Published in final edited form as:

Am J Cardiol. 2014 September 15; 114(6): 843–848. doi:10.1016/j.amjcard.2014.06.012.

Plasma Free Fatty Acids, Fatty Acid-binding Protein 4, and Mortality in Older Adults (From the Cardiovascular Health Study)

Michael D. Miedema, MD MPH^{a,b}, Marlena Maziarz, MSc^c, Mary L. Biggs, PhD^c, Susan J. Ziemann, MD^d, Jorge R. Kizer, MD MSc^e, Joachim H. Ix, MD MAS^f, Dariush Mozaffarian, MD DrPH^g, Russell P. Tracy, PhD^h, Bruce M. Psaty, MD PhD^{i,j}, David S. Siscovick, MDⁱ, Kenneth J. Mukamal, MD^k, and Luc Djousse, MD ScD^a

^aDivision of Aging, Brigham and Women's Hospital, Harvard Medical School, Boston, MA and Boston Veterans Affairs Healthcare System, Boston, MA

^bMinneapolis Heart Institute and Minneapolis Heart Institute Foundation, Minneapolis MN

^cDepartment of Biostatistics, School of Public Health, University of Washington, Seattle WA

^dNational Institute on Aging, National Institutes of Health, Bethesda, MD

^eDepartment of Medicine, and Department of Epidemiology and Population Health, Albert Einstein College of Medicine, Bronx, NY

^fNephrology Section, Veterans Affairs San Diego Healthcare System and Divisions of Nephrology and Preventive Medicine, University of California-San Diego, San Diego CA

^gDivision of Cardiovascular Medicine, Brigham and Women's Hospital, Boston, MA

^hDepartment of Pathology, Colchester Research Facility, University of Vermont, Colchester VA

ⁱCardiovascular Health Research Unit, Departments of Medicine and Epidemiology, University of Washington, Seattle WA

^jDepartment of Health Services, University of Washington and Group Health Research Institute, Group Health Cooperative, Seattle WA

^kBeth Israel Deaconess Medical Center, Boston MA

Abstract

Plasma free fatty acids (FFA) are largely derived from adipose tissue. Elevated levels of FFA and fatty acid-binding protein 4 (FABP4), a key cytoplasmic chaperone of fatty acids, have been associated with adverse cardiovascular outcomes but limited data are available on the relation of these biomarkers with cardiovascular and total mortality. We studied 4,707 participants with a mean age of 75 years who had plasma FFA and FABP4 measured in 1992–1993 as part of the

© 2014 Excerpta Medica, Inc. All rights reserved.

Corresponding author: Michael Miedema MD, Phone: 612.863.3900; Fax 612.863.4073; mdm307@mail.harvard.edu; Minneapolis Heart Institute, 920 East 28th Street, Suite 300, Minneapolis, MN 55407.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Cardiovascular Health Study, an observational cohort of community dwelling older adults. Over a median follow-up of 11.8 years, 3,555 participants died. Cox proportional hazard regression was used to determine the association between FFA, FABP4, and mortality. In fully adjusted models, FFA were associated with dose-dependent significantly higher total mortality (hazard ratio (HR) per standard deviation (SD): 1.14, 95% confidence interval (CI) 1.09–1.18), but FABP4 levels were not (HR 1.04, 95% CI 0.98–1.09). In a cause-specific mortality analysis, higher concentrations of FFA were associated with significantly higher risk of death due to cardiovascular disease, dementia, infection, and respiratory causes, but not cancer or trauma. We did not find evidence of an interaction between FFA and FABP4 ($p=0.45$), but FABP4 appeared to be associated with total mortality differentially among men and women (HR 1.17 (1.08–1.26) for men, HR 1.02 (0.96–1.07) for women, interaction p -value <0.001). In conclusion, in a cohort of community-dwelling older individuals, elevated plasma concentrations of FFA, but not FABP4, were associated with cardiovascular and non-cardiovascular mortality.

Keywords

Fatty acids; Mortality; Epidemiology

Plasma free fatty acids (FFA), a byproduct of lipolysis, are largely derived from adipose tissue. Several studies have shown that elevated levels of FFA are associated with insulin resistance and diabetes (1–3). In addition to diabetes, FFA have also been associated with hypertension, atrial fibrillation, coronary heart disease, and cardiovascular disease (CVD) (4–7). Fatty acid binding protein 4 (FABP4) serves as a carrier protein in the transport of FFA and other lipophilic substances (8). FABP4 has also been associated with insulin resistance and diabetes (9,10), as well as incident heart failure (11), and poor outcomes after acute ischemic stroke (12). Despite the association of FFA and FABP4 with cardiovascular risk factors and CVD, their relationship with mortality in older adults is unclear. Studies evaluating FFA and mortality have produced conflicting results for CVD mortality and one study indicated an increase in non-cardiovascular deaths (13,14). Studies analyzing the association between FABP4 and mortality have been limited to people with ischemic stroke and end-stage renal disease (12,15). The current study aimed to evaluate the association of FFA and FABP4 with total and cause-specific mortality in a cohort of community-dwelling older men and women.

Methods

The Cardiovascular Health Study (CHS) is a prospective, population-based cohort consisting of 5,888 men and women aged 65 who were recruited from a random sample of Medicare-eligible residents from 4 United States (US) communities (Forsyth County, NC; Sacramento County, CA; Washington County, MD; and Allegheny County, PA). A detailed description of the methods and procedures has previously been published (16). From 1989–1990, 5,201 participants were enrolled and in 1992–1993, a supplemental cohort of 687 predominantly African-Americans was recruited at 3 of the original sites (except for Washington County). Individuals were eligible if they were not wheelchair dependent or institutionalized, did not require a proxy for consent, were not receiving treatment for cancer, and were expected to

remain in their respective region for the upcoming 3 years. Participants were contacted every 6 months for follow-up, alternating between a telephone interview and a clinic visit until 1989–1999, and by telephone interview only after that. Each participant gave informed consent and the institutional review board at each center approved the study. For this analysis, the 1992–1993 clinic visit was used as baseline. Of the 5,265 participants who participated in the 1992–1993 exam, 558 subjects had missing data on FFA and/or FABP4 and were excluded from the analysis. Thus, 4,707 participants were included in the analysis.

Plasma samples collected at the 1992–1993 exam were stored at -70°C in the central laboratory at the University of Vermont. Plasma FFA concentration was measured by the Wako enzymatic method, which requires the acylation of CoA 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 and in the presence of peroxidase permits the oxidative condensation of 3-methy-N-ethyl-N(B-hydroxyethyl)-aniline with 4-aminoantipyrine to form a purple-colored adduct. The latter is then measured colorimetrically at 550 nm. Two measurements were taken and the average was used in the current analysis. The interassay coefficient of variation (CV) was 3.54–8.17% (detectable range 0.0156–1.50 mEq/L). Plasma FABP4 concentration was measured using standard enzyme-linked immunosorbent assay kits (Biovendor ELISA). The interassay CV was 2.61–5.32% (detectable range 5–250 ng/ml).

Surveillance for mortality occurred during alternating telephone interviews and clinical examinations every 6 months through 1999 and then exclusively via telephone contacts every 6 months thereafter as previously described (17). Briefly, deaths were confirmed and classified by a mortality review committee using information from hospital records, death certificates, autopsy and coroner reports, insurance records, obituaries, and interviews with physicians or next of kin. By these methods, ascertainment of vital status was complete for 100% of participants.

Covariate data from the 1992–1993 exam was used in the analysis. Information on age, sex, race, educational attainment, physical activity, hormone replacement therapy, alcohol consumption, and smoking status were based on self-report. Weight, height and waist circumference were measured using standardized protocols. Leisure-time activity (kcal/wk) was assessed using a modified Minnesota Leisure-Time Activities questionnaire (18). Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Missing values for smoking and height were carried forward from previous years if available. Seated resting blood pressure was measured in the right arm using a habitus appropriate cuff. Diabetes was defined as a use of insulin or oral hypoglycemic agents, a fasting glucose level of ≥ 7 mmol/L (126mg/dL), or a non-fasting glucose level of ≥ 11.1 mmol/L (200mg/dL). Lipids, fasting glucose, C-reactive protein, Cystatin C, and albumin were measured in fasting blood specimens as previously described (19).

Baseline characteristics of the study participants were summarized according to quartiles of FFA; continuous variables are presented as mean $\pm$ SD and categorical variables as percentages. Incidence rates for mortality were calculated per 1,000 person-years. Cox proportional hazards regression was used to estimate the association of FFA and FABP4

with mortality to allow adjustment for covariates. Individuals were censored for death, loss to follow-up, or end of mortality ascertainment (December 31, 2010). Cubic splines were used to assess the form of the association of FFA and FABP4 with mortality. Because the association of both biomarkers with mortality appeared approximately linear, FFA and FABP4 were modeled continuously. Confounders included in model 1 were age, sex, race, clinic, and education (less than high school versus high school or more). Model 2 included the variables in model 1 with the addition of BMI, cystatin C, albumin, kilocalories of physical activity, alcohol intake (0, <7, 7 drinks/week), smoking status (never, former, current), hormone replacement therapy for women, and self-reported health status (excellent, very good, good, fair, poor). The correlation between FFA and FABP4 was determined using Spearman's rank correlation coefficient. To evaluate intermediate pathways by which FFA and FABP4 might lead to mortality, we fit additional models that included diabetes, prevalent CVD, and C-reactive protein. We tested for interaction between FFA and FABP4 by including both FFA and FABP4 and their cross-product term (FFAxFABP4) in the model. We also looked for evidence of effect modification of the relationship between each biomarker and mortality by testing interaction terms for age (continuous), sex, prevalent CVD, diabetes, and BMI (continuous). We also evaluated the association between FFA and cause-specific mortality, including deaths due to CVD, cancer (any type), dementia, infection (pneumonia, sepsis, or other infection), respiratory disease, trauma (including fractures), and other (liver disease, gastrointestinal disease, renal failure, amyotrophic lateral sclerosis (ALS), Parkinson's disease, bladder disease, metabolic conditions, amyloid, failure to thrive, myelodysplastic syndrome, and other musculoskeletal disease). Schoenfeld residuals and plots of the residuals over time were used to evaluate proportional hazard assumptions; there were no meaningful violations.

Results

The mean age of the study participants was 75 years (range 65–98) and 58.3% were women. Over a median follow-up of 11.8 years, 3,555 participants died. Baseline characteristics of the study population are shown in Table 1. FFA and FABP4 levels had a modest correlation ($r=0.18$). Mortality rates per 1,000 person-years were 61.6, 62.9, 67.4, and 71.3 across consecutive quartiles of FFA. Corresponding values for FABP4 quartiles were 65.7, 65.4, 61.1, and 70.7, respectively.

In a multivariable Cox proportional hazards model, FFA but not FABP4 was associated with total mortality (Table 2). The addition of components of the metabolic syndrome not already in the model (triglycerides, fasting glucose, waist circumference, diastolic blood pressure) did not appreciably alter the results (results not shown). Simultaneous adjustment for both FFA and FABP4 did not appreciably alter either variable's association with mortality in the fully adjusted model (HR 1.13 (1.09–1.18) for FFA, HR 1.02 (0.96–1.07) for FABP4). We did not find evidence of an interaction between FFA and FABP4 ($p=0.45$).

For FFA, there was no evidence of effect modification by age, sex, prevalent CVD, diabetes, or BMI, and addition of potential mediators (diabetes, prevalent CVD, and C-reactive protein) did not attenuate the relationship between FFA and total mortality (results not shown). For FABP4, there was evidence of effect modification by sex, with a positive

association between FABP4 and mortality in men but not women (HR 1.17 (1.08–1.26) for men, HR 1.02 (0.96–1.07) for women, interaction p-value <0.001). There was no evidence of effect modification by age, prevalent CVD, diabetes, or BMI (results not shown). An analysis of the association of FFA and cause-specific mortality revealed higher concentrations of FFA were associated with a greater risk of death due to multiple causes (Table 3).

Discussion

In this prospective study of community-dwelling older individuals, higher levels of FFA, but not FABP4, were associated with mortality. Controlling for multiple potential confounders, including FABP4, did not attenuate this association. An analysis of cause-specific mortality revealed significant associations for CVD as well as non-cardiovascular diseases, including dementia, infection, and respiratory etiologies in individuals with higher concentrations of FFA. Additional adjustment for potential mediators, including diabetes and C-reactive protein, did not attenuate the relationship between FFA and mortality.

The association of FFA and mortality has been studied in other populations. Data from the Paris Prospective Study did not show an association between FFA and CVD mortality, though an unexpected association was seen between FFA and cancer mortality (13). This study population was limited to healthy middle-aged men (mean age 48.8 years) with relatively low rates of coronary heart disease. Conversely, data from the Ludwigshafen Risk and CHS, a study of individuals with known coronary heart disease (age range 60–71), showed a positive relationship between FFA and CVD mortality (HR 1.83 for 4th quartile compared to 1st quartile, p-value 0.001) (14).

There are several biologic mechanisms by which higher concentrations of FFA could lead to increased mortality. Circulating FFA increase insulin resistance and glucose production (20,21), and higher levels of FFA are seen in individuals with increased abdominal adiposity, a known CVD risk factor (22,23). However, adjusting for BMI and diabetes in our study did not significantly attenuate the relationship between FFA and mortality, suggesting other biologic mechanisms may be important (24). Prior studies evaluating FFA have largely focused on cardiometabolic outcomes, so the finding of a strong association between FFA and multiple non-cardiovascular causes of mortality is surprising. The older population and long duration of follow-up in our study allowed for accrual of a significant number of cardiovascular and non-cardiovascular deaths. While elevated FFA levels may have a pathophysiologic role in multiple disease states, it would seem more likely that elevated FFA levels are simply be a marker of poor overall health, associated with an increased risk of both cardiovascular and non-cardiovascular disease. There is prior evidence that FFA stimulate polymerization of the dysfunctional proteins that serve as the pathogenesis for Alzheimer's disease (25), which would support the increase in deaths due to dementia seen in our study. Additionally, FFA may be a marker of dysfunctional adipocytes, increased adiposity, and an increased inflammatory state (26); known risk factors for infectious and respiratory diseases (27–29).

We found no association between FABP4 and mortality in the primary analysis although we did find evidence of effect modification by sex with a positive association in men but not women. We previously studied the association between FABP4 and diabetes and also found evidence of effect modification by sex as FABP4 was found to be more strongly associated with diabetes in men compared to women, especially in those with BMI less than 25 kg/m¹¹. FABP4 has been associated with mortality in specific populations such as patients with stroke, end-stage renal disease, and coronary heart disease (12,15,30). These studies, although based on small sample sizes, did not show evidence of effect modification by gender. As such, the finding of a positive association between FABP4 and mortality in men but not women in our study requires further confirmation despite a highly significant p-value.

The clinical implications of our findings may be limited, as the effect estimates for FFA are likely too small to allow it to serve as a risk stratification tool for clinical use. However, this study does have research implications as it provides further support for the association between FFA and cardiovascular disease as well as the novel finding that higher FFA levels are associated with multiple non-cardiovascular causes of mortality as well.

Our study has several limitations. We cannot exclude the possibility of residual or unmeasured confounding given the observational nature of our study. Generalizability to other ethnicities may also be limited since most participants in our study were Caucasian. We have only a single measurement of FFA and FABP4 so changes in levels of these biomarkers over time could not be accounted for in this study. Our study also has strengths including a large sample, adequate numbers of both men and women, a representative sample of the older US population, a valid and reproducible method for measuring FFA and FABP4, availability of multiple covariates to limit confounding, and long-term follow-up with standardized and thorough adjudication of mortality.

Acknowledgments

We are indebted to the participants and staff of the CHS. MLB and MM had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. A full list of principal CHS investigators and institutions can be found at <http://www.chsnhlbi.org/pi.htm>

This research was supported by contracts HHSN268201200036C, HHSN268200800007C, N01 HC55222, N01HC85079, N01HC85080, N01HC85081, N01HC85082, N01HC85083, N01HC85086, and grants HL080295 and HL0945565 from the National Heart, Lung, and Blood Institute (NHLBI), with additional contribution from the National Institute of Neurological Disorders and Stroke (NINDS). Additional support was provided by AG023629 from the National Institute on Aging (NIA). A full list of principal CHS investigators and institutions can be found at <http://www.chs-nhlbi.org/PI.htm>. Funding agencies did not have any role in the design and conduct of the study; collection, management, analysis, and interpretation of the data.

References

1. Delarue J, Magnan C. Free fatty acids and insulin resistance. *Curr Opin Clin Nutr Metab Care*. 2007; 10:142–148. [PubMed: 17285001]
2. Bergman RN, Ader M. Free fatty acids and pathogenesis of type 2 diabetes mellitus. *Trends Endocrinol Metab*. 2000; 11:351–356. [PubMed: 11042464]
3. Pankow JS, Duncan BB, Schmidt MI, Ballantyne CM, Couper DJ, Hoogeveen RC, Golden SH. Fasting plasma free fatty acids and risk of type 2 diabetes: the atherosclerosis risk in communities study. *Diabetes Care*. 2004; 27:77–82. [PubMed: 14693970]

4. Fagot-Campagna A, Balkau B, Simon D, Warnett JM, Claude JR, Ducimetiere P, Eschwege E. High free fatty acid concentration: an independent risk factor for hypertension in the Paris Prospective Study. *Int J Epidemiol.* 1998; 27:808–813. [PubMed: 9839737]
5. Khawaja O, Bartz TM, Ix JH, Heckbert SR, Kizer JR, Zieman SJ, Mukamal KJ, Tracy RP, Siscovick DS, Djousse L. Plasma free fatty acids and risk of atrial fibrillation (from the Cardiovascular Health Study). *Am J Cardiol.* 2012; 110:212–216. [PubMed: 22503582]
6. Pirro M, Mauriège P, Tchernof A, Cantin B, Dagenais GR, Despres JP, Lamarche B. Plasma free fatty acid levels and the risk of ischemic heart disease in men: prospective results from the Québec Cardiovascular Study. *Atherosclerosis.* 2002; 160:377–384. [PubMed: 11849661]
7. Carlsson M, Wessman Y, Almgren P, Groop L. High levels of nonesterified fatty acids are associated with increased familial risk of cardiovascular disease. *Arterioscler Thromb Vasc Biol.* 2000; 20:1588–1594. [PubMed: 10845876]
8. Makowski L, Hotamisligil GS. Fatty acid binding proteins—the evolutionary crossroads of inflammatory and metabolic responses. *J Nutr.* 2004; 134:2464S–2468S. [PubMed: 15333743]
9. Djousse L, Khawaja O, Bartz TM, Biggs ML, Ix JH, Zieman SJ, Kizer JR, Tracy RP, Siscovick DS, Mukamal KJ. Plasma fatty acid-binding protein 4, nonesterified fatty acids, and incident diabetes in older adults. *Diabetes Care.* 2012; 35:1701–1707. [PubMed: 22584136]
10. Xu A, Tso AW, Cheung BM, Wang Y, Wat NM, Fong CH, Yeung DC, Janus ED, Sham PC, Lam KS. Circulating adipocyte-fatty acid binding protein levels predict the development of the metabolic syndrome: a 5-year prospective study. *Circulation.* 2007; 115:1537–1543. [PubMed: 17389279]
11. Djousse L, Bartz TM, Ix JH, Kochar J, Kizer JR, Gottdiener JS, Tracy RP, Mozaffarian D, Siscovick DS, Mukamal KJ, Zieman SJ. Fatty acid-binding protein 4 and incident heart failure: the Cardiovascular Health Study. *Eur J Heart Fail.* 2013; 15:394–399. [PubMed: 23223158]
12. Holm S, Ueland T, Dahl TB, Michelsen AE, Skjelland M, Russell D, Nymo SH, Krohg-Sorensen K, Clausen OP, Atar D, Januzzi JL, Aukrust P, Jensen JK, Halvorsen B. Fatty acid binding protein 4 is associated with carotid atherosclerosis and outcome in patients with acute ischemic stroke. *PLoS One.* 2011; 6:e28785. [PubMed: 22174896]
13. Charles MA, Fontbonne A, Thibault N, Claude JR, Warnet JM, Rosselin G, Ducimetière P, Eschwege E. High plasma nonesterified fatty acids are predictive of cancer mortality but not of coronary heart disease mortality: results from the Paris Prospective Study. *Am J Epidemiol.* 2001; 153:292–298. [PubMed: 11157417]
14. Pilz S, Schrnagl H, Tiran B, Wellnitz B, Seelhorst U, Boehm BO, Marz W. Free fatty acids are independently associated with all-cause and cardiovascular mortality in subjects with coronary artery disease. *J Clin Endocrinol Metab.* 2006; 91:2542–2547. [PubMed: 16595593]
15. Furuhashi M, Ishimura S, Ota H, Hayashi M, Nishitani T, Tanaka M, Yoshida H, Shimamoto K. Serum fatty acid-binding protein 4 is a predictor of cardiovascular events in end-stage renal disease. *PLoS One.* 2011; 6:e27356. [PubMed: 22102888]
16. Fried LP, Borhani NO, Enright P, Furberg CD, Gardin JM, Kronmal RA, Kuller LH, Manolio TA, Mittelmark MB, Newman A, for the Cardiovascular Health Study (CHS Collaborative Research Group. The Cardiovascular Health Study: design and rationale. *Ann Epidemiol.* 1991; 1:263–276. [PubMed: 1669507]
17. Ives DG, Fitzpatrick AL, Bild DE, Psaty BM, Kuller LH, Crowley PM, Cruise RG, Theroux S. Surveillance and ascertainment of cardiovascular events: the Cardiovascular Health Study. *Ann Epidemiol.* 1995; 5:278–285. [PubMed: 8520709]
18. Taylor HL, Jacobs DR Jr, Schucker B, Knudson J, Leon AS, Debacker G. A questionnaire for the assessment of leisure time physical activities. *J Chronic Dis.* 1978; 31:741–755. [PubMed: 748370]
19. Cushman M, Cornell ES, Howard PR, Bovill EG, Tracy RP. Laboratory methods and quality assurance in the Cardiovascular Health Study. *Clin Chem.* 1995; 41:264–270. [PubMed: 7874780]
20. Randle PJ. Regulatory interactions between lipids and carbohydrates: the glucose fatty acid cycle after 35 years. *Diabetes Metab Rev.* 1998; 14:263–283. [PubMed: 10095997]
21. Rebrin K, Steil GM, Getty L, Bergman RN. Free fatty acid as a link in the regulation of hepatic glucose output by peripheral insulin. *Diabetes.* 1995; 44:1038–1045. [PubMed: 7657026]

22. Casassus P, Fontbonne A, Thibault N, Ducimetiere P, Richard JL, Claude JR, Warnett JM, Rosselin G, Eschwege E. Upper-body fat distribution: a hyperinsulinemia-independent predictor of coronary heart disease mortality. The Paris Prospective Study. *Arterioscler Thromb Vasc Biol.* 1992; 12:1387–1392.
23. Freedman DS, Williamson DF, Croft JB, Balieu C, Byers T. Relation of body fat distribution to ischemic heart disease. The National Health and Nutrition Examination Survey I (NHANES I) epidemiologic follow-up study. *Am J Epidemiol.* 1995; 142:53–63. [PubMed: 7785674]
24. Kurien VA, Oliver MF. A metabolic cause for arrhythmias during acute myocardial hypoxia. *Lancet.* 1970; 1:813–815. [PubMed: 4191438]
25. Wilson DM, Binder LI. Free fatty acids stimulate the polymerization of tau and amyloid beta peptides. In vitro evidence for a common effector of pathogenesis in Alzheimer's disease. *Am J Pathol.* 1997; 150:2181–2195. [PubMed: 9176408]
26. Tripathy D, Mohanty P, Dhindsa S, Syed T, Ghanim H, Aljada A, Dandona P. Elevation of free fatty acids induces inflammation and impairs vascular reactivity in healthy subjects. *Diabetes.* 2003; 52:2882–2887. [PubMed: 14633847]
27. Karlsson EA, Beck MA. The burden of obesity on infectious disease. *Exp Biol Med.* 2010; 235:1412–1424.
28. Murugan AT, Sharma G. Obesity and respiratory diseases. *Chron Respir Dis.* 2008; 5:233–242. [PubMed: 19029235]
29. Boden G. Obesity and free fatty acids. *Endocrinol Metab Clin North Am.* 2008; 37:635–646. [PubMed: 18775356]
30. von Eynatten M, Breitling LP, Roos M, Baumann M, Rothenbacher D, Brenner H. Circulating adipocyte fatty acid-binding protein levels and cardiovascular morbidity and mortality in patients with coronary heart disease: a 10-year prospective study. *Arterioscler Thromb Vasc Biol.* 2012; 32:2327–2335. [PubMed: 22679309]

Table 1

Characteristics of the 4,707 participants of the Cardiovascular Health Study according to quartiles of plasma free fatty acids¹

Variable	Quartiles of FFA (mEq/L)			
	Q1 (1180)	Q2 (1177)	Q3 (1173)	Q4 (1177)
Age (years)	74.1 ± 4.9	74.7 ± 5.3	75.2 ± 5.4	75.4 ± 5.6
Men	61.2%	46.1%	34.5%	25.1%
African American	14.9%	17.0%	17.5%	17.5%
Education: High School or >	77.0%	72.5%	73.8%	68.8%
Body mass index (kg/m ²)	26.2 ± 4.0	26.8 ± 4.6	27.1 ± 5.0	27.3 ± 5.3
Waist circumference (cm)	96.3 ± 11.3	97.1 ± 13.4	97.9 ± 13.6	98.4 ± 14.3
Glucose (mg/dl)	104.7 ± 30.7	105.4 ± 29.3	106.9 ± 31.8	116.4 ± 46.0
Cystatin-C (mg/L)	1.14 ± 0.38	1.11 ± 0.29	1.12 ± 0.33	1.11 ± 0.37
Albumin (g/dl)f	3.92 ± 0.27	3.96 ± 0.27	3.98 ± 0.27	4.02 ± 0.27
Physical activity (kcal/d)	1642 ± 1920	1462 ± 1787	1329 ± 1612	1290 ± 1687
Alcoholic drinks/week				
None	50.6%	54.5%	55.0%	58.9%
7	38.7%	35.3%	36.2%	29.1%
>7	10.7%	10.2%	8.8%	12.0%
Smoking				
Never	38.1%	44.4%	47%	52.5%
Former	51.1%	44.3%	43.8%	39.3%
Current	10.9%	11.3%	9.2%	8.2%
Estrogen Use	9.2%	12.9%	13.0%	16.7%
Health Status				
Excellent	9.2%	7.6%	4.8%	4.8%
Very good	35.3%	32.8%	29.7%	25.5%
Good	37.1%	40.7%	45.1%	43.8%
Fair	16.4%	17.5%	18.2%	22.6%
Poor	2.0%	1.4%	2.2%	3.2%
Prevalent diabetes mellitus	12.0%	12.9%	14.6%	21.7%
Metabolic syndrome	38.5%	43.6%	48.2%	55.9%
Systolic blood pressure (mmHg)	131.7 ± 20.9	135.6 ± 21.6	137.2 ± 21.4	140.8 ± 21.4
Diastolic blood pressure (mmHg)	70.5 ± 10.8	71.7 ± 11.2	71.2 ± 12.2	71.7 ± 11.5
Low-density lipoprotein cholesterol (mg/dl)	119 ± 3	121 ± 3	121 ± 4	118 ± 4
High-density lipoprotein cholesterol (mg/dl)	50 ± 1	52 ± 1	54 ± 2	57 ± 2
Triglycerides (mg/dl)	131.6 ± 73.5	141.1 ± 79.1	148.1 ± 94.9	155.7 ± 91.9
Free fatty acids (mEq/L)	0.27 ± 0.06	0.41 ± 0.03	0.54 ± 0.04	0.76 ± 0.14
Fatty acid binding protein-4 (ng/ml)	29.7 ± 17.5	32.4 ± 17.1	35.3 ± 17.7	39.5 ± 21.6

Table 2

Hazard ratios for total mortality according to quartiles and per SD of FFA and FABP4 in Cardiovascular Health Study

	Number of Events	Model 1		Model 2	
		HR	95% CI	HR	95% CI
FFA Q1 (Reference)	3555	1.00		1.00	
FFA Q2		1.02	(0.93–1.12)	1.05	(0.95–1.15)
FFA Q3		1.16	(1.06–1.28)	1.20	(1.08–1.32)
FFA Q4		1.30	(1.18–1.43)	1.34	(1.21–1.48)
FFA per SD		1.12	(1.08–1.16)	1.14	(1.09–1.18)
FABP4 Q1 (Reference)	3555	1.00		1.00	
FABP4 Q2		1.16	(1.05–1.27)	1.07	(0.97–1.18)
FABP4 Q3		1.19	(1.08–1.32)	1.05	(0.94–1.17)
FABP4 Q4		1.51	(1.36–1.68)	1.13	(0.99–1.28)
FABP4 per SD		1.21	(1.17–1.25)	1.04	(0.98–1.09)

FABP4=fatty acid-binding protein 4; FFA=free fatty acids, HDL=high density lipoprotein; HR=hazard ratio; LDL=low density lipoprotein; SD=standard deviation SD of FFA = 0.2mEq/L. SD of FABP4 = 18.9ng/mL. Total person-years = 54167. Model 1: adjusted for age, sex, race, clinic and education (n = 4707). Model 2: adjusted for age, sex, race, clinic, education, BMI, cystatin C, albumin, physical activity, alcohol intake, smoking, hormone replacement therapy, self-reported health status, systolic blood pressure, LDL cholesterol, HDL cholesterol, hypertensive medication and lipid-lowering medication (n=4620).

Table 3

Hazard ratios for cause-specific mortality per SD of FFA in Cardiovascular Health Study

	Number of vents	HR	Model 1 95% CI	p-value	HR	Model 2 95% CI	p-value
Cardiovascular	1,155	1.08	(1.01, 1.14)	0.02	1.08	(1.01, 1.15)	0.02
Cancer	729	1.02	(0.95, 1.11)	0.55	1.05	(0.96, 1.14)	0.29
Dementia	502	1.27	(1.16, 1.39)	<0.001	1.32	(1.20, 1.44)	<0.001
Infection	288	1.23	(1.09, 1.38)	0.001	1.25	(1.10, 1.41)	0.001
Respiratory	199	1.26	(1.10, 1.45)	0.001	1.23	(1.06, 1.43)	0.007
Trauma	156	0.97	(0.82, 1.16)	0.77	0.99	(0.83, 1.19)	0.93
Other	322	1.11	(0.99, 1.24)	0.08	1.16	(1.03, 1.31)	0.02

CI=confidence interval; FFA=free fatty acids, HR=hazard ratio; SD=standard deviation SD of FFA = 0.2mEq/L. Total person-years = 54,167. Model 1: adjusted for age, sex, race, clinic and education (n = 4707). Model 2: adjusted for age, sex, race, clinic, education, BMI, cystatin C, albumin, physical activity, alcohol intake, smoking, hormone replacement therapy, self-reported health status, systolic blood pressure, LDL cholesterol, HDL cholesterol, hypertensive medication and lipid-lowering medication (n = 4620). Types of mortality were defined as: cardiovascular (coronary heart disease, stroke, or other atherosclerotic disease), cancer (any type), dementia, infection (pneumonia, sepsis or other infection), respiratory, trauma (includes fractures) or other (liver disease, gastrointestinal disease, renal failure, ALS, Parkinson's disease, bladder disease, metabolic conditions, amyloid, failure to thrive, myelodysplastic syndrome, and other musculoskeletal diseases).