Society for Endocrinology
European Society of Endocrinology

Endocrine Connections (2024) **13** e240343
https://doi.org/10.1530/EC-24-0343

Received 11 July 2024
Accepted 8 August 2024
Available online 8 August 2024
Version of Record published 14 October 2024

RESEARCH

Associations between circulating levels of FABP4 and TNF receptors are more evident in patients with type 2 diabetes mellitus than in patients with type 1 diabetes mellitus

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Abstract

Background: Fatty acid-binding protein 4 (FABP4) is an adipokine that plays significant roles in the development of insulin resistance and atherosclerosis. High levels of soluble tumor necrosis factor receptors (TNFRs) including TNFR1 and TNFR2 are associated with renal dysfunction and increased mortality in patients with diabetes mellitus (DM). However, the association between circulating levels of FABP4 and TNFRs remains unclear.

Methods: We investigated the associations of FABP4 with TNFRs and metabolic markers in Japanese patients with type 1 DM (T1DM, $n = 76$, men/women: 31/45) and type 2 DM (T2DM, $n = 575$, men/women: 312/263).

Results: FABP4 concentration was positively correlated with levels of TNFR1 and TNFR2 in both patients with T1DM and those with T2DM. Multivariable regression analyses showed that there were independent associations of FABP4 concentration with body mass index (BMI) and estimated glomerular filtration rate (eGFR) after adjustment for age and sex in both patients with T1DM and those with T2DM. FABP4 concentration was independently associated with circulating levels of TNFR1 and TNFR2 after adjustment for the confounders in patients with T2DM but not in those with T1DM. Similarly, levels of TNFR1 and TNFR2 were independently associated with FABP4 concentration after adjustment for age, sex, systolic blood pressure, duration of DM and levels of eGFR, high-density lipoprotein cholesterol, and C-reactive protein in patients with T2DM but not in those with T1DM.

Conclusion: FABP4 concentration is independently associated with levels of TNFRs in patients with DM, but the association is more evident in patients with T2DM than in those with T1DM.

Keywords: adiposcience; diabetes mellitus; fatty acid-binding protein 4; tumor necrosis factor receptor

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Introduction

Diabetes mellitus (DM) is a critical issue in modern public health, and the prevalence of DM is continuously increasing worldwide (1). It has been reported that major causes of death, including cardiovascular disease, stroke, renal dysfunction, and cancers, are triggered by the presence of DM (2, 3). In a recent research field called 'adiposcience', new scientific concepts including 'adipokines' as various biologically active substances derived from adipose tissue, and metabolically driven chronic and low-grade inflammation referred to as 'metaflammation', have emerged to define the unique combination of overlapping mediators and/or states that contribute to immunometabolism and metabolic homeostasis (4, 5).

Type 1 DM (T1DM) is characterized by pancreatic β-cell destruction and absolute insulin deficiency, while type 2 DM (T2DM) is mainly characterized by the presence of insulin resistance and relative insulin insufficiency, which are induced by genetic factors and metabolic dysfunction including obesity (3). Several adipokines and metaflammation in adipose tissue have been reported to reflect inflammatory processes in T2DM (6). However, there has been little investigation of the metaflammation in T1DM (7).

Fatty acid-binding protein 4 (FABP4) is expressed in adipocytes, macrophages, and capillary and injured arterial endothelial cells, and excessive expression of FABP4 is associated with the development of insulin resistance and atherosclerosis, leading to cardiovascular diseases (8, 9, 10). FABP4 has been shown to be secreted from adipocytes in association with lipolysis via a non-classical pathway and acts as an adipokine, though there are no typical secretory signal peptides in the sequence of FABP4 (11). Recently, the endothelium has also been shown to be a major source of circulating FABP4 (12). It has been reported that circulating levels of FABP4 are associated with the development of cardiovascular disease (13, 14, 15), renal dysfunction (16, 17), and cancer (18) and that an elevated circulating level of FABP4 can be a prognostic predictor for cardiovascular death in the general population (19). Furthermore, there are distinct independent associations of FABP4 with renal dysfunction, adiposity, and hypertriglyceridemia in patients with T2DM (20). However, the effects of FABP4 on DM-associated complications have not been elucidated in patients with T1DM.

Tumor necrosis factor α (TNFα) has been shown to be an important cytokine derived from adipose tissue (5). In addition to the ligand itself, it has also been reported that tumor necrosis factor receptors (TNFRs), including TNFR1 and TNFR2, which are cell membrane-bound receptors involved in inflammation and immune response, are released into the extracellular space by enzymatic cleavage as solubilized forms (5). Recent studies have shown that elevated circulating levels of TNFR1 and TNFR2 are associated with renal dysfunction (21, 22, 23, 24, 25, 26) and can be prognostic factors for mortality in patients with T2DM (22, 27). However, the relationships between FABP4 and TNFRs, including TNFR1 and TNFR2, have not been fully addressed despite the fact that their upstream and downstream factors overlap (28, 29). In the present study, we aimed to elucidate the associations of FABP4 with TNFRs and metabolic markers including renal dysfunction in a real-world clinical setting in Japanese patients with T1DM and those with T2DM, two types of DM that have different pathophysiological phenotypes in whole body metabolism.

Materials and methods

The present study was a cross-sectional, single-center study conducted to assess the relationship between pathological conditions and several biomarkers in patients with DM who received standard and homogeneous treatment by diabetologists in Japan. The study conformed to the principles outlined in the Declaration of Helsinki and was approved by the ethics committees of Kure Medical Center and Chugoku Cancer Center (26-06) and Sapporo Medical University (3-1-77). Written informed consent was obtained from all of the subjects.

Study subjects

Japanese patients with DM were recruited at Kure Medical Center and Chugoku Cancer Center during the period from July 1, 2014, to March 31, 2016 ($n = 738$). Management of diabetes therapy including the use of medication for anti-diabetic drugs was performed by each attending physician. A flow chart of the study patients is shown in Supplementary Figure 1 (see section on supplementary materials given at the end of this article). Prespecified exclusion criteria were patients with DM due to specific causes, gestational DM, and those who were treated with pioglitazone, a peroxisome proliferator-activated receptor γ (PPARγ) agonist, since the FABP4 gene is a target of PPARγ. Patients with T1DM were defined as those diagnosed with T1DM by their attending physicians and those receiving at least insulin therapy. Patients with T2DM were defined as those having DM except for T1DM and DM caused by other factors. After exclusion, a total of 651 patients with T1DM ($n = 76$) and T2DM ($n = 575$) were enrolled in the present study.

Measurements

Blood and urine samples were obtained and stored at −80°C until biochemical analyses. Concentrations of FABP4, TNFR1, and TNFR2 were measured using enzyme-linked immunosorbent assay kits for FABP4 (BioVendor, Modrice, Czech Republic), TNFR1 (DRT100;

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R&D Systems), and TNFR2 (DRT200; R&D Systems), respectively. Two internal serum controls were included in each assay to estimate the inter-assay coefficients of variation. The inter-assay coefficients of variation for FABP4, TNFR1, and TNFR2 were 3.7, 8.0, and 9.4%, respectively (25, 30, 31). Variables of liver function, renal function, and glucose and lipid metabolism were measured as previously described (27). The estimated glomerular filtration rate (eGFR) for Japanese individuals was calculated using an equation (32). A self-administered questionnaire survey was performed to obtain information on current smoking habit, alcohol drinking habit, and family history of DM.

Statistical analysis

Variables are presented as means ± S.D. for normal distributions or medians (interquartile ranges) for skewed variables. The normality of each variable was tested by the Shapiro–Wilk test. Comparisons between two groups for parametric and nonparametric parameters were performed by the Student's *t*-test and the Mann–Whitney *U* test, respectively. The chi-square test was performed for intergroup differences in the percentages of parameters. Pearson's correlation analysis was performed for the correlation between two variables. Non-normally distributed variables were logarithmically transformed for regression analyses. Multivariable regression analyses were performed to identify independent associations of FABP4, TNFR1, and TNFR2 after adjustment for age, sex, and variables with significant correlations determined by Pearson's coefficients after consideration of multicollinearity, showing the standardized regression coefficient (β), the percentage of variance for the selected independent predictors explained (R^2), and Akaike's information criterion. A *P*-value of less than 0.05 was considered statistically significant. The statistical power ($1 - \beta$ error) as a *post hoc* analysis was calculated by G*Power 3.1 (33, 34) using sample size, the number of predictors, and the effect size of f^2. The f^2 was calculated by the adjusted coefficient of determination R^2: $f^2 = R^2/(1 - R^2)$. All data were analyzed using EZR (35), R version 3.6.1., and JMP15.2.1 for Windows (SAS Institute, Cary, NC, USA).

Results

Characteristics of the studied patients with DM

Basal characteristics of the enrolled ($n = 651$) and excluded ($n = 87$) patients with DM are shown in Supplementary Table 1. The enrolled patients had a significantly higher rate of use of insulin and a lower rate of use of sulfonylureas and α-glucosidase inhibitors than did the excluded subjects. The FABP4 level was significantly lower in the enrolled patients than in the excluded patients. There were no significant differences in the levels of TNFR1 and TNFR2 between the excluded and enrolled patients.

Basal characteristics of the enrolled patients with T1DM and T2DM are shown in Table 1, and those divided by sex are shown in Supplementary Table 2. The patients with T2DM were significantly older than those with T1DM and included a significantly higher percentage of men than did patients with T1DM. Body mass index (BMI) and levels of triglycerides, alanine transaminase (ALT), and γ-glutamyl transpeptidase (γGTP) were significantly higher in patients with T2DM than in patients with T1DM, and fasting glucose level was significantly lower in patients with T2DM than in those with T1DM. There were no significant differences in levels of hemoglobin A1c and aspartate transaminase (AST) between patients with T1DM and those with T2DM.

Levels of FABP4 and TNFRs in patients with T1DM and T2DM

Levels of FABP4 (Fig. 1A), TNFR1 (Fig. 1B), and TNFR2 (Fig. 1C) were significantly higher in patients with T2DM than in those with T1DM. When sex was separately analyzed in the T1DM and T2DM groups, FABP4 concentration was significantly lower in men than in women (Fig. 1D). There were no significant differences in levels of TNFR1 (Fig. 1E) and TNFR2 (Fig. 1F) between men and women in patients with T1DM. In patients with T2DM, levels of TNFR1 and TNFR2 were significantly higher in men than in women.

Correlation analyses for FABP4 in patients with T1DM and T2DM

In both patients with T1DM and those with T2DM, FABP4 concentration was positively correlated with BMI and triglyceride levels and was negatively correlated with eGFR (Table 2). The FABP4 concentration was positively correlated with levels of AST, ALT, and γGTP in patients with T1DM but not in those with T2DM. There were significant positive correlations of FABP4 concentration with levels of TNFR1 and TNFR2 in both patients with T1DM and those with T2DM (Table 2).

When sex was separately analyzed in patients with T1DM ($n = 76$, men/women: 31/45), FABP4 concentration was significantly correlated with BMI (Fig. 2A) and with levels of eGFR (Fig. 2B), uric acid, triglycerides, TNFR1 (Fig. 2C) and TNFR2 (Fig. 2D) in women but not in men (Supplementary Table 3). On the other hand, FABP4 concentration was significantly correlated with systolic and diastolic blood pressures and levels of AST, ALT, and γGTP in men but not in women (Supplementary Table 3).

In patients with T2DM ($n = 575$, men/women: 312/263), FABP4 concentration was significantly correlated with BMI (Fig. 2E) and levels of eGFR (Fig. 2F), uric acid, triglycerides, TNFR1 (Fig. 2G), and TNFR2 (Fig. 2H) in both men and women (Supplementary Table 3).

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https://doi.org/10.1530/EC-24-0343

Table 1 Characteristics of the enrolled patients with DM. Variables are expressed as number (%), means ± S.D., or medians (interquartile ranges).

	T1DM (*n* = 76)	**T2DM** (*n* = 575)	***P***
Age, years	60 ± 14	65 ± 13	0.001
Sex, men	31 (40.8)	312 (54.3)	0.028
BMI	23 ± 4	25 ± 4	<0.001
Systolic BP, mmHg	135 ± 16	139 ± 18	0.044
Diastolic BP, mmHg	77 ± 11	78 ± 12	0.381
Heart rate, per min	81 ± 13	81 ± 14	0.956
Current smoking habit	10 (13.5)	111 (17.5)	0.510
Alcohol drinking habit	17 (23.0)	160 (25.6)	0.672
Duration of DM, years	12 (5–26)	14 (6–22)	0.939
Family history			
DM	36 (47.4)	339 (59.0)	0.064
Comorbidity			
Hypertension	47 (61.8)	422 (73.4)	0.041
Cardiovascular disease	15 (19.7)	209 (36.4)	0.014
Medication			
Anti-diabetic drugs			
Insulin	76 (100)	212 (36.9)	<0.001
GLP-1 analogs	1 (1.3)	31 (5.4)	0.160
Sulfonylureas	0 (0)	166 (28.9)	<0.001
Biguanides	11 (14.5)	309 (53.7)	<0.001
α-glucosidase inhibitors	5 (6.6)	95 (16.5)	0.026
DPP-4 inhibitors	18 (23.7)	414 (72.0)	<0.001
Glinides	0 (0)	49 (8.5)	0.004
SGLT2 inhibitors	0 (0)	10 (1.7)	0.616
Anti-dyslipidemic drugs			
Statin	29 (38.2)	302 (52.5)	0.020
Biochemical data			
Total protein, g/dL	7.2 ± 0.4	7.3 ± 0.5	0.018
AST, IU/L	21 (17–25)	21 (17–28)	0.378
ALT, IU/L	18 (13–23)	19 (14–28)	0.017
γGTP, IU/L	21 (15–39)	25 (18–46)	0.007
BUN, mg/dL	17 ± 7	17 ± 8	0.977
Creatinine, mg/dL	0.72 (0.60–0.90)	0.80 (0.64–1.00)	0.114
eGFR, mL/min/1.73 m^2	70 ± 25	67 ± 23	0.274
Uric acid, mg/dL	4.8 ± 1.5	5.4 ± 1.3	<0.001
TC, mg/dL	190 ± 30	182 ± 36	0.050
LDL-C, mg/dL	112 ± 23	105 ± 29	0.073
HDL-C, mg/dL	63 ± 17	51 ± 14	< 0.001
Non-HDL-C, mg/dL	127 ± 26	130 ± 33	0.430
Triglycerides, mg/dL	75 (52–100)	109 (77–169)	<0.001
CRP, mg/dL	0.07 (0.05–0.13)	0.11 (0.06–0.20)	0.009
Glucose, mg/dL	173 (115–218)	140 (118–171)	0.047
Hemoglobin A1c, %	7.4 (6.7–8.1)	7.1 (6.5–7.9)	0.097
C-peptide, ng/mL	0.15 (0.15–0.71)	1.69 (1.12–2.54)	<0.001
TNFR1, ng/mL	1.33 (108–1.79)	1.61 (1.28–2.18)	<0.001
TNFR2, ng/mL	2.95 (2.38–4.04)	3.43 (2.74–4.56)	0.005
FABP4, ng/mL	14.1 (9.0–17.3)	17.6 (11.6–24.4)	0.001

ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; BP, blood pressure; BUN, blood urea nitrogen; CRP, C-reactive protein; DM, diabetes mellitus; DPP-4, dipeptidyl peptidase-4; eGFR, estimated glomerular filtration rate; FABP4, fatty acid-binding protein 4; GLP-1, glucagon-like peptide-1; γGTP, γ-glutamyl transpeptidase; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SGLT2, sodium-glucose co-transporter-2; TC, total cholesterol; TNFR, tumor necrosis factor receptor; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus.

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https://doi.org/10.1530/EC-24-0343

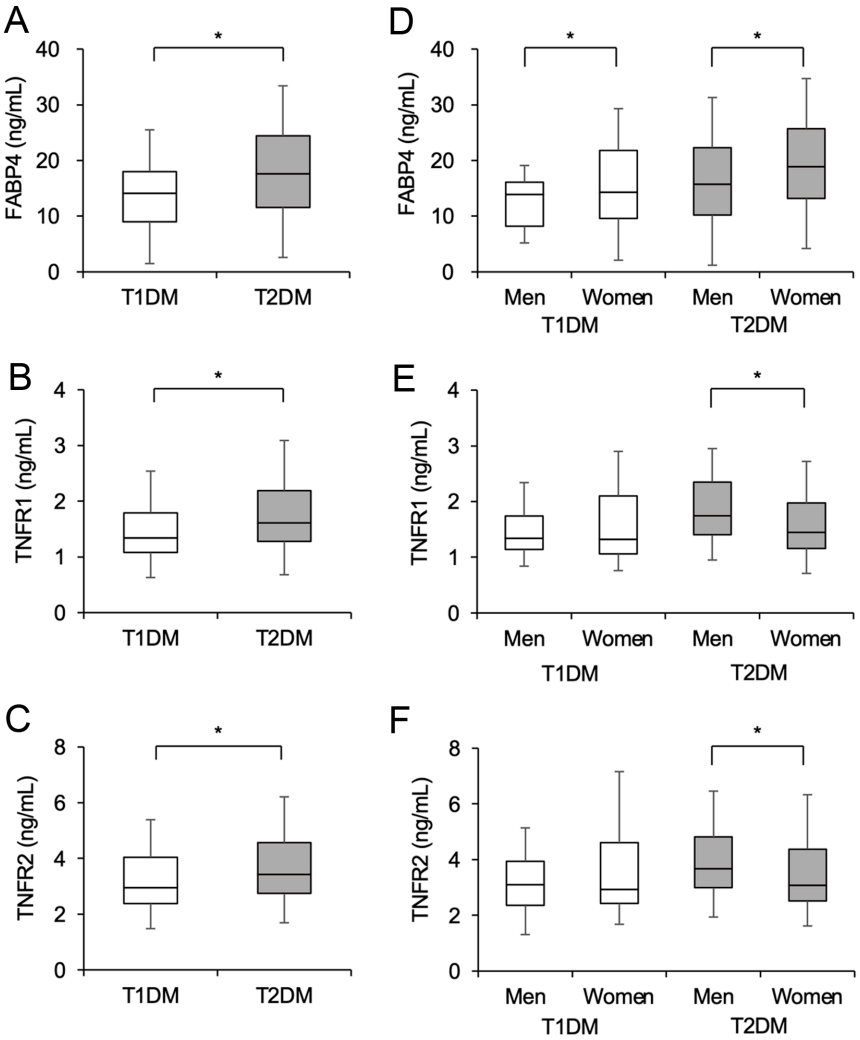

Figure 1

Concentrations of FABP4, TNFR1, and TNFR2. (A–C) Concentrations of fatty acid-binding protein 4 (FABP4) (A), tumor necrosis factor receptor (TNFR) 1 (B), and TNFR2 (C) in patients with type 1 diabetes mellitus (T1DM, $n = 76$) and those with type 2 diabetes mellitus (T2DM, $n = 575$). (D–F) Concentrations of FABP4 (D), TNFR1 (E), and TNFR2 (F) in patients with T1DM (men/women: 31/45) and those with T2DM (men/women: 312/263) divided by sex. Box-and-whisker plots show the median, the first quartile (Q1), the third quartile (Q3), lower outlier (Q1 – 1.5 × interquartile range (IQR)), and higher outlier (Q3 + 1.5 × IQR). *$P < 0.05$.

Multivariable regression analyses for FABP4 in patients with T1DM and T2DM

Multivariable regression analyses using age, sex, and variables with significant correlations after consideration of multicollinearity, including BMI as a marker of obesity, eGFR as an indicator of renal function, and triglycerides as a lipid marker, showed that FABP4 level was independently associated with eGFR but with the level of TNFR1 (Model 1) or TNFR2 (Model 2) in patients with T1DM (Table 3). There was no significant interaction of age, sex, or duration of DM for the association of FABP4 level with the level of TNFR1 (*P* values: 0.470/0.612/0.563, respectively) or TNFR2 (*P* values: 0.215/0.620/0.276, respectively) in patients with T1DM. The statistical powers of Models 1 and 2 for FABP4 were both 0.999 in patients with T1DM. When additionally testing at a significance level of 0.01 or 0.001, the statistical powers of multivariable regression analyses for FABP4 in Models 1 and 2 were 0.995/0.967 and 0.995/0.968, respectively, in patients with T1DM.

In patients with T2DM, the FABP4 level was independently associated with the level of TNFR1 ($\beta = 0.281$, $P < 0.001$) (Model 1) or TNFR2 ($\beta = 0.247$, $P < 0.001$) (Model 2) after adjustment for confounders including age, sex, BMI, and levels of eGFR and triglycerides (Table 3). There was no significant interaction of age, sex, or duration of DM for the association of FABP4 level with the level of TNFR1 (*P* values: 0.351/0.462/0.141, respectively) or TNFR2 (*P* values: 0.436/0.441/0.226, respectively) in patients with T2DM. The statistical powers of Models 1 and 2 for FABP4 were all the same (1.000) in patients with T2DM.

After additional adjustment of smoking habits and medications for DM, the FABP4 level was not independently associated with the level of TNFR1 (Model 3) or TNFR2 (Model 4) in patients with T1DM (Supplementary Table 4). On the other hand, the FABP4 level was independently associated with the level of TNFR1 (Model 3) or TNFR2 (Model 4) in patients with T2DM.

Moreover, multivariable regression analyses after adjustment for glucose, C-reactive protein (CRP), cardiovascular disease, and use of statin, in addition to age, sex, BMI, eGFR, and triglycerides,

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https://doi.org/10.1530/EC-24-0343

Table 2 Correlation analyses for FABP4 in patients with DM.

	Log FABP4			
	T1DM (n = 76)		T2DM (n = 575)	
	r	P	r	P
Age	0.029	0.802	−0.005	0.913
BMI	0.392	<0.001	0.316	<0.001
Systolic BP	0.159	0.170	0.058	0.160
Diastolic BP	0.013	0.914	0.021	0.611
Heart rate	−0.077	0.510	0.076	0.068
Log (duration of DM)	0.218	0.061	0.062	0.144
Total protein	0.004	0.716	−0.043	0.305
Log AST	0.273	0.017	0.036	0.386
Log ALT	0.248	0.031	0.062	0.124
Log γGTP	0.270	0.019	0.054	0.191
BUN	0.459	<0.001	0.231	<0.001
Log creatinine	0.506	<0.001	0.272	<0.001
eGFR	−0.510	<0.001	−0.297	<0.001
Uric acid	0.187	0.106	0.203	<0.001
TC	0.001	0.935	0.001	0.996
LDL-C	0.015	0.898	0.006	0.887
HDL-C	−0.178	0.250	−0.124	0.003
Non-HDL-C	0.126	0.277	0.051	0.222
Log triglycerides	0.344	0.002	0.128	0.002
Log CRP	0.202	0.081	0.106	0.012
Log glucose	0.078	0.503	−0.023	0.576
Log (hemoglobin A1c)	0.221	0.054	0.072	0.086
Log C-peptide	0.184	0.112	0.202	<0.001
Log TNFR1	0.546	<0.001	0.366	<0.001
Log TNFR2	0.519	<0.001	0.334	<0.001

ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; BP, blood pressure; BUN, blood urea nitrogen; CRP, C-reactive protein; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; FABP4, fatty acid-binding protein 4; γGTP, γ-glutamyl transpeptidase; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TNFR, tumor necrosis factor receptor; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus.

were analyzed to investigate potential confounders. The results (Models 5 and 6) (Supplementary Table 5) were similar to the results of Models 1 and 2 (Table 3).

Correlation and multivariable regression analyses for TNFRs in patients with T1DM and T2DM

There was a strong correlation between concentrations of TNFR1 and TNFR2 in both patients with T1DM (r = 0.956, P < 0.001) and those with T2DM (r = 0.940, P < 0.001) (Table 4). Levels of TNFR1 and TNFR2 were positively correlated with systolic blood pressure, duration of DM, and level of CRP and were negatively correlated with levels of eGFR and high-density lipoprotein cholesterol (HDL-C) in both patients with T1DM and those with T2DM (Table 4).

Multivariable regression analyses showed that FABP4 concentration was not an independent predictor for TNFR1 or TNFR2 after adjustment for age, sex, and variables with significant correlations after consideration of multicollinearity, including systolic blood pressure, duration of DM, and levels of eGFR, HDL-C, and CRP in patients with T1DM (Table 5). The statistical powers for TNFR1 or TNFR2 were 0.999 in patients with T1DM. In patients with T2DM, FABP4 concentration was independently associated with levels of TNFR1 and TNFR2 after adjustment of the confounders. The statistical powers for TNFR1 or TNFR2 were 1.000 in patients with T2DM.

Discussion

The present study showed that circulating FABP4 concentration was independently associated with levels of TNFR1 and TNFR2 after adjustment for age, sex, BMI, and levels of eGFR and triglycerides in patients with T2DM. Similarly, both levels of TNFR1 and TNFR2 were independently associated with FABP4 levels after adjustment for age, sex, systolic blood pressure, duration of DM, and levels of eGFR, HDL-C, and CRP in patients with T2DM. We confirmed our hypothesis that FABP4 and TNFRs are associated with each other independent of metabolic markers, including renal dysfunction. To the best of our knowledge, there were two previous studies that focused on the association between levels of FABP4 and TNFR1. In a study using 81 Spanish women, including 43 morbidly obese patients (BMI > 40), circulating FABP4 levels were significantly higher in obese subjects than in non-obese subjects and were associated with TNFR1 levels after adjustment for age and BMI (28). The other study, using 282 human immunodeficiency virus 1-infected patients, showed that circulating FABP4 levels were positively correlated with TNFR1 levels (29). In the present study, there was a strong correlation between levels of TNFR1 and TNFR2. Furthermore, FABP4 concentration was independently associated with levels of not only TNFR1 but also TNFR2 in patients with T2DM. Taken together, these findings support the notion that circulating FABP4 is significantly associated with soluble TNFRs in patients with metabolic disorders, suggesting a link between FABP4 and TNF bioactivity, including insulin resistance, lipolysis, and metaflammation (5).

Distinct independent associations of FABP4 with adiposity and renal dysfunction were confirmed in both patients with T1DM and those with T2DM (Table 3). However, the association between FABP4 and TNFRs was not found in patients with T1DM after adjustment for the confounders. Although the precise reasons for the discrepant results in the T1DM and T2DM groups were not elucidated in the present study, there are at least two possibilities. First, since the number of patients with T1DM (n = 76) and the number of patients with T2DM

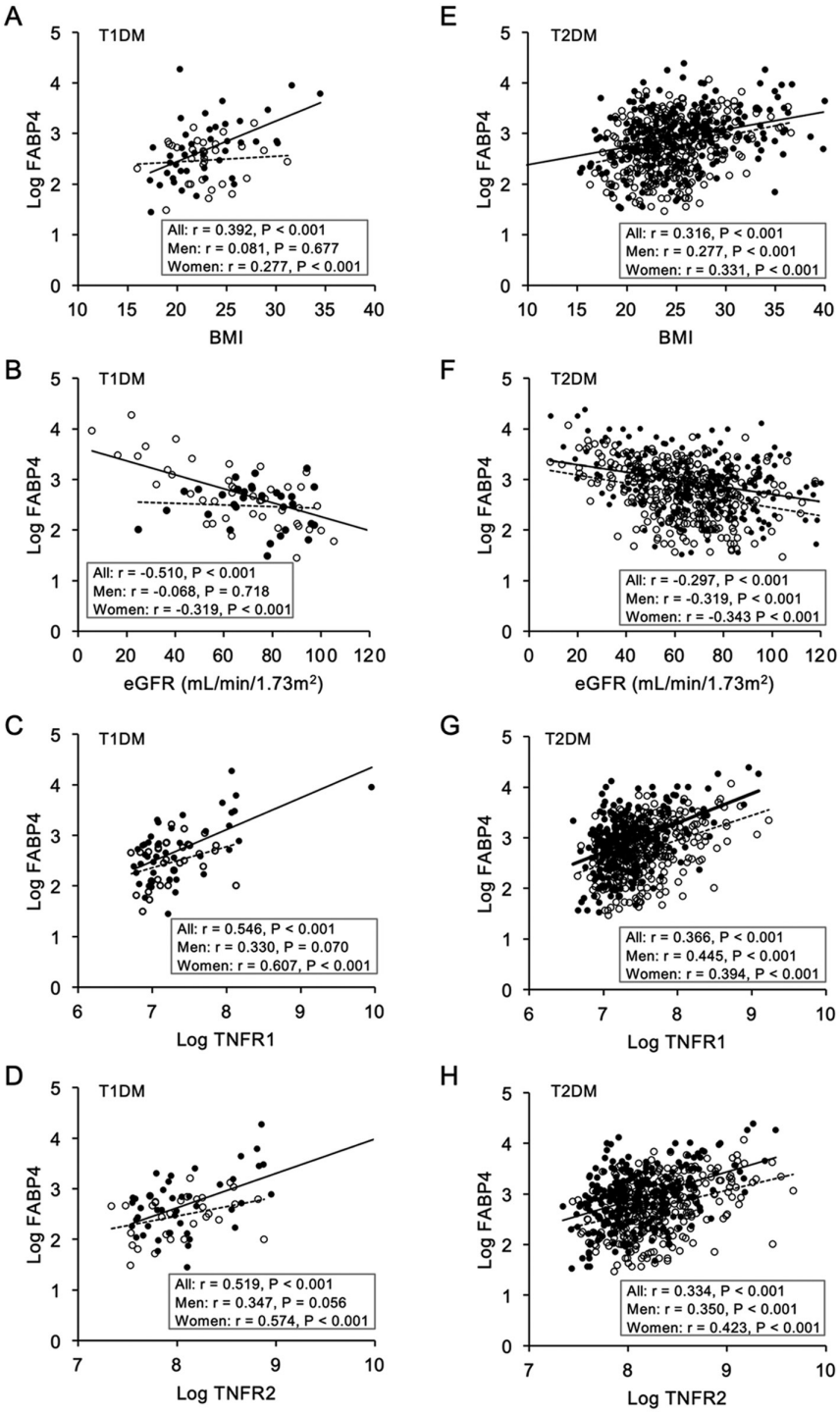

Figure 2

Correlations of FABP4 levels with metabolic parameters. (A–D) Levels of logarithmically transformed (Log) fatty acid-binding protein 4 (FABP4) were plotted against levels of body mass index (BMI) (A), estimated glomerular filtration rate (eGFR) (B), Log tumor necrosis factor receptor (TNFR)1 (C) and TNFR2 (D) in patients with type 1 diabetes mellitus (T1DM, $n = 76$). (E–H) Levels of Log FABP4 were plotted against levels of BMI (E), eGFR (F), Log TNFR1 (G), and Log TNFR2 (H) in patients with type 2 diabetes mellitus (T2DM, $n = 575$). Open circles and broken regression lines: men. Closed circles and solid regression lines: women.

($n = 575$) who were enrolled in this study were different, the difference of statistical power may be involved in the discrepant results. Indeed, the correlation coefficients of FABP4 with TNFR1 (T1DM vs T2DM: $r = 0.546$ vs $r = 0.366$) and TNFR2 (T1DM vs T2DM: $r = 0.519$ vs $r = 0.334$) were even larger in patients with T1DM than in those with T2DM (Table 2). The small number of patients with T1DM may also have influenced the discrepant results between men ($n = 31$) and women ($n = 45$) in simple correlation analyses for FABP4 (Fig. 2, Supplementary Table 3). Second, although T1DM and T2DM share the same aspects of impaired insulin action, their upstream pathophysiological mechanisms are substantially different, with insulin deficiency underlying T1DM and insulin resistance being central to the pathogenesis in most patients with T2DM. Considering that FABP4 is

Table 3 Multivariable regression analyses for Log FABP4 in patients with DM.

	T1DM ($n = 76$)				T2DM ($n = 575$)			
	Model 1		Model 2		Model 1		Model 2	
	β	P	β	P	β	P	β	P
Age	−0.126	0.288	−0.143	0.216	−0.107	0.013	−0.125	0.004
Sex (men)	−0.125	0.198	−0.119	0.221	−0.232	<0.001	−0.228	<0.001
BMI	0.207	0.079	0.206	0.079	0.280	<0.001	0.286	<0.001
eGFR	−0.335	0.048	−0.366	0.016	−0.238	<0.001	−0.273	<0.001
Log triglycerides	0.128	0.260	0.136	0.231	0.003	0.930	0.007	0.851
Log TNFR1	0.106	0.180	–	–	0.281	<0.001	–	–
Log TNFR2	–	–	0.189	0.169	–	–	0.247	<0.001
AIC	99		99		736		742	
R^2	0.403		0.419		0.303		0.295	
Adjusted R^2	0.367		0.368		0.296		0.288	
Statistical power (1 − β error)	0.999		0.999		1.000		1.000	

AIC, Akaike's information criterion; β, standardized regression coefficient; BMI, body mass index; eGFR, estimated glomerular filtration rate; FABP4, fatty acid-binding protein 4; TNFR, tumor necrosis factor receptor; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus.

Table 4 Correlation analyses for TNFRs in patients with DM.

	Log TNFR1				Log TNFR2			
	T1DM ($n = 76$)		T2DM ($n = 575$)		T1DM ($n = 76$)		T2DM ($n = 575$)	
	r	P	r	P	r	P	r	P
Age	0.172	0.138	0.280	<0.001	0.196	0.090	0.326	<0.001
BMI	0.289	0.012	0.025	0.550	0.260	0.024	−0.002	0.968
Systolic BP	0.395	<0.001	0.134	0.001	0.315	0.006	0.126	0.025
Diastolic BP	0.037	0.748	−0.128	0.002	0.018	0.876	−0.125	0.003
Heart rate	−0.030	0.796	−0.001	0.866	0.040	0.732	−0.043	0.308
Log (duration of DM)	0.302	0.008	0.238	<0.001	0.264	0.023	0.244	<0.001
Total protein	−0.178	<0.001	0.103	0.374	−0.165	<0.001	0.102	0.381
Log AST	0.339	0.003	0.900	0.005	0.340	0.003	0.079	0.058
Log ALT	0.268	0.020	−0.105	0.012	0.235	0.041	−0.062	0.134
Log γGTP	0.449	<0.001	0.040	0.344	0.421	<0.001	0.071	0.088
BUN	0.716	<0.001	0.661	<0.001	0.691	<0.001	0.613	<0.001
Log creatinine	0.852	<0.001	0.747	<0.001	0.776	<0.001	0.709	<0.001
eGFR	−0.762	<0.001	−0.655	<0.001	−0.713	<0.001	−0.641	<0.001
Uric acid	0.321	0.004	0.328	<0.001	0.320	0.005	0.328	<0.001
TC	−0.167	0.149	−0.250	<0.001	−0.246	0.036	−0.287	0.089
LDL-C	−0.120	0.301	−0.245	<0.001	−0.158	0.174	−0.279	<0.001
HDL-C	−0.235	0.041	−0.268	<0.001	−0.312	0.006	−0.268	<0.001
Non-HDL-C	−0.042	0.716	−0.157	<0.001	−0.084	0.470	−0.196	<0.001
Log triglycerides	0.228	0.048	0.102	0.015	0.191	0.098	0.078	0.062
Log CRP	0.486	<0.001	0.254	<0.001	0.471	<0.001	0.253	<0.001
Log glucose	0.014	0.908	0.012	0.769	0.028	0.809	0.001	0.992
Log (hemoglobin A1c)	−0.029	0.807	−0.034	0.420	−0.001	0.996	−0.056	0.180
Log C-peptide	0.385	<0.001	0.096	0.022	0.283	0.013	0.070	0.093
Log TNFR1	–	–	–	–	0.956	<0.001	0.940	<0.001
Log TNFR2	0.956	<0.001	0.940	<0.001	–	–	–	–
Log FABP4	0.546	<0.001	0.366	<0.001	0.519	<0.001	0.334	<0.001

ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; BP, blood pressure; BUN, blood urea nitrogen; CRP, C-reactive protein; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; FABP4, fatty acid-binding protein 4; γGTP, γ-glutamyl transpeptidase; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus; TNFR, tumor necrosis factor receptor.

Table 5 Multivariable regression analyses for Log TNFRs in patients with DM.

	T1DM ($n = 76$)				T2DM ($n = 575$)			
	Log TNFR1		Log TNFR2		Log TNFR1		Log TNFR2	
	β	P	β	P	β	P	β	P
Age	−0.258	<0.001	−0.179	0.040	−0.029	0.417	0.051	0.177
Sex (men)	0.087	0.166	0.068	0.376	0.110	<0.001	0.111	<0.001
Systolic BP	0.224	<0.001	0.141	0.073	0.071	0.015	0.054	0.071
Log (duration of DM)	0.107	0.085	0.096	0.116	0.045	0.152	0.034	0.295
eGFR	−0.685	<0.001	−0.600	<0.001	−0.561	<0.001	−0.516	<0.001
HDL-C	−0.067	0.288	−0.167	0.034	−0.124	<0.001	−0.134	<0.001
Log CRP	0.242	<0.001	0.229	0.009	0.193	<0.001	0.200	<0.001
Log FABP4	0.085	0.247	0.104	0.249	0.187	<0.001	0.173	<0.001
AIC	38		40		212		156	
R^2	0.781		0.669		0.551		0.540	
Adjusted R^2	0.754		0.629		0.545		0.519	
Statistical power (1 − β error)	0.999		0.999		1.000		1.000	

AIC, Akaike's information criterion; β, standardized regression coefficient; BP, blood pressure; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; FABP4, fatty acid-binding protein 4; HDL-C, high-density lipoprotein cholesterol; TNFR, tumor necrosis factor receptor; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus.

particularly associated with insulin resistance (8, 9, 10), it is plausible that differences in these correlations are due to differences in glucose metabolism and insulin resistance. Nevertheless, further studies are needed to clarify the association between FABP4 and TNFRs using a sufficient number of patients with T1DM.

Regarding insights into possible biological mechanisms linking FABP4 and TNF-related molecules, it has been reported that the inhibition of FABP4 decreased the gene expression of TNFα in the adipose tissue of obese mice (36) and that the knockdown of FABP4 suppressed the inflammatory response by downregulating the elevated levels of cellular inflammatory factors including TNFα in cigarette smoke extract-mediated 16HBE cells (37). FABP4 has also been shown to promote lipolysis and inflammation in differentiated 3T3-L1 adipocytes with elevated gene expression of TNFα (38). Since chronic inflammation has been reported to be one of the leading features of DM and DM-related complications (39, 40), inflammation and/or metaflammation in patients with DM may be a pivotal common pathway linking circulating FABP4 and TNFRs. Indeed, we previously showed that adenovirus-mediated overexpression of FABP4 in human coronary artery endothelial cells increased inflammatory cytokines, including TNFα (41). Furthermore, treatment of human renal glomerular endothelial cells or mouse podocytes with palmitate-bound recombinant FABP4 significantly increased the gene expression of inflammatory cytokines, including TNFα, and the effects of FABP4 in podocytes were attenuated in the presence of an anti-FABP4 antibody (42). The findings of significant correlations between circulating levels of FABP4 and TNFRs in patients with DM may be implicated in underlying mechanisms other than adipocyte-derived pathophysiological mechanisms. Since the distinct mechanisms of the association between circulating levels of FABP4 with TNFRs have not yet been elucidated, more detailed *in vivo* and *in vitro* studies are needed.

FABP4 possibly passes through the glomerulus filtration barrier since the molecular mass of FABP4 is about 15 kDa (10). On the other hand, the molecular weights of TNFR1 and TNFR2 are 55 kDa and 75 kDa, respectively (21), which are similar to that of albumin (69 kDa). Hence, the metabolic kinetics and excretion pathways of FABP4 and TNFRs regarding renal dysfunction are considered to be different. It has recently been reported that ectopic expression of FABP4 in glomerular endothelial cells is associated with proteinuria and renal dysfunction (16) and that urinary FABP4 is a possible biomarker of glomerular damage (17, 42). It has also been reported that exposure of kidney organ culture to TNFR1 or TNFR2 increases apoptosis mainly in tubules (43) and that individual knockout mouse models of TNFR1 or TNFR2 have a delay in the fibrotic response in a mouse model of tubulointerstitial fibrosis (44). Elucidation of the upstream molecular mechanisms between elevated FABP4 and TNFRs with renal dysfunction in patients with DM may contribute to the development of novel approaches against DM-associated renal damage.

The present study has several limitations. First, since the present study was a cross-sectional study, the association of FABP4 with TNFRs does not prove causality. Second, since patients were recruited from a single hospital, the possibility of sample selection bias cannot be ruled out. Third, since only Japanese people were enrolled, the results obtained in the present study might not be applicable to other races. Fourth, since only patients with DM were enrolled in the present study, comparisons of subjects with and those without

DM were not investigated. Finally, other TNF-related proteins including TNFα could not be measured in the present study.

In conclusion, FABP4 concentration is independently associated with levels of TNFRs in patients with DM, but the association is more evident in patients with T2DM than in those with T1DM. Measurement of FABP4 and TNFRs in patients with DM might be useful for predicting prognosis, including renal dysfunction. Furthermore, understanding the mechanisms behind the link between FABP4 and TNFRs may enable the development of new therapeutic strategies for DM-associated complications, including renal dysfunction.

Supplementary materials

This is linked to the online version of the paper at https://doi.org/10.1530/EC-24-0343.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the study reported.

Funding

MT (22K08313), TG (23K07681), and MF (23K07993) were supported by grants from the Japan Society for the Promotion of Science.

Author contribution statement

MT: conceptualization, data curation, investigation, formal analysis, visualization, roles/writing – original draft. TG: investigation, resources. NK: investigation, resources. MM: investigation, resources. TS: investigation, resources. MK: investigation, resources. EI: investigation, resources. KE: investigation, resources. YS: supervision. MF: conceptualization, data curation; formal analysis, supervision, visualization, roles/writing – original draft, and writing – review and editing. All authors read and approve the final version of the manuscript.

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