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Plasma Levels of Fatty Acid-Binding Protein 4, Retinol-Binding Protein 4, High-Molecular Weight Adiponectin, and Cardiovascular Mortality among Men with Type 2 Diabetes: A 22-Year Prospective Study

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Abstract

Objective—To examine select adipokines, including fatty acid-binding protein 4 (FABP4), retinol-binding protein 4 (RBP4), and high-molecular weight (HMW) adiponectin in relation to cardiovascular disease (CVD) mortality among patients with type 2 diabetes (T2D).

Approach and Results—Plasma levels of FABP4, RBP4, and HMW adiponectin were measured in 950 men with T2D in the Health Professionals Follow-up Study. After an average of 22 years of follow up (1993–2015), 580 deaths occurred, of whom 220 died of CVD. After multivariate adjustment for covariates, higher levels of FABP4 were significantly associated with a higher CVD mortality: comparing extreme tertiles, the hazard ratio (HR) and 95% confidence interval (CI) of CVD mortality was 1.78 (1.22, 2.59; *P* trend=0.001). A positive association was also observed for HMW adiponectin: the HR (95% CI) was 2.07 (1.42, 3.06; *P* trend=0.0002),

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Disclosure

None.

comparing extreme tertiles, whereas higher RBP4 levels were non-significantly associated with a decreased CVD mortality with an HR (95% CI) of 0.73 (0.50, 1.07; *P* trend=0.09). A Mendelian randomization (MR) analysis suggested that the causal relationships of HMW adiponectin and RBP4 would be directionally opposite to those observed based on the biomarkers, although none of the MR associations achieved statistical significance.

Conclusions—These data suggest that higher levels of FABP4 and HMW adiponectin are associated with elevated CVD mortality among men with T2D. Biological mechanisms underlying these observations deserve elucidation, but the associations of HMW adiponectin may partially reflect altered adipose tissue functionality among T2D patients.

Keywords

cardiovascular disease; mortality; adipokines; fatty acid-binding protein; retinol-binding protein 4; high-molecular weight adiponectin

Introduction

Type 2 diabetes (T2D) has become a global public health challenge with tremendous social and economic burden.[1] Compared with the non-diabetic population, individuals with T2D have a two- to four-fold higher risk of developing cardiovascular disease (CVD), which is the leading cause of death in diabetes patients.[2–4] Although the exact underlying mechanisms are unclear, accumulating evidence indicates that adipokines may be involved in the pathogenesis of CVD and mortality among general populations and diabetes patients.[5–8]

In particular, fatty acid binding protein (FABP4; also known as aP2), an adipokine primarily expressed in adipocytes and also in macrophages,[9–13] plays a pivotal role in coordinating and integrating metabolic and inflammatory signaling in the setting of insulin resistance and obesity in mouse models.[14–17] Moreover, animal experiments have shown that FABP4, when expressed in macrophages, contributes particularly to the etiology of atherosclerosis.[18,19] In addition, epidemiological data supported a positive association between circulating FABP4 levels and the risk of T2D, heart failure, and stroke in the general population.[6,20–22] Overall, the accumulating evidence suggests a possible role of FABP4 in the etiology of not only diabetes but also its CVD complications, although few studies have been conducted to evaluate whether FABP4 is associated with CVD mortality among patients with T2D.

Similarly, levels of retinol-binding protein 4 (RBP4), another cytokine primarily produced by adipose tissue, were associated with an elevated risk of developing coronary heart disease (CHD) among generally healthy population,[7,23] whereas it is largely unexplored regarding whether this adipokine predicts CVD mortality among T2D patients. In contrast to the lack of data for FABP4 and RBP4, Menzaghi et al.[24] recently reported a positive association between circulating levels of total adiponectin and CVD mortality among T2D patients in three prospective cohort studies, especially among men with T2D. The biological mechanisms underlying this positive association are unclear, although this observation suggests that the potentially beneficial effects of high circulating adiponectin,[25] especially

high-molecular weight (HMW) adiponectin, on cardiometabolic risk may not manifest among T2D patients.

To further elucidate associations between these adipokines and CVD mortality among T2D patients, we evaluated prospective relationships between levels of FABP4, RBP4, and HMW adiponectin and CVD mortality among men with T2D in the Health Professionals Follow-up Study (HPFS). We hypothesized that FABP4 and RBP4 levels were positively, while HMW adiponectin levels were inversely, associated with CVD mortality in diabetics.

Materials and Methods

Materials and Methods are available in the online-only Data Supplement.

Results

Table 1 shows the baseline characteristics of the study population. During 13,103 person-years of follow up (1993–2015), 220 CVD deaths occurred with an incidence rate of 168/10,000 person-years. Participants with CVD death were older, had a higher prevalence of smoking, hypertension, angina, CABG, myocardial infarction, and were more likely to take aspirin and cholesterol-lowering drugs at baseline. They also had lower levels of eGFR, longer diabetes duration, and higher concentrations of hsCRP, TNFR2, FABP4, and HMW adiponectin when compared with the remaining participants (all $P<0.05$).

The Spearman partial correlations between FABP4, RBP4, HMW adiponectin, BMI, and CVD risk markers are shown in Supplementary Table I. In general, of the three adipokines, FABP4 was most strongly associated with an adverse profile of CVD risk factors, such as positive correlations with BMI at blood collection, BMI at age 21, TG, hsCRP, and TNFR2 (r_S 0.19–0.40), and inverse correlations with HDL and eGFR (r_S −0.23 and −0.25, respectively). RBP4 was positively associated with TG (r_S=0.27) and inversely associated with eGFR (r_S=−0.31), but not significantly associated with BMI at blood collection or BMI at age 21; HMW adiponectin was positively associated with HDL (r_S=0.37) and inversely associated with BMI at blood collection (r_S=−0.16) and TG (r_S=−0.32). Other correlations between adipokines and CVD risk factors were weaker. Between adipokines, FABP4 and RBP4 levels were correlated (r_S=0.26), and other correlations were much weaker (r_S <0.10)

Elevated FABP4 levels were associated with an elevated CVD mortality (Table 2). After multivariate adjustment of covariates, including age, BMI at age 21, physical activity, smoking status, alcohol consumption, diabetes duration, family history of MI, aHEI score, use of aspirin or cholesterol-lowering medication, baseline morbidity, the hazard ratio (HR) and 95% confidence interval (CI) of CVD mortality was 1.80 (1.27, 2.57) (P trend<0.001), comparing extreme tertiles of FABP4. Further adjustment of TG, HDL, LDL, eGFR, and hsCRP, did not change this association materially: the HR (95% CI) was 1.78 (1.22, 2.59; P trend=0.001) for CVD mortality. For RBP4, after multivariate adjustment of the abovementioned covariates, a non-significant, inverse association with CVD mortality was observed (HR [95% CI] was 0.73 [0.50, 1.07]; P trend=0.09). For HMW adiponectin, a positive association was observed in relation to CVD mortality. Comparing men in the lowest tertile of HMW adiponectin, men in the highest tertile had an HR of 2.07 (1.42, 3.06;

P trend=0.0002) for CVD mortality after multivariate adjustment. In a secondary analysis, when weight changes (weight change between age 21 and blood collection, or weight change between 1986 and blood collection) were further adjusted in models, the results remained similar. Furthermore, when FABP4, RBP4, and HMW adiponectin were mutually adjusted, the results did not change significantly. After excluding participants with myocardial infarction, stroke, angina, or CABG at baseline (479 participants), a similar trend was observed, although the associations did not reach statistical significance due to reduced statistical power. After multivariate adjustment, the risk of CVD mortality for the diabetic men in the highest tertile (compared with the lowest tertile) was 1.59 (0.53, 4.76) for FABP4 (*P* trend=0.44), 0.87 (0.64, 1.03) for RBP4 (*P* trend=0.65), and 1.39 (0.54, 3.61) for HMW adiponectin (*P* trend=0.48).

In stratified analyses, the associations of plasma FABP4 and HMW adiponectin with risk of CVD mortality remained positive across most of the subgroups defined by age at blood draw, BMI at age 21, and current smoking status, although some of the associations did not reach statistical significance due to diminished statistical power (Table 3).

In the Mendelian randomization analysis, instrumental variables were found for RBP4 and HMW adiponectin, whereas no plausible instrumental variable was available for FABP4 (Supplementary Table IV). We observed an inverse trend of association between HMW adiponectin and CVD mortality (the causal estimate coefficient was −1.9, *P*=0.15), and a positive trend of association between RBP4 and CVD mortality (the causal estimate coefficient was 19.0, *P*=0.11) (Table 4), but none of the associations achieved statistical significance.

As secondary analyses, we also examined these adipokines in relation to total and cancer-related mortality. Higher FABP4 and HMW adiponectin levels were associated with an increased all-cause mortality, while RBP4 was not significantly associated with all-cause mortality (Supplementary Table II). FABP4 was positively associated with cancer mortality, while RBP4 and HMW were not (Supplementary Table III).

Discussion

In this prospective study among U.S. men with T2D, after adjustment of demographics, lifestyle, diet, blood lipids, and inflammatory markers, we found a significant association between elevated levels of FABP4 and increased total and CVD mortality. In contrast, contrary to our hypotheses, higher plasma levels of HMW adiponectin were associated with a higher CVD mortality, and elevated RBP4 levels were non-significantly associated with a lower CVD mortality. These associations largely persisted within subgroups defined by various CVD risk factors.

To our knowledge, this is among the first investigations that estimated the association of FABP4 with CVD mortality in diabetes patients. Previous studies conducted in the general population found that FABP4 might be an early predictor of developing cardiometabolic conditions, including metabolic syndrome and T2D,[20,26] heart failure, and stroke.[12,13] Another study found that circulating FABP4 predicted the risk of adverse cerebrovascular or

cardiovascular events in patients with acute coronary syndrome but not in asymptomatic individuals.[27] The underlying mechanisms linking FABP4 with cardiometabolic conditions are illustrated in animal studies. In obese mice, the use of a small molecular inhibitor of FABP4 or targeted deletion of FABP4 could protect against the development of insulin resistance and lipid dysregulation.[16,17] Moreover, unlike RBP4 and adiponectin, FABP4 is not only expressed in adipocytes but also in macrophages. In ApoE−/− mice the deletion of FABP4 genes results in protection from atherosclerosis without significant change in blood lipids or insulin sensitivity.[18,19] Interestingly, bone-marrow transplantation experiments demonstrated that these effects were solely due to FABP4 expressed in macrophages.[19] These experiments clearly indicate that FABP4 expressed in macrophages causes atherosclerosis through pathways other than insulin resistance. FABP4 is highly expressed *in vivo* in foam cells of human atherosclerotic plaques.[28] These form cells, when exposed to oxidized LDL (ox-LDL), demonstrated elevated expression levels of FABP4.[28] Expression of FABP4 in macrophages, in turn, significantly increases the triacylglycerol and cholesterol accumulation in the macrophages through downregulation of genes for cholesterol efflux and cholesterol ester hydrolysis.[29,30] Overall, existing evidence from these animal experiments may likely explain the positive association between FABP4 levels and CVD mortality among T2D patients. We also observed a positive association between FABP4 and cancer mortality in this analysis. The mechanisms underlying this observation are unclear, and the role of residual confounding and/or chance cannot be excluded. On the other hand, several studies demonstrated that elevated expression of FABP4 might be involved in tumor growth and metastasis.[31–33]

The associations for RBP4 and HMW adiponectin observed in the current investigation were not consistent with findings in generally healthy populations. For example, circulating RBP4 levels were positively associated with the risk of diabetes, CHD, and other CVD events in the general population.[7,23,34] Likewise, in human studies high adiponectin levels were inversely associated with risk of developing T2D and CHD.[25,35,36] Of note, based on data from previous studies in the HPFS,[24,35] we also observed that diabetes patients had lower total adiponectin levels than healthy controls who were free of CVD and T2D (the median [interquartile range] was 14.1 10.1–19.6] μg/ml *vs* 16.7 [11.8, 22.9] μg/ml). Neverthelss, our findings regarding RBP4 and HMW adiponectin were consistent with those in previous studies conducted among some high-risk populations. For example, serum RBP4 levels were significantly associated with reduced mortality in elderly patients with acute exacerbations of chronic obstructive pulmonary disease.[37] Total adiponectin levels predicted mortality or heart failure among elderly participants or patients with carotid atherosclerosis, type 1 diabetes, and CVD.[8,38–42] More recently, in another prospective study consisted of 2,094 diabetes patients, including the HPFS participants involved in the current analysis, high circulating total adiponectin levels predicted increased CVD mortality in men, but not in women.[19]

These unexpected observations cannot be explained by evidence from animal studies, suggesting collectively that high RBP4 levels and low adiponectin levels contribute to the etiology of obesity, diabetes, and CVD.[43,44] These unexpected findings might be explained by potential functional changes of adipose tissue after the development of overt insulin resistance and T2D or the use of diabetes medication, and thus these observed associations

with mortality may be due to reverse causation bias rendered by existing diseases. For example, a higher pre-diabetes BMI predicted higher mortality whereas BMI adjacent to diabetes diagnosis was no longer associated with excess mortality.[45] Moreover, Nilsson et al.[46] demonstrated altered DNA methylation and differential expression of genes related to metabolism and inflammation in adipose tissue from patients with T2D. Tiikkainen et al.[47] found that the use of rosiglitazone and metformin could influence hepatic insulin resistance and gene expression in adipose tissue in patients with T2D. Several studies suggested that adiponectin resistance might lead to elevated levels of adiponectin in patients with heart failure, which would in turn predict high CVD mortality.[48,49] In addition, the natriuretic peptide system including B-type natriuretic peptide (BNP) and the N-terminal fragment of its prohormone (NT-pro BNP) plays an important role in adipose tissue metabolism,[50,51] which might influence the secretion of adiponectin. Some studies demonstrated that NT-pro BNP and adiponectin had a significantly positive correlation and both could predict a high mortality in participants with chronic heart failure or chronic kidney disease.[52–56] Therefore, another possible explanation was that the positive association between adiponectin and CVD mortality was due to elevated levels of BNP or NT-pro BNP that predispose patients to a higher CVD-related mortality. Clearly, more studies in this regard are needed to further elucidate differential biological pathways linking these adipokines with mortality among healthy population versus populations with existing chronic diseases that may lead to weight change and altered adipose tissue function.

The current study has several strengths. This is the first prospective study estimating the associations of plasma FABP4, RBP4, and HMW adiponectin with CVD mortality in men with T2D. Other strengths include a long follow-up period, use of CVD end-points validated by medical records or death certificates, and adjustments for a multitude of potential risk factors. There are a few limitations as well. First, the study participants are all male health professions, and most of them are Caucasians. Although our study potentially minimizes residual confounding by socioeconomic status, this relative homogeneity reduces the generalizability to other populations or ethnic groups. Second, we only measured baseline plasma FABP4, RBP4, and HMW adiponectin, which might not represent the long-term levels of these markers. Nonetheless, plasma adipokine levels might remain stable over time.[57] Third, the validity of the ELISA assay of adiponectin was questioned in a previous study by Bluher et al that reported significant differences between different commercially available assays,[58] Meanwhile, another study among 204 individuals showed that the results of HWM adiponectin by two commercial ELISAs (ALPCO and Millipore) were significantly correlated with those obtained by quantitative Western blotting (both $r>0.75$, $P<0.001$).[59] Given our prospective study design, the misclassification of true adiponectin levels is likely to be non-differential between CVD deaths and the rest of study participants, and thus the true associations are more likely to be biased toward the null. Fourth, we did not collect data regarding left ventricular ejection fraction (LVEF) which could impact adiponectin levels, although several previous studies in patients with chronic heart failure suggested that LVEF might not significantly influence the association of adiponectin with mortality.[53,60] In addition, we cannot exclude the possibility that these findings are due to chance, especially since we examined three adipokines simultaneously. Lastly, the role of residual confounding could not be entirely ruled out in observational studies.

In conclusion, in this prospective study, elevated levels of FABP4 are associated with higher CVD mortality among men with T2D. The unexpected associations of HMW adiponectin and RBP4 with CVD mortality suggest that the findings regarding these adipokines in the general population might not directly apply to diseased populations probably due to weight change or alterations of adipose tissue functionality subsequent to disease pathophysiology. Nonetheless, the underlying mechanisms require further exploration.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Nonstandard Abbreviations and Acronyms

FABP4	Fatty acid-binding protein 4
RBP4	Retinol-binding protein 4
HMW	High-molecular weight
T2D	Type 2 diabetes
CVD	Cardiovascular disease

References

1. Lam DW, LeRoith D. The worldwide diabetes epidemic. Curr Opin Endocrinol Diabetes Obes. 2012; 19:93–96. [PubMed: 22262000]
2. Engelgau MM, Geiss LS, Saaddine JB, Boyle JP, Benjamin SM, Gregg EW, Tierney EF, Rios-Burrows N, Mokdad AH, Ford ES, Imperatore G, Narayan KM. The evolving diabetes burden in the United States. Ann Intern Med. 2004; 140:945–950. [PubMed: 15172919]
3. Hu FB, Stampfer MJ, Solomon CG, Liu S, Willett WC, Speizer FE, Nathan DM, Manson JE. The impact of diabetes mellitus on mortality from all causes and coronary heart disease in women: 20 years of follow-up. Arch Intern Med. 2001; 161:1717–1723. [PubMed: 11485504]
4. Fox CS, Coady S, Sorlie PD, Levy D, Meigs JB, D'Agostino RB Sr, Wilson PW, Savage PJ. Trends in cardiovascular complications of diabetes. JAMA. 2004; 292:2495–2499. [PubMed: 15562129]
5. Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. Nat Rev Immunol. 2011; 11:85–97. [PubMed: 21252989]
6. Tso AW, Lam TK, Xu A, Yiu KH, Tse HF, Li LS, Law LS, Cheung BM, Cheung RT, Lam KS. Serum adipocyte fatty acid-binding protein associated with ischemic stroke and early death. Neurology. 2011; 76:1968–1975. [PubMed: 21562251]

7. Ingelsson E, Sundstrom J, Melhus H, Michaelsson K, Berne C, Vasan RS, Riserus U, Blomhoff R, Lind L, Arnlov J. Circulating retinol-binding protein 4, cardiovascular risk factors and prevalent cardiovascular disease in elderly. Atherosclerosis. 2009; 206:239–244. [PubMed: 19339013]
8. Poehls J, Wassel CL, Harris TB, Havel PJ, Swarbrick MM, Cummings SR, Newman AB, Satterfield S, Kanaya AM. Association of adiponectin with mortality in older adults: the Health, Aging, and Body Composition Study. Diabetologia. 2009; 52:591–595. [PubMed: 19159917]
9. Saksi J, Ijas P, Mayranpaa MI, et al. Low-expression variant of fatty acid-binding protein 4 favors reduced manifestations of atherosclerotic disease and increased plaque stability. Circ Cardiovasc Genet. 2014; 7:588–598. [PubMed: 25122052]
10. Cao H, Sekiya M, Ertunc ME, Burak MF, Mayers JR, White A, Inouye K, Rickey LM, Ercal BC, Furuhashi M, Tuncman G, Hotamisligil GS. Adipocyte lipid chaperone AP2 is a secreted adipokine regulating hepatic glucose production. Cell Metab. 2013; 17:768–778. [PubMed: 23663740]
11. Furuhashi M, Hotamisligil GS. Fatty acid-binding proteins: role in metabolic diseases and potential as drug targets. Nat Rev Drug Discov. 2008; 7:489–503. [PubMed: 18511927]
12. Shum BO, Mackay CR, Gorgun CZ, Frost MJ, Kumar RK, Hotamisligil GS, Rolph MS. The adipocyte fatty acid-binding protein aP2 is required in allergic airway inflammation. J Clin Invest. 2006; 116:2183–2192. [PubMed: 16841093]
13. Ertunc ME, Sikkeland J, Fenaroli F, Griffiths G, Daniels MP, Cao H, Saatcioglu F, Hotamisligil GS. Secretion of fatty acid binding protein aP2 from adipocytes through a nonclassical pathway in response to adipocyte lipase activity. J Lipid Res. 2015; 56:423–434. [PubMed: 25535287]
14. Burak MF, Inouye KE, White A, et al. Development of a therapeutic monoclonal antibody that targets secreted fatty acid-binding protein aP2 to treat type 2 diabetes. Sci Transl Med. 2015; 7:319ra205.
15. Tuncman G, Erbay E, Hom X, De Vivo I, Campos H, Rimm EB, Hotamisligil GS. A genetic variant at the fatty acid-binding protein aP2 locus reduces the risk for hypertriglyceridemia, type 2 diabetes, and cardiovascular disease. Proc Natl Acad Sci U S A. 2006; 103:6970–6975. [PubMed: 16641093]
16. Hotamisligil GS, Johnson RS, Distel RJ, Ellis R, Papaioannou VE, Spiegelman BM. Uncoupling of obesity from insulin resistance through a targeted mutation in aP2, the adipocyte fatty acid binding protein. Science. 1996; 274:1377–1379. [PubMed: 8910278]
17. Furuhashi M, Tuncman G, Gorgun CZ, Makowski L, Atsumi G, Vaillancourt E, Kono K, Babaev VR, Fazio S, Linton MF, Sulsky R, Robl JA, Parker RA, Hotamisligil GS. Treatment of diabetes and atherosclerosis by inhibiting fatty-acid-binding protein aP2. Nature. 2007; 447:959–965. [PubMed: 17554340]
18. Boord JB, Maeda K, Makowski L, Babaev VR, Fazio S, Linton MF, Hotamisligil GS. Adipocyte fatty acid- binding protein, aP2, alters late atherosclerotic lesion formation in severe hypercholesterolemia. Arterioscler Thromb Vasc Biol. 2002; 22:1686–1691. [PubMed: 12377750]
19. Makowski L, Boord JB, Maeda K, Babaev VR, Uysal KT, Morgan MA, Parker RA, Suttles J, Fazio S, Hotamisligil GS, Linton MF. Lack of macrophage fatty-acid-binding protein aP2 protects mice deficient in apolipoprotein E against atherosclerosis. Nat Med. 2001; 7:699–705. [PubMed: 11385507]
20. Tso AW, Xu A, Sham PC, Wat NM, Wang Y, Fong CH, Cheung BM, Janus ED, Lam KS. Serum adipocyte fatty acid binding protein as a new biomarker predicting the development of type 2 diabetes: a 10-year prospective study in a Chinese cohort. Diabetes Care. 2007; 30:2667–2672. [PubMed: 17620449]
21. Chow WS, Tso AW, Xu A, Yuen MM, Fong CH, Lam TH, Lo SV, Tse HF, Woo YC, Yeung CY, Cheung BM, Lam KS. Elevated circulating adipocyte-fatty acid binding protein levels predict incident cardiovascular events in a community-based cohort: a 12-year prospective study. J Am Heart Assoc. 2013; 2:e004176. [PubMed: 23525430]
22. 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]

23. Sun Q, Kiernan UA, Shi L, Phillips DA, Kahn BB, Hu FB, Manson JE, Albert CM, Rexrode KM. Plasma retinol-binding protein 4 (RBP4) levels and risk of coronary heart disease: a prospective analysis among women in the nurses' health study. Circulation. 2013; 127:1938–1947. [PubMed: 23584360]
24. Menzaghi C, Xu M, Salvemini L, De Bonis C, Palladino G, Huang T, Copetti M, Zheng Y, Li Y, Fini G, Hu FB, Bacci S, Qi L, Trischitta V. Circulating adiponectin and cardiovascular mortality in patients with type 2 diabetes mellitus: evidence of sexual dimorphism. Cardiovasc Diabetol. 2014; 13:130. [PubMed: 25200659]
25. Li S, Shin HJ, Ding EL, van Dam RM. Adiponectin levels and risk of type 2 diabetes: a systematic review and meta-analysis. JAMA. 2009; 302:179–188. [PubMed: 19584347]
26. Xu A, Wang Y, Xu JY, Stejskal D, Tam S, Zhang J, Wat NM, Wong WK, Lam KS. Adipocyte fatty acid-binding protein is a plasma biomarker closely associated with obesity and metabolic syndrome. Clin Chem. 2006; 52:405–413. [PubMed: 16423904]
27. Reiser H, Klingenberg R, Hof D, et al. Circulating FABP4 is a prognostic biomarker in patients with acute coronary syndrome but not in asymptomatic individuals. Arterioscler Thromb Vasc Biol. 2015; 35:1872–1879. [PubMed: 26069234]
28. Fu Y, Luo N, Lopes-Virella MF, Garvey WT. The adipocyte lipid binding protein (ALBP/aP2) gene facilitates foam cell formation in human THP-1 macrophages. Atherosclerosis. 2002; 165:259–269. [PubMed: 12417276]
29. Fu Y, Luo L, Luo N, Garvey WT. Lipid metabolism mediated by adipocyte lipid binding protein (ALBP/aP2) gene expression in human THP-1 macrophages. Atherosclerosis. 2006; 188:102–111. [PubMed: 16313911]
30. Makowski L, Hotamisligil GS. The role of fatty acid binding proteins in metabolic syndrome and atherosclerosis. Curr Opin Lipidol. 2005; 16:543–548. [PubMed: 16148539]
31. Tang Z, Shen Q, Xie H, Zhou X, Li J, Feng J, Liu H, Wang W, Zhang S, Ni S. Elevated expression of FABP3 and FABP4 cooperatively correlates with poor prognosis in non-small cell lung cancer (NSCLC). Oncotarget. 2016 Jun 15. [Epub ahead of print]. doi: 10.18632/oncotarget.10086
32. Nieman KM, Romero IL, Van Houten B, Lengyel E. Adipose tissue and adipocytes support tumorigenesis and metastasis. Biochim Biophys Acta. 2013; 1831:1533–1541. [PubMed: 23500888]
33. Guaita-Esteruelas S, Bosquet A, Saavedra P, Guma J, Girona J, Lam EW, Amillano K, Borras J, Masana L. Exogenous FABP4 increases breast cancer cell proliferation and activates the expression of fatty acid transport proteins. Mol Carcinog. 2016 Apr 6. [Epub ahead of print]. doi: 10.1002/mc.22485
34. Meisinger C, Ruckert IM, Rathmann W, Doring A, Thorand B, Huth C, Kowall B, Koenig W. Retinol-binding protein 4 is associated with prediabetes in adults from the general population: the Cooperative Health Research in the Region of Augsburg (KORA) F4 Study. Diabetes Care. 2011; 34:1648–1650. [PubMed: 21617096]
35. Pischon T, Girman CJ, Hotamisligil GS, Rifai N, Hu FB, Rimm EB. Plasma adiponectin levels and risk of myocardial infarction in men. JAMA. 2004; 291:1730–1737. [PubMed: 15082700]
36. Sattar N, Wannamethee G, Sarwar N, Tchernova J, Cherry L, Wallace AM, Danesh J, Whincup PH. Adiponectin and coronary heart disease: a prospective study and meta-analysis. Circulation. 2006; 114:623–629. [PubMed: 16894037]
37. Jin Q, Chen Y, Lou Y, He X. Low Serum retinol-binding protein-4 levels in acute exacerbations of chronic obstructive pulmonary disease at intensive care unit admission is a predictor of mortality in elderly patients. J Inflamm (Lond). 2013; 10:31. [PubMed: 24099047]
38. Dekker JM, Funahashi T, Nijpels G, Pilz S, Stehouwer CD, Snijder MB, Bouter LM, Matsuzawa Y, Shimomura I, Heine RJ. Prognostic value of adiponectin for cardiovascular disease and mortality. J Clin Endocrinol Metab. 2008; 93:1489–1496. [PubMed: 18211973]
39. Kizer JR, Benkeser D, Arnold AM, Mukamal KJ, Ix JH, Zieman SJ, Siscovick DS, Tracy RP, Mantzoros CS, Defilippi CR, Newman AB, Djousse L. Associations of total and high-molecular-weight adiponectin with all-cause and cardiovascular mortality in older persons: the Cardiovascular Health Study. Circulation. 2012; 126:2951–2961. [PubMed: 23159554]

40. Persson J, Folkersen L, Ekstrand J, Helleberg J, Gabrielsen A, Lundman P, Hedin U, Paulsson-Berne G. High plasma adiponectin concentration is associated with all-cause mortality in patients with carotid atherosclerosis. Atherosclerosis. 2012; 225:491–496. [PubMed: 23092825]
41. Wu ZJ, Cheng YJ, Gu WJ, Aung LH. Adiponectin is associated with increased mortality in patients with already established cardiovascular disease: a systematic review and meta-analysis. Metabolism. 2014; 63:1157–1166. [PubMed: 24933398]
42. Forsblom C, Thomas MC, Moran J, Saraheimo M, Thorn L, Waden J, Gordin D, Frystyk J, Flyvbjerg A, Groop PH. Serum adiponectin concentration is a positive predictor of all-cause and cardiovascular mortality in type 1 diabetes. J Intern Med. 2011; 270:346–355. [PubMed: 21615808]
43. Liao Y, Takashima S, Maeda N, Ouchi N, Komamura K, Shimomura I, Hori M, Matsuzawa Y, Funahashi T, Kitakaze M. Exacerbation of heart failure in adiponectin-deficient mice due to impaired regulation of AMPK and glucose metabolism. Cardiovasc Res. 2005; 67:705–713. [PubMed: 15907819]
44. Yang Q, Graham TE, Mody N, Preitner F, Peroni OD, Zabolotny JM, Kotani K, Quadro L, Kahn BB. Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. Nature. 2005; 436:356–362. [PubMed: 16034410]
45. Tobias D, Pan A, Hu FB. BMI and mortality among adults with incident type 2 diabetes. N Engl J Med. 2014; 370:1363–1364. [PubMed: 24693908]
46. Nilsson E, Jansson PA, Perfilyev A, et al. Altered DNA methylation and differential expression of genes influencing metabolism and inflammation in adipose tissue from subjects with type 2 diabetes. Diabetes. 2014; 63:2962–2976. [PubMed: 24812430]
47. Tiikkainen M, Hakkinen AM, Korsheninnikova E, Nyman T, Makimattila S, Yki-Jarvinen H. Effects of rosiglitazone and metformin on liver fat content, hepatic insulin resistance, insulin clearance, and gene expression in adipose tissue in patients with type 2 diabetes. Diabetes. 2004; 53:2169–2176. [PubMed: 15277403]
48. Khan RS, Kato TS, Chokshi A, et al. Adipose tissue inflammation and adiponectin resistance in patients with advanced heart failure: correction after ventricular assist device implantation. Circ Heart Fail. 2012; 5:340–348. [PubMed: 22379072]
49. Van Berendoncks AM, Garnier A, Beckers P, Hoymans VY, Possemiers N, Fortin D, Martinet W, Van Hoof V, Vrints CJ, Ventura-Clapier R, Conraads VM. Functional adiponectin resistance at the level of the skeletal muscle in mild to moderate chronic heart failure. Circ Heart Fail. 2010; 3:185–194. [PubMed: 20103776]
50. Collins S. A heart-adipose tissue connection in the regulation of energy metabolism. Nat Rev Endocrinol. 2014; 10:157–163. [PubMed: 24296515]
51. Kovacova Z, Tharp WG, Liu D, Wei W, Xie H, Collins S, Pratley RE. Adipose tissue natriuretic peptide receptor expression is related to insulin sensitivity in obesity and diabetes. Obesity (Silver Spring). 2016; 24:820–828. [PubMed: 26887289]
52. Kragelund C, Gronning B, Kober L, Hildebrandt P, Steffensen R. N-terminal pro-B-type natriuretic peptide and long-term mortality in stable coronary heart disease. N Engl J Med. 2005; 352:666–675. [PubMed: 15716560]
53. Kistorp C, Faber J, Galatius S, Gustafsson F, Frystyk J, Flyvbjerg A, Hildebrandt P. Plasma adiponectin, body mass index, and mortality in patients with chronic heart failure. Circulation. 2005; 112:1756–1762. [PubMed: 16157772]
54. von Eynatten M, Hamann A, Twardella D, Nawroth PP, Brenner H, Rothenbacher D. Relationship of adiponectin with markers of systemic inflammation, atherogenic dyslipidemia, and heart failure in patients with coronary heart disease. Clin Chem. 2006; 52:853–859. [PubMed: 16556684]
55. Menon V, Li L, Wang X, Greene T, Balakrishnan V, Madero M, Pereira AA, Beck GJ, Kusek JW, Collins AJ, Levey AS, Sarnak MJ. Adiponectin and mortality in patients with chronic kidney disease. J Am Soc Nephrol. 2006; 17:2599–2606. [PubMed: 16885405]
56. Austin WJ, Bhalla V, Hernandez-Arce I, Isakson SR, Beede J, Clopton P, Maisel AS, Fitzgerald RL. Correlation and prognostic utility of B-type natriuretic peptide and its amino-terminal fragment in patients with chronic kidney disease. Am J Clin Pathol. 2006; 126:506–512. [PubMed: 16938661]

57. Lee SA, Kallianpur A, Xiang YB, Wen W, Cai Q, Liu D, Fazio S, Linton MF, Zheng W, Shu XO. Intra-individual variation of plasma adipokine levels and utility of single measurement of these biomarkers in population-based studies. Cancer Epidemiol Biomarkers Prev. 2007; 16:2464–2470. [PubMed: 18006938]

58. Bluher M, Brennan AM, Kelesidis T, Kratzsch J, Fasshauer M, Kralisch S, Williams CJ, Mantzoros CS. Total and high-molecular weight adiponectin in relation to metabolic variables at baseline and in response to an exercise treatment program: comparative evaluation of three assays. Diabetes Care. 2007; 30:280–285. [PubMed: 17259495]

59. Liu D, Schuster T, Baumann M, Roos M, Sollinger D, Lutz J, Heemann U, von Eynatten M. Comparison of immunoassays for the selective measurement of human high-molecular weight adiponectin. Clin Chem. 2009; 55:568–572. [PubMed: 19168560]

60. Tamura T, Furukawa Y, Taniguchi R, Sato Y, Ono K, Horiuchi H, Nakagawa Y, Kita T, Kimura T. Serum adiponectin level as an independent predictor of mortality in patients with congestive heart failure. Circ J. 2007; 71:623–630. [PubMed: 17456982]

Highlights

- Elevated levels of plasma fatty acid-binding protein 4 (FABP4) were significantly associated with an increased cardiovascular disease (CVD) mortality in men with type 2 diabetes (T2D).
- Higher levels of high-molecular weight (HMW) adiponectin were associated with a higher risk of CVD mortality among men with T2D.
- Our findings suggested that elevated levels of FABP4 might predict higher CVD mortality among men with T2D, while the findings regarding HMW adiponection in the general population might not directly extrapolate to diseased populations probably due to weight change or alterations of adipose tissue functionality subsequent to disease pathophysiology.

Table 1

Baseline characteristics of participants

	CVD death N = 220	Others N = 730	*P* value
Age at blood draw (yr)	68.6 ± 7.0	63.1 ± 8.3	<0.001
Body mass index at baseline (kg/m^2)	27.9 ± 4.3	27.8 ± 4.3	0.61
Body mass index at age 21 (kg/m^2)†	23.9 ± 3.8	23.7 ± 3.4	0.31
Physical activity (MET-hr/week)†	16.3 (6.0, 35.1)	18.0 (6.5, 35.6)	0.62
Alcohol (g/d)†	2.1 (0, 12.1)	2.9 (0, 12.1)	0.38
Alternative healthy eating index score	53.9 ± 10.5	53.9 ± 12.0	0.97
Diabetes duration (months)	76.5 (14, 142)	48.5 (0, 115)	0.01
Smoking status‡, %			<0.001
≥45 pack-years	21.1	12.6	
<45 pack-years	49.8	44.4	
Never Smoked	29.1	43.0	
Medical history			
Hypertension, %	65.0	44.3	<0.001
Hypercholesterolemia, %	49.6	46.4	0.42
Angina, %	30.5	11.4	<0.001
Coronary Artery Bypass Grafting, %	24.6	8.9	<0.001
Myocardial infarction, %	25.0	7.7	<0.001
Stroke, %	1.4	1.5	0.88
Parental MI before age 65 years, %	37.7	37.4	0.93
Use of aspirin, %	51.4	41.0	0.006
Use of cholesterol-lowering drug, %	15.9	10.1	0.02
eGFR (mg/dL)	74.8 ± 20.7	80.2 ± 17.6	<0.001
TG (mg/dL)	183 (129, 250)	175 (117, 256)	0.67
LDL (mg/dL)	125.5 ± 39.4	125.8 ± 35.9	0.92
HDL (mg/dL)	38.7 ± 11.8	40.2 ± 10.7	0.08
hsCRP (mg/dL)	0.22 (0.13, 0.42)	0.16 (0.09, 0.33)	<0.001
TNFR2 (ng/mL)	3.2 (2.6, 3.9)	2.8 (2.3, 3.3)	<0.001
FABP4 (ng/mL)	20.9 (15.7, 27.3)	18.2 (14.0, 20.7)	<0.001
RBP4 (μg/mL)	36.6 (30.9, 44.1)	36.2 (30.8, 42.5)	0.41
HMW adiponectin (μg/mL)	1.5 (1.1, 2.6)	1.4 (0.9, 2.2)	0.04

Data are mean ± SD, median (interquartile range), or percentage (%). Abbreviation: eGFR, estimated glomerular filtration rate; TG, triacylglycerol; LDL, low-density lipoprotein; HDL, high-density lipoprotein; hsCRP, C-reactive protein; TNFR2, soluble tumor necrosis factor-α receptor 2; FABP4, fatty acid-binding protein 4; RBP4, retinol-binding protein 4; HMW, high-molecular weight; *P* value was calculated by using *t* test for continuous variables or the Chi-Square test for categorical variables.

† Data were missing for body mass index at age 21 in 45 participants; physical activity in 42 participants; and alcohol intake in 74 participants.

‡ Based on data in 897 participants.

Table 2

Hazard ratio (95% CI) of cardiovascular mortality associated with FABP4, RBP4, and HMW adiponectin levels

	Tertiles levels			
	1	**2**	**3**	***P* for trend**
FABP4				
Case/person years	54/4681	67/4451	99/3971	
Model 1*	1.0	1.20 (0.84, 1.72)	2.13 (1.53, 2.97)	<0.0001
Model 2†	1.0	1.16 (0.80, 1.68)	1.80 (1.27, 2.57)	0.0004
Model 3‡	1.0	1.13 (0.77, 1.65)	1.78 (1.22, 2.59)	0.001
RBP4				
Case/person years	72/4349	72/4441	76/4312	
Model 1*	1.0	0.93 (0.67, 1.29)	0.95 (0.69, 1.31)	0.76
Model 2†	1.0	1.00 (0.71, 1.40)	0.87 (0.62, 1.23)	0.43
Model 3‡	1.0	0.92 (0.65, 1.30)	0.73 (0.50, 1.07)	0.09
HMW adiponectin				
Case/person years	57/4567	78/4456	85/4080	
Model 1*	1.0	1.27 (0.90, 1.79)	1.45 (1.03, 2.03)	0.04
Model 2†	1.0	1.30 (0.91, 1.86)	1.76 (1.23, 2.51)	0.002
Model 3‡	1.0	1.40 (0.97, 2.01)	2.07 (1.42, 3.06)	0.0002

*Model 1 was adjusted for age at blood draw.

†Based on Model 1, Model 2 was further adjusted for date of blood draw (yr), body mass index at age 21 (kg/m^2), physical activity (in quintiles), smoking status (never smoked, <10 pack-years, 10–24 pack-years, 25–44 pack-years, 45–64 pack-years, 65+ pack-years, missing), alcohol consumption (non-drinker, <5.0 g/day, 5.0–9.9 g/day, 10.0–14.9 g/day, 15.0–29.9 g/day, ≥30.0 g/day, missing), diabetes duration (yr), family history of myocardial infarction, aHEI score (in tertiles), use of aspirin or cholesterol-lowering medication (yes, no), baseline history of hypertension, high cholesterol, angina, CABG, myocardial infarction, and stroke (yes, no).

‡Based on Model 2, Model 3 was further adjusted for eGFR, triacylglycerol levels (mg/dL), high-density lipoprotein cholesterol (mg/dL), low-density lipoprotein cholesterol (mg/dL), and hsCRP.

Table 3

Stratified analysis of the associations [hazard ratio (95% CI)] between high and low tertiles of FABP4, RBP4, and HMW adiponectin and risk of cardiovascular mortality

		FABP4		RBP4		HMW Adiponectin
	Case/Total	**HR (95% CI)**	***P* interaction**	**HR (95% CI)**	***P* interaction**	***P* interaction HR (95% CI)**
Age at blood draw (yr)			0.03		0.91	0.18
<65	58/435	4.37 (1.85, 10.3)		0.55 (0.23, 1.31)		1.76 (0.72, 4.33)
≥65	162/515	1.36 (0.88, 2.12)		0.82 (0.53, 1.28)		2.25 (1.38, 3.65)
BMI at age 21 (kg/m^2) *			0.44		0.95	0.87
<25	134/598	1.30 (0.79, 2.13)		0.71 (0.43, 1.18)		1.97 (1.21, 3.22)
≥25	71/307	2.65 (1.22, 5.80)		0.85 (0.41, 1.74)		2.92 (1.17, 7.32)
Current smoking status, % †			0.99		0.92	0.96
No	61/342	1.30 (0.59, 2.89)		0.51 (0.23, 1.13)		2.30 (1.01, 5.25)
Yes	155/561	1.81 (1.16, 2.81)		0.72 (0.45, 1.15)		2.26 (1.42, 3.60)

Hazard ratio, comparing extreme tertiles, was adjusted for age at blood draw (yr), date of blood draw (yr), body mass index at age 21 (kg/m2), physical activity (in quintiles), smoking status (never smoked, <10 pack-years, 10–24 pack-years, 25–44 pack-years, 45–64 pack-years, 65+ pack-years, missing), alcohol consumption (non-drinker, <5.0 g/day, 5.0–9.9 g/day, 10.0–14.9 g/day, 15.0–29.9 g/day, ≥30.0 g/day, missing), diabetes duration (yr), family history of myocardial infarction, aHEI score (in tertiles), use of aspirin or cholesterol-lowering medication (yes, no), baseline history of hypertension, high cholesterol, angina, CABG, myocardial infarction, stroke (yes, no), triacylglycerol levels (mg/dL), high-density lipoprotein cholesterol (mg/dL), low-density lipoprotein cholesterol (mg/dL), and eGFR. Stratifying variables were not included in the model when analyses were stratified by these variables individually.

* Data were missing for body mass index at age 21 in 45 participants;

† Data were missing for smoking status in 44 participants.

Table 4

Associations between SNPs, adipokines, and CVD mortality

	Biomarker (RBP4/HMW adiponectin)		CVD mortality	
SNPs	**Estimate**	***P***	**Estimate**	***P***
RBP4				
LogRBP4 (Observational) *			0.7	0.37
rs10882272_C	−0.01	0.03		
LogRBP4 (Causal)			15. 7	0.10
rs17108993_G	−0.003	0.94		
LogRBP4 (Causal)			130.0	0.65
rs3758538_G	−0.004	0.65		
LogRBP4 (Causal)			36.3	0.44
RBP4_SNP_Score †	−0.02	0.05		
LogRBP4 (Causal)			19.0	0.11
HMW adiponectin				
Log HMW (Observational) *			0.53	0.07
rs1342387_T	−0.003	0.83		
Log HMW (Causal)			40.0	0.28
rs12733285_T	0.006	0.71		
Log HMW (Causal)			−23.6	0.24
rs822354_A	0.01	0.54		
Log HMW (Causal)			−29.4	0.03
rs1426810_G	0.04	0.02		
Log HMW (Causal)			0.80	0.82
rs266717_T	0.04	0.006		
Log HMW (Causal)			−0.55	0.85
rs6810075_C	0.03	0.05		
Log HMW (Causal)			−9.90	0.02
rs16861194_G	−0.03	0.38		
Log HMW (Causal)			−11.0	0.21
rs17300539_A	0.08	0.009		
Log HMW (Causal)			−1.66	0.60
rs266729_G	−0.04	0.03		
Log HMW (Causal)			−7.5	0.03
rs822394_A	−0.001	0.97		
Log HMW (Causal)			178.9	0.49
rs17366568_A	−0.08	0.0008		
Log HMW (Causal)			−2.6	0.26
rs1501299_T	0.06	0.0007		
Log HMW (Causal)			−0.93	0.69
rs3774262_A	−0.01	0.57		

	Biomarker (RBP4/HMW adiponectin)		CVD mortality	
SNPs	**Estimate**	***P***	**Estimate**	***P***
Log HMW (Causal)			13.7	0.33
rs17366743_C	0.05	0.25		
Log HMW (Causal)			7.1	0.26
rs6773957_A	0.04	0.005		
Log HMW (Causal)			−3.01	0.28
rs1063538_T	0.04	0.007		
Log HMW (Causal)			−3.3	0.25
rs1063539_C	−0.02	0.45		
Log HMW (Causal)			7.6	0.47
rs6444175_A	0.05	0.002		
Log HMW (Causal)			−0.42	0.86
rs7615090_G	−0.06	0.06		
Log HMW (Causal)			−0.69	0.87
HMW_SNP_Score[†]	0.14	0.02		
Log HMW (Causal)			−1.9	0.15

[*] Crude estimates without adjustment for any covariates.

[†] The unweighted score of each individual was calculated by summing the number of rsik alleles.