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Plasma Free Fatty Acids and Risk of Stroke in the Cardiovascular Health Study

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Abstract

Background—While free fatty acids (FFA) have been positively associated with risk factors for stroke, the role of plasma FFA in the development of stroke has not been elucidated in older adults.

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Disclosures:
None.

Aims—We sought to examine the association between plasma FFA and incident stroke.

Methods—Prospective cohort of 4,369 men and women ≥65 years of age in the Cardiovascular Health Study. Plasma levels of FFA were measured at the 1992–93 examination and stroke events were adjudicated by a committee of experts including neurologists and neuroradiologists. Cox regression was used to estimate the relative risk of stroke associated with FFA concentrations.

Results—The average age among participants was 75 ± 5.2 years. During a median follow-up of 11.4 years, 732 incident strokes occurred. The crude incidence rates of stroke were 14.5, 14.9, and 17.6 per 1,000 person-years across increasing tertiles of plasma FFA. The adjusted hazard ratio (95% CI) for incident stroke was 1.05 (0.97–1.14) per standard deviation (SD) increase in plasma FFA. Restriction to ischemic stroke did not alter the results [hazard ratio (95% CI): 1.04 (0.96–1.14) per SD higher FFA] and there was no effect modification by adiposity (p interaction = 0.18) or by diabetes (p interaction = 0.15).

Conclusion—Our data did not show an association of plasma FFA with incident stroke among community dwelling older adults.

Introduction

Stroke is a major cause of functional impairment. Approximately 795,000 Americans suffer from stroke each year with 134,000 deaths annually [1]. The prevalence of stroke among US adults is approximately 3% of which 87% are ischemic in nature [2]. Incidence of stroke doubles with each decade among those over 55 years of age [3].

Recently, INTERSTROKE study identified ten risk factors i.e. diet, physical inactivity, abdominal obesity, current smoking, alcohol consumption, hypertension (HTN), diabetes mellitus (DM), dyslipidemia, cardiac causes, and psychosocial stress/depression which could explain 90% of stroke events [4]. Elevated plasma free fatty acids (FFA) levels have also been associated with inflammation [5], physical inactivity [5], adiposity [6], HTN [7], DM [8], and cardiac arrhythmias/sudden cardiac death [9, 10]. However, the association between plasma FFA and incident stroke has not been investigated in a prospective cohort of older adults.

Aims

Given the high risk of stroke in the extremely vulnerable population of older adults, it is critical to examine whether plasma FFA are associated with the development of stroke. We therefore sought to examine the association between plasma FFA and incident stroke. Since plasma FFA levels can be modified through pharmacological or lifestyle interventions, findings of the current project may have clinical significance.

Methods

Study population

Detailed descriptions of the CHS have been published elsewhere [11]. Briefly, CHS is a prospective, population-based cohort study of cardiovascular disease in older adults. In 1989–1990, 5,201 men and women aged ≥65 years were recruited from a random sample of

Medicare-eligible residents in the following 4 US communities: Forsyth County, NC; Sacramento County, CA; Washington County, MD; and Allegheny County, PA. A supplemental cohort of 687 predominantly African American men and women was recruited in 1992–1993 from 3 of the same communities (excepting Washington County) by using the same sampling and recruitment methods. The 1992–93 examination served as the baseline for the current analysis. Of the 4,456 participants free of stroke for whom FFA measurements were available, we excluded 87 subjects with missing covariates. Thus, a final sample of 4,369 participants was used for the current analyses. The institutional review board of each center approved the study, and all participants gave informed written consent to participate in the study.

Measurement of FFA

Plasma samples collected at the 1992–1993 examination were stored at −70°C until analyzed at the Central Laboratory at the University of Vermont. FFA concentrations in plasma were measured in duplicates by the Wako enzymatic method and the average of the 2 measurements was used for analyses. This technique relies on the acylation of coenzyme A by the fatty acids in the presence of added acyl-CoA synthetase. Acyl-CoA produced is oxidized by added acyl-CoA oxidase with generation of hydrogen peroxide, which, in the presence of peroxidase, permits the oxidative condensation of 3-methy-N-ethyl-N(β-hydroxyethyl)-aniline with 4-aminoantipyrine to form a purple-colored adduct. The latter is then measured colorimetrically at 550 nm. The intra-assay coefficient of variation was 5%.

Ascertainment of stroke

Detailed description of stroke ascertainment in CHS has been previously published [12]. Briefly, strokes were identified during annual follow-up examinations and at 6-month telephone contacts, as described previously [13, 14].

Records for all reported stroke and non stroke hospitalizations with International Classification of Diseases, 9th Revision (codes 430 through 438 identifying cerebrovascular disease), were abstracted and reviewed by a neurologist at each field center. Physician questionnaire was used to obtain information on reported non-hospitalized stroke patients. The information was then reviewed by a CHS neurologist at each field center, and any inconsistencies were discussed with the participant's physician. Stroke cases were adjudicated with information from patient interviews, medical records, and brain-imaging studies (available in 87% of adjudicated strokes) by a committee of neurologists, neuroradiologists, and internists. Strokes were classified as ischemic if there was a rapid onset of focal neurological deficit without evidence of hemorrhage by neuroimaging, lumbar puncture, or autopsy. Hemorrhagic strokes were defined based on the presence of blood in the subarachnoid space, ventricles, or parenchyma by neuroimaging not attributable to secondary hemorrhage into an infarct or if bloody spinal fluid or evidence of hemorrhage was found on autopsy. Participants who died <24 hours post stroke onset were assumed to have hemorrhagic stroke if they did not undergo lumbar puncture, neuroimaging, or autopsy. The stroke type was classified as unknown if information was insufficient to define stroke as hemorrhagic or ischemic. A detailed criterion for stroke classification and subtyping has also been described previously [15].

Other variables

Comprehensive information on health-related factors was collected at the baseline and annually thereafter in a standardized fashion. All covariates used in the analysis were based on information collected at the 1992–93 examination except as specified in the statistical methods section. Age, sex, race, years of education, smoking status, alcohol consumption, and health status were self-reported. Leisure-time activity (kcal/week) was assessed using a modified Minnesota Leisure-Time Activities questionnaire. Weight, height, and waist circumference were measured using standardized protocols. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Medication use was ascertained using a medication inventory [16]. Fasting glucose, lipids, albumin, high-sensitivity C-reactive protein (hsCRP, mg/l), and cystatin-C were measured in fasting blood specimens [17–19]. Glomerular filtration rate (GFR) was estimated using serum cystatin C measurements [20].

Statistical methods

The functional form of the association between FFA and incident stroke was assessed using restricted cubic splines. FFA were standardized by their standard deviation (SD) and treated as a continuous covariate. Covariates for multivariable adjustment were selected *a priori* based on previous knowledge of characteristics likely to be associated with both FFA and incident stroke.

Baseline characteristics of study participants were summarized according to the tertiles of FFA. Continuous variables were presented as means ± SD or medians [inter-quartile range (IQ)] if the distribution was skewed. Categorical variables were presented as N (%).

Cox proportional hazards models were used to estimate the association of FFA with incident stroke. We computed time at risk as the interval between the 1992–93 examination and the first occurrence of: 1) incident stroke, 2) death, or 3) end of follow-up. To evaluate whether the association between FFA and stroke varied by stroke type, we repeated the analysis after restricting events to ischemic stroke. In an additional analysis, we examined the association between FFA and ischemic stroke subtypes [cardioembolic (CE) and non CE strokes]. Because a single measurement of FFA may not be a good estimate of exposure over the long term, we repeated the analysis after restricting follow-up time to the first 5 years of observation.

As a part of the secondary analysis, we evaluated whether there were statistically significant interactions between FFA and adiposity (BMI ≥30 kg/m^2) or DM by creating cross-product terms and comparing models with and without the product terms. The validity of the proportional hazards assumption was evaluated by using Schoenfeld residuals with no meaningful departures found. Statistical analysis was performed in R 2.13.0 (www.r-project.org).

Results

The mean age of the study participants was 75 ± 5.2 years. The mean fasting time was 13.9 ± 2.3 hours with only <2% having fasted <8 hours. During a mean follow up of 10.7 years

(median 11.4 years), 732 incident cases of stroke were identified. Table 1 presents the baseline characteristics of study participants according to the tertiles of plasma FFA. Participants in the highest FFA tertile were more likely to be female, African American, less educated, never smokers, non-drinkers, and had higher measures of triglycerides, high density lipoprotein, systolic blood pressure, and hsCRP. Higher FFA levels were also associated with prevalent DM.

In a multivariate model, the following covariates were independently associated with significantly higher FFA levels: age, BMI, hypertension, CRP, and serum albumin. Covariates associated with significantly lower FFA levels were male sex, prevalent CHD, cystatin-C, and LDL cholesterol.

The crude incidence rates of stroke were 14.5, 14.9, and 17.6 per 1,000 person-years from lowest to the highest tertile of FFA. Each SD (SD=0.20 mEq/l) increment of FFA was not associated with a statistically significant higher risk of stroke [5% (95% CI: −3% to 14%)] in the fully adjusted model (Table 2). Results did not change when the analysis was limited to ischemic strokes [n=592; HR (95% CI): 1.04 (0.96–1.14) per SD higher FFA]. On further stratification of ischemic strokes, FFA were not associated with CE [n=182; HR (95% CI): 1.03 (0.87, 1.21)] or non-CE strokes [n= 137; HR: 1.01 (0.83, 1.23) per SD higher FFA]. Restriction of main analysis to the first 5 years of follow up did not alter the conclusions [HR (95% CI): 1.04 (0.92, 1.18) per SD higher FFA]. Lastly, there was no evidence of effect modification by adiposity (p interaction = 0.18) or by diabetes (p interaction = 0.15).

Discussion

In this cohort of older adults, we found that higher concentrations of plasma FFA measured late in life were not associated with the incidence of stroke. In a secondary analysis, there was no statistically significant interaction between FFA and adiposity or FFA and DM. To the best of our knowledge, this is the first large prospective study to assess the association between plasma FFA and incident stroke in community living older adults.

In a recent cross sectional analysis, a significant association was demonstrated between plasma FFA and ischemic stroke of CE subtype [21]; the same study also noted a higher prevalence of AF in association with higher levels of plasma FFA, suggesting that AF may be a mediating factor. In a prior study by our group, we prospectively demonstrated an association between higher plasma FFA levels and incident AF in the same cohort [22]. The difference between our study and that of Seo et al [21] could be partially explained by the prospective design, a long duration of follow up (10.7 years), and older age among CHS study participants (mean age 75 years).

Elevated plasma free fatty acids (FFA) levels have also been associated with inflammation [5], physical inactivity [5], adiposity [6], HTN [7], DM [8], and cardiac arrhythmias/sudden cardiac death [9, 10], all of which are considered to be important risk factors for developing stroke. In our analysis, despite the existence of possible biologic mechanisms, we were not able to show an association between elevated FFA levels and stroke.

Our study has some limitations. Study participants were aged ≥65 years at baseline, thereby limiting the generalizability of these findings to younger adults. With a single measure of plasma FFA late in life, we were unable to account for longitudinal changes in plasma FFA levels over time. Although we had information on stroke subtypes, we had limited statistical power when strokes were further classified by subtype. We did not have data on specific FFA including trans fatty acids known to adversely affect cardiovascular risk. It is possible that unmeasured or residual confounding could partially explain our results. Despite above limitations, our study has numerous strengths including its prospective design, a large sample size, inclusion of both men and women, lengthy follow up, availability of data on numerous potential confounders, review of medical records to validate stroke as well as a valid and reproducible way to measure the levels of plasma FFA.

In summary, our study does not provide evidence in support of a significant association between plasma FFA and incident stroke in older adults.

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Table 1

Baseline characteristic by tertiles of plasma free fatty acids

Free Fatty Acids Range (mEq/L)	T1 (0.00,0.39) (n=1,457)	T2 (0.39,0.55) (n=1,463)	T3 (0.55, 1.38) (n=1,449)
Age (years)	74 ± 4.87	75 ± 5.22	75 ± 5.50
Male (%)	852 (58)	554 (38)	369 (25)
African American (%)	219 (15)	237 (16)	259 (18)
High school vs < High School (%)	1110 (76)	1077 (74)	1036 (72)
Smoking (%)			
--> Never	584 (40)	669 (46)	750 (52)
--> Former	710 (49)	642 (45)	576 (40)
--> Current	163 (11)	152 (10)	123 (8)
Alcohol (%)			
--> None	743 (51)	792 (54)	844 (58)
--> 1–7 drinks/week	555 (38)	538 (37)	442 (31)
--> more than 7 drinks/week	159 (11)	133 (9)	163 (11)
Physical activity [kcal/week (median, IQR)]	1053 (405, 2165)	855 (286, 1905)	695 (236, 1693)
Body Mass Index (kg/m^2)	26 ± 4.06	27 ± 4.8	27 ± 5.34
High Density Lipoprotein (mg/dL)	50 ± 13	53 ± 14	57 ± 16
Low Density Lipoprotein (mg/dL)	120 ± 32	122 ± 34	119 ± 36
Triglycerides [mg/dL (median, IQR)]	115 (84, 157)	127 (90, 173)	130 (94, 187)
Systolic Blood Pressure (mmHg)	132 ± 21	136 ± 21	140 ± 21
Diastolic Blood Pressure (mmHg)	71 ± 11	71 ± 12	72 ± 12
GFR-Cystatin C (ml/min/1.73 m^2)	1.11 ± 0.32	1.11 ± 0.30	1.1 ± 0.33
High- Sensitivity C-Reactive Protein [mg/L (median, IQR)]	2.2 (1.0, 5.0)	2.7 (1.3, 5.8)	3.0 (1.3, 6.6)
Albumin (mg/L)	3.99 ± 0.29	4.00 ± 0.28	4.02 ± 0.28
Prevalent Coronary Heart Disease (%)	346 (24)	285 (19)	271 (19)
Prevalent Congestive Heart Failure (%)	78 (5)	78 (5)	77 (5)
Atrial Fibrillation (%)	34 (2)	37 (3)	40 (3)
Antihypertensive Medications (%)	665 (46)	694 (47)	810 (56)
Prevalent Diabetes Mellitus (%)	164 (11)	195 (13)	287 (20)

Table 2

Hazard ratios (95% CI) of incident stroke per one standard deviation increment in plasma free fatty acids

Model	Hazard Ratio	95% Confidence Interval	p value
1	1.07	(0.99, 1.15)	0.10
2	1.05	(0.97, 1.14)	0.20

Model 1: Adjusted for age, sex, race, clinic, and education.

Model 2: Adjusted for age, sex, race, clinic, education, alcohol, smoking, physical activity (log transformed), antihypertensive meds, coronary heart disease, congestive heart failure, cystatin C (log transformed), high sensitivity C-reactive protein (log transformed), low density lipoprotein, serum albumin, and systolic blood pressure.