effects of obesity on health. Similarly, the findings for hypertension showed a protective association with Ttau protein levels, suggesting beneficial effects. Moreover, our study further refines the understanding of these associations by suggesting that detailed analyses of dietary patterns and physical activity specifics could illuminate their direct and indirect impacts on AD biomarkers.

The relationship between non-smoking status and elevated Aβ42 levels, though counterintuitive, may point to potential confounders or reflect the residual impacts of past smoking habits, which could affect both cardiovascular and cognitive health. Incorporating objective measures of smoking exposure, such as cotinine levels, could provide a clearer picture of how smoking and its cessation influence AD pathophysiology. Cigarette smoking has been shown to impact neuroinflammation and other pathophysiological processes related to AD [14], showing the complexity of its relationship with neurodegenerative markers. Interestingly, the association of a higher diet score with increased tau biomarker concentrations suggests that even healthy dietary patterns might have complex interactions with AD biomarkers. To further explore these associations, future studies could implement stratified or sensitivity analyses using robust statistical models and potentially more comprehensive dietary assessment tools.

## Strengths

Our study uses extensive data from two large, community-based cohorts, the EHAS and EHBS, which enhance the robustness and generalizability of our findings. The inclusion of diverse

participants across a broad geographic and demographic spectrum allows our results to reflect a wide range of population characteristics, enhancing their applicability to public health scenarios.

Furthermore, we adopted a structured framework using AHA guidelines for evaluating cardiovascular health, ensuring a comprehensive assessment of exposure variables relevant to cardiovascular health. This framework guided our categorization of lifestyle and health metrics, providing a consistent basis for our analysis. The use of advanced statistical analyses to adjust for covariates, strengthens the reliability of our associations. By controlling these variables, we could isolate the effects of specific cardiovascular risk factors on AD biomarkers, thereby enhancing the credibility of our findings.

## Limitations

Despite these strengths, our study faces several limitations that must be considered. The cross-sectional design of our research limits our ability to establish causality between cardiovascular risk factors and AD biomarkers. Implementing a longitudinal study could also help show a temporal relationship between lifestyle changes and alterations in AD biomarkers, providing a stronger basis for causal inference [16]. Moreover, integrating genetic and epigenetic data could offer insights into how individual biological differences affect the relationship between cardiometabolic health and AD, enabling more personalized preventive strategies [17].

Our findings might also have limited applicability to other populations due to the specific selection criteria and demographic focus on individuals aged 50–75. The reliance on self-reported measures for diet, physical activity, and medical history introduces potential biases. These self-reported data are subject to recall inaccuracies and personal bias, which could affect the reliability of our risk factor assessments.

Additionally, the exclusion of cholesterol data due to measurement inaccuracies removed a significant cardiovascular risk factor from our analysis, potentially skewing the associations with AD. Potential collinearity among independent variables presents another challenge, complicating the interpretation of their individual contributions to the observed outcomes. This issue, along with residual confounding by variables not adequately measured or controlled for, could distort the true effects of the studied factors.

Lastly, the relatively small final sample size (N=826), while sufficient for broad analyses, limits the statistical power to detect more subtle associations. This constraint is critical when considering the variability of biomarkers and the complex interplay of multiple risk factors influencing AD.

## Conclusion

This study provides useful and interesting insights to help us understand how CVD risk factors, as outlined by the AHA's Life's Essential 8, come into play with biomarker profiles for AD in the Emory Healthy Brain Study (EHBS) cohort. Our findings suggest that lifestyle and health behaviors may be associated with the biomarker profile for preclinical AD. These include physical activity, BMI, hypertension, adherence to dietary guidelines, optimal sleep patterns, and smoking status.

Such modifications align with preventive health strategies that might facilitate early diagnosis and impede the disease's progression in its asymptomatic stages. Our research underscores the imperative for ongoing investigations that could inform clinical practices and public health

policies aimed at mitigating the burgeoning impact of AD. Thereby improving quality of life, by reducing the burden on healthcare systems and resources, particularly in the low- and middle-income countries where AD is a looming crisis [1].

# Appendix

## Table 1: Summary of baseline demographic, lifestyle, and clinical characteristics of the study population.

Baseline Characteristics of Study Participants

Summary of demographic, lifestyle and clinical characteristics of study population stratified by gender

| Variable | Female, N = 571[1] | Male, N = 255[1] |
|---|---|---|
| **Age (Years)** | 62 (7) | 63 (7) |
| **Race** | | |
| Black | 79 (14%) | 24 (9.4%) |
| White | 470 (82%) | 223 (87%) |
| Other | 22 (3.9%) | 8 (3.1%) |
| **BMI (kg/m²)** | 25.9 (3.9) | 26.9 (3.0) |
| **Education Level** | | |
| Less than HS | 1 (0.2%) | 0 (0%) |
| HS graduate (GED) | 8 (1.4%) | 5 (2.0%) |
| Associate degree | 42 (7.4%) | 14 (5.5%) |
| College graduate | 294 (51%) | 125 (49%) |
| Master's degree | 156 (27%) | 67 (26%) |
| Professional degree | 70 (12%) | 44 (17%) |
| Doctorate | 0 (0%) | 0 (0%) |
| **Income Level** | | |
| Low (Under $30,000) | 57 (10.0%) | 12 (4.7%) |
| Middle ($30,000 - $59,999) | 71 (12%) | 30 (12%) |
| Upper-middle ($60,000 - $100,000) | 149 (26%) | 47 (18%) |
| High (Over $100,000) | 294 (51%) | 166 (65%) |
| **Smoking Status** | | |
| Never Smoker | 415 (73%) | 178 (70%) |
| Current Smoker | 11 (1.9%) | 8 (3.1%) |
| Former Smoker | 145 (25%) | 69 (27%) |

[1] Mean (SD); n (%)

**Table 1** (Continued)

| Baseline Characteristics of Study Participants<br>Summary of demographic, lifestyle and clinical characteristics of study population stratified by gender | | |
|---|---|---|
| **Variable** | **Female, N = 571**[1] | **Male, N = 255**[1] |
| **Sleep (hrs/day)** | | |
| <5 | 7 (1.2%) | 2 (0.8%) |
| 5-6 | 158 (28%) | 66 (26%) |
| 7-8 | 367 (64%) | 177 (69%) |
| ≥9 | 39 (6.8%) | 10 (3.9%) |
| **Diet Score (AHA guidelines)** | | |
| 0 | 336 (59%) | 136 (53%) |
| 1 | 107 (19%) | 62 (24%) |
| 2 | 85 (15%) | 38 (15%) |
| 3 | 43 (7.5%) | 19 (7.5%) |
| 4 | 0 (0%) | 0 (0%) |
| **Exercise (mins/wk)** | 116 (118) | 132 (108) |
| **Cholesterol (mg/dL)** | 269 (46) | 236 (44) |
| **Triglycerides (mg/dL)** | 65 (36) | 71 (36) |
| **Systolic BP (mmHg)** | 136 (18) | 139 (15) |
| **Diastolic BP (mmHg)** | 76 (10) | 82 (8) |
| **Heart Rate (beats/min)** | 65 (10) | 61 (12) |
| **Hypertension** | 160 (28%) | 91 (36%) |
| **High Cholesterol** | 259 (45%) | 144 (56%) |
| **Diabetes** | 32 (5.6%) | 27 (11%) |
| **Beta-Amyloid 42 (pg/dL)** | 1,077 (331) | 1,082 (324) |
| **Total-Tau (pg/dL)** | 179 (78) | 164 (58) |
| **Phosphorylated-Tau (pg/dL)** | 16.1 (8.2) | 14.8 (6.4) |

[1] Mean (SD); n (%)

**Table 2: Regression Analysis of Cardiovascular Disease Risk Factor and Their Association with Beta-Amyloid 42 Levels in Preclinical Alzheimer's Disease Across Different Exposure Levels (BMI, Hypertension, Physical activity, Smoking status, Diet Score and Sleep).**

| Exposure Variable | Levels | Aβ42 | | | | | |
|---|---|---|---|---|---|---|---|
| | | Model 1 | | | Model 2 | | |
| | | Estimate (95% CI) | Std. Error | P-value | Estimate (95% CI) | Std. Error | P-value |
| **BMI** | | -5.81 (-12.18 , 0.56) | 3.24 | 0.074 | -4.42 (-12.23 , 1.25) | 3.47 | 0.203 |
| **Hypertension** | | 10.29 (-36.66 , 57.24) | 23.92 | 0.667 | 12.79 (-36.05 , 61.63) | 24.88 | 0.607 |
| **Physical Activity** | | 0.31 (0.11 , 0.50) | 0.1 | 0.003* | 0.30(0.09 , 0.50) | 0.1 | 0.005* |
| **Smoking** | **Non-smoker (Ref)** | - | - | - | - | - | - |
| | Smoker Current | 126.67 (-25.55, 278.90) | 77.55 | 0.103 | 129.37 (-23.44, 282.17) | 77.85 | 0.097 |
| | Smoker Former | 36.73 (-15.42, 88.87) | 26.57 | 0.167 | 26.78 (-26.50, 80.06) | 27.14 | 0.324 |
| **Diet** | **Diet Score 0 (Ref)** | - | - | - | - | - | - |
| | Diet Score 1 | -19.67 (-77.77, 38.42) | 29.6 | 0.506 | -15.68 (-73.91, 42.55) | 29.67 | 0.597 |
| | Diet Score 2 | -17.32 (-82.83, 48.19) | 33.37 | 0.604 | -22.65 (-88.03, 42.73) | 33.31 | 0.497 |
| | Diet Score 3 | -23.57 (-111.36, 64.22) | 44.72 | 0.598 | -42.44 (-130.74, 45.86) | 44.99 | 0.346 |
| **Sleep** | **Sleep Hrs 7.5 (Ref)** | - | - | | - | - | - |
| | Sleep Hrs 5.5 | -0.09 (-52.96, 52.79) | 26.94 | 0.997 | 9.47 (-43.90, 62.85) | 27.19 | 0.728 |
| | Sleep Hrs 3.5 | 183.02 (-34.07, 400.11) | 110.6 | 0.098 | 194.59 (-22.13, 411.30) | 110.4 | 0.078 |
| | Sleep Hrs 9.5 | -1.97 (-98.39, 94.45) | 49.12 | 0.968 | -9.46 (-106.18, 87.26) | 49.27 | 0.848 |

Footnote:
Asterisks (*) indicate p-values < 0.05, indicating statistical significance.
Model 1: Exposure variable ; Gender, Age, Race, Income, and Education.
Model 2: All Model 1 variables ; Hypertension, Diabetes, High Cholesterol ; medication use for Hypertension, Diabetes and High Cholesterol.

**Table 3: Regression Analysis of Cardiovascular Disease Risk Factor and Their Association with Total-tau Levels in Preclinical Alzheimer's Disease Across Different Exposure Levels (BMI, Hypertension, Physical activity, Smoking status, Diet Score and Sleep).**

| Exposure Variable | Levels | Ttau | | | | | |
|---|---|---|---|---|---|---|---|
| | | Model 1 | | | Model 2 | | |
| | | Estimate (95% CI) | Std. Error | P-value | Estimate (95% CI) | Std. Error | P-value |
| **BMI** | | -1.42 (-2.78 , -0.05) | 78.19 | 0.042 | -1.78 (-3.24 , -0.33) | 0.74 | 0.016* |
| **Hypertension** | | -10.22 ( -20.26 , -0.17) | 5.12 | 0.046* | -9.55 (-19.97 , 0.88) | 5.31 | 0.073 |
| **Physical Activity** | | 0.01(-0.03 , 0.05) | 0.02 | 0.634 | -0.01(-0.05 , 0.04) | 0.02 | 0.78 |
| **Smoking** | **Non-smoker (Ref)** | - | - | - | - | - | - |
| | Smoker Current | -15.82 (-48.44, 16.80) | 16.62 | 0.341 | -14.79 (-47.42, 17.84) | 16.62 | 0.374 |
| | Smoker Former | 11.15 (-0.02, 22.33) | 5.69 | 0.05* | 14.30 (2.92, 25.68) | 5.8 | 0.014* |
| **Diet** | **Diet Score 0 (Ref)** | - | - | - | - | - | - |
| | Diet Score 1 | 5.89 (-6.53, 18.30) | 6.32 | 0.352 | 5.73 (-6.70, 18.16) | 6.33 | 0.366 |
| | Diet Score 2 | -1.18 (-15.18, 12.81) | 7.13 | 0.868 | -2.84 (-16.79, 11.12) | 7.11 | 0.69 |
| | Diet Score 3 | 22.77 (4.02 , 41.53) | 9.56 | 0.017* | 19.85 (1.00 , 38.71) | 9.61 | 0.039* |
| **Sleep** | **Sleep Hrs 7.5 (Ref)** | - | - | - | - | - | - |
| | Sleep Hrs 5.5 | 7.62 (-3.72, 18.96) | 5.78 | 0.187 | 7.81 (-3.62, 19.23) | 5.82 | 0.18 |
| | Sleep Hrs 3.5 | 8.36 (-38.21, 54.93) | 23.72 | 0.725 | 7.24 (-39.17, 53.65) | 23.64 | 0.759 |
| | Sleep Hrs 9.5 | -3.17 (-23.85, 17.51) | 10.54 | 0.763 | -1.56 (-22.24, 19.13) | 10.54 | 0.883 |

**Table 4: Regression Analysis of Cardiovascular Disease Risk Factor and Their Association with Phosphorylated-tau Levels in Preclinical Alzheimer's Disease Across Different Exposure Levels (BMI, Hypertension, Physical activity, Smoking status, Diet Score and Sleep).**

| Exposure Variable | Levels | Ptau | | | | | |
|---|---|---|---|---|---|---|---|
| | | Model 1 | | | Model 2 | | |
| | | Estimate (95% CI) | Std. Error | P-value | Estimate (95% CI) | Std. Error | P-value |
| **BMI** | | -0.13 (-0.27 , 0.02) | 0.07 | 0.086 | -0.16 (-0.31 , 0.0) | 0.08 | 0.048 |
| **Hypertension** | | -1.09 (-2.15 , -0.03) | 0.54 | 0.045* | -1.04 (-2.14 , 0.07) | 0.56 | 0.065 |
| **Physical Activity** | | 0.00 (0.00, 0.01) | 0 | 0.645 | -0.01 (-0.05, 0.04) | 0.02 | 0.78 |
| **Smoking** | **Non-smoker (Ref)** | - | - | - | - | - | - |
| | Smoker Current | -2.06 (-5.51, 1.40) | 1.76 | 0.243 | -1.91 (-5.37, 1.55) | 1.76 | 0.278 |
| | Smoker Former | 1.28 (0.10, 2.46) | 0.6 | 0.034* | 1.62 (0.41, 2.82) | 0.61 | 0.009* |
| **Diet** | **Diet Score 0 (Ref)** | - | - | - | - | - | - |
| | Diet Score 1 | 0.80 (-0.51, 2.12) | 0.67 | 0.232 | 0.79 (-0.86, 26.25) | 0.67 | 0.237 |
| | Diet Score 2 | 0.04 (-1.44, 1.52 | 0.76 | 0.959 | -0.13 (-1.61, 1.35) | 0.75 | 0.867 |
| | Diet Score 3 | 2.67 (0.68, 4.65) | 1.01 | 0.009* | 2.40 (0.40, 4.40) | 1.02 | 0.019* |
| **Sleep** | **Sleep Hrs 7.5 (Ref)** | - | - | - | - | - | - |
| | Sleep Hrs 5.5 | 0.83 (-0.38, 2.03) | 0.61 | 0.178 | 0.81 (-0.40, 2.03) | 0.62 | 0.187 |
| | Sleep Hrs 3.5 | 0.78 (-4.16, 5.72) | 2.51 | 0.757 | 0.63 (-4.29, 5.55) | 2.51 | 0.802 |
| | Sleep Hrs 9.5 | -0.36 (-2.55, 1.83) | 1.12 | 0.746 | -0.18 (-2.37, 2.02) | 1.12 | 0.875 |

Footnote:
Asterisks (*) indicate p-values < 0.05, indicating statistical significance.
Model 1: Exposure variable ; Gender, Age, Race, Income, and Education.
Model 2: All Model 1 variables ; Hypertension, Diabetes, High Cholesterol ; medication use for Hypertension, Diabetes and High Cholesterol.

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