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Circulating level of fatty acid-binding protein 4 is an independent predictor of metabolic dysfunction-associated fatty liver disease in middle-aged and elderly individuals

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Keywords
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ABSTRACT

Aims/Introduction: Metabolic dysfunction-associated fatty liver disease (MAFLD), defined as hepatosteatosis with type 2 diabetes mellitus, overweight/obesity or metabolic dysregulation, has been proposed as a new feature of chronic liver disease. Fatty acid-binding protein 4 (FABP4) is expressed in adipose tissue, and secreted FABP4 is associated with the development of insulin resistance and atherosclerosis. However, the relationship between MAFLD and FABP4 has not been fully addressed.

Materials and Methods: Associations of MAFLD with metabolic markers, including FABP4, fibroblast growth factor 21 and adiponectin, were investigated in 627 individuals (men/women 292/335) in the Tanno-Sobetsu Study, a population-based cohort.

Results: The mean age was 65 years (range 19–98 years, median [interquartile range] 68 [56–76] years). Hepatosteatosis was determined by the fatty liver index (FLI), and FLI ≥35 for men and FLI ≥16 for women were used for detection of fatty liver, as previously reported using 14,471 Japanese individuals. FLI was positively correlated with systolic blood pressure and levels of FABP4 ($r = 0.331$, $P < 0.001$), fibroblast growth factor 21, homeostasis model assessment of insulin resistance as an insulin resistance index and uric acid, and was negatively correlated with levels of high-density lipoprotein cholesterol and adiponectin. FABP4 concentration was independently associated with FLI after adjustment of age, sex, systolic blood pressure and levels of uric acid, high-density lipoprotein cholesterol, homeostasis model assessment of insulin resistance, adiponectin and fibroblast growth factor 21 in multivariable regression analysis. Logistic regression analysis showed that FABP4 was an independent predictor of MAFLD after adjustment of age, sex, presence of diabetes mellitus, hypertension and dyslipidemia, and levels of uric acid, homeostasis model assessment of insulin resistance, adiponectin and fibroblast growth factor 21.

Conclusions: FABP4 concentration is independently associated with FLI and is an independent predictor of MAFLD in middle-aged and elderly individuals.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a prevalent chronic liver disease and is closely related to obesity and lifestyle-related diseases[1]. The frequency of NAFLD in adults who received health examinations has been reported to be 9–30% in Japan, and has been increasing in recent years[2]. NAFLD is a multisystem disease affecting extrahepatic organs and regulatory pathways in association with increased risks of

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insulin resistance, type 2 diabetes mellitus, hypertension, cardiovascular disease and chronic kidney disease, as well as the development of liver cirrhosis and liver cancer[3]. It is noteworthy that a new concept of metabolic dysfunction-associated fatty liver disease has been proposed regardless of alcohol consumption[4].

MAFLD is defined as criteria based on evidence of hepatosteatosis in addition to one of the following three criteria: overweight/obesity, presence of type 2 diabetes mellitus and evidence of metabolic dysregulation[4]. In epidemiological studies, several non-invasive biochemical indicators other than abdominal ultrasonography and liver biopsy are used for the diagnosis of NAFLD/MAFLD. Among the biomarkers, fatty liver index (FLI)[5], which is calculated by using waist circumference (WC), body mass index (BMI), and levels of triglycerides and γ-glutamyl transferase (GGT), has been recommended as a biomarker for detection of fatty liver in MAFLD[4]. FLI was originally reported in Italy as an index for the prediction of fatty liver detected by abdominal ultrasonography, and the cut-off value was reported to be FLI ≥60[5]. The ability of FLI to predict fatty liver has been verified, and its usefulness has been reported in several countries[5,6]. However, sex and racial differences in FLI level were not taken into consideration in most of the studies. We recently showed that simple and useful cut-off values for prediction of NAFLD in Japanese men and women were FLI ≥35 and FLI ≥16, respectively[6].

It has been shown that several humoral factors, including adipokines and hepatokines, are associated with metabolic syndrome and its related pathological conditions[7]. Fatty acid-binding protein 4 (FABP4), also known as adipocyte FABP, is

Table 1 | Characteristics of the studied participants

	All (n = 627)	Men (n = 292)	Women (n = 335)	P
Age (years)	65 ± 15	64 ± 16	65 ± 15	0.460
Body mass index	23.5 ± 3.8	24.0 ± 3.6	23.0 ± 3.8	0.001
Waist circumference (cm)	85.6 ± 10.9	86.9 ± 10.5	84.5 ± 11.2	0.006
Systolic blood pressure (mmHg)	135 ± 22	136 ± 19	134 ± 23	0.316
Diastolic blood pressure (mmHg)	76 ± 11	77 ± 11	75 ± 12	0.044
Current smoking habit	105 (16.7)	70 (24.0)	35 (10.4)	<0.001
Alcohol drinking habit	261 (41.6)	172 (58.9)	89 (26.6)	<0.001
Comorbidity				
Hypertension	358 (57.1)	171 (58.6)	187 (55.8)	0.489
Diabetes mellitus	69 (11.0)	42 (14.4)	27 (8.1)	0.012
Dyslipidemia	336 (53.6)	149 (51.0)	187 (55.8)	0.230
MAFLD	268 (42.7)	116 (39.7)	152 (45.4)	0.154
Biochemical data				
AST (IU/L)	22 (20–27)	24 (20–29)	22 (19–26)	<0.001
ALT (IU/L)	18 (14–24)	21 (16–28)	16 (13–21)	<0.001
GGT (IU/L)	22 (16–33)	28 (20–41)	18 (14–25)	<0.001
FLI	21.2 (8.8–41.5)	27.4 (12.1–49.1)	14.4 (7.2–33.2)	<0.001
Blood urea nitrogen (mmol/L)	5.7 ± 1.7	6.0 ± 1.9	5.5 ± 1.5	<0.001
Creatinine (μmol/L)	72 ± 19	81 ± 20	63 ± 13	<0.001
eGFR (mL/min/1.73 m^2)	67.1 ± 15.0	68.7 ± 15.9	65.7 ± 14.1	0.011
Uric acid (μmol/L)	320 ± 78	359 ± 73	286 ± 66	<0.001
Total cholesterol (mmol/L)	5.4 ± 0.9	5.2 ± 0.9	5.6 ± 0.9	<0.001
LDL cholesterol (mmol/L)	3.1 ± 0.8	3.0 ± 0.8	3.3 ± 0.8	<0.001
HDL cholesterol (mmol/L)	1.6 ± 0.4	1.5 ± 0.4	1.7 ± 0.4	<0.001
Triglycerides (mmol/L)	1.0 (0.7–1.5)	1.1 (0.8–1.6)	1.0 (0.7–1.3)	0.003
Fasting glucose (mmol/L)	5.2 (4.8–5.7)	5.3 (4.9–6.0)	5.1 (4.7–5.5)	<0.001
Hemoglobin A1c (%)	5.5 (5.2–5.8)	5.5 (5.2–5.9)	5.5 (5.2–5.7)	0.138
Insulin (pmol/L)	59 (31–119)	65 (30–126)	58 (31–112)	0.320
HOMA-R	1.96 (0.93–4.03)	2.17 (0.96–4.39)	1.82 (0.92–3.61)	0.113
FABP4 (μg/L)	11.7 (7.3–17.9)	10.5 (6.1–16.4)	13.0 (8.3–19.4)	<0.001
Adiponectin (mg/L)	7.3 (4.8–10.9)	5.6 (4.0–9.4)	8.9 (6.0–12.2)	<0.001
FGF21 (ng/L)	105 (69–158)	118 (80–170)	97 (62–151)	0.001

Variables are expressed as number (%), mean ± standard deviations or median (interquartile range). AST, aspartate transaminase; ALT, alanine transaminase; eGFR, estimated glomerular filtration rate; FABP4, fatty acid-binding protein 4; FGF21, fibroblast growth factor 21; FLI, fatty liver index; GGT, γ-glutamyl transpeptidase; HDL, high-density lipoprotein; HOMA-R, homeostasis model assessment of insulin resistance; LDL, low-density lipoprotein; MAFLD, metabolic dysfunction-associated fatty liver disease.

expressed in adipocytes, macrophages and capillary and injured endothelial cells, and is related to the development of insulin resistance and atherosclerosis[8–10]. FABP4 is secreted from adipocytes through a non-classical pathway in relation to lipolysis[11,12], although there are no typical secretory signal peptides in the sequence of FABP4[9,10]. Circulating FABP4 has been reported to act as an adipokine for the development of insulin resistance[12], atherosclerosis[13] and vascular remodeling[14] in experimental models, although potential FABP4 receptors have still not been identified[10]. Furthermore, it has been reported that the use of FABP4 neutralizing antibodies and/or small molecule-specific FABP4 inhibitors can be novel therapeutic strategies for treatment of metabolic dysfunction and vascular injury[12,14–16].

Cross-sectional studies have shown that that an elevated circulating FABP4 level is associated with insulin resistance estimated by the hyperinsulinemic glucose clamp method[17–19], as well as obesity, hypertension, type 2 diabetes mellitus, dyslipidemia, dysregulation of purine metabolism, atherosclerosis, and disturbance of the liver, heart and kidney[20–25]. It has also been shown that FABP4 level is a predictor for the development of metabolic syndrome[26], type 2 diabetes mellitus[27], atherosclerosis[28] and cardiovascular events[29].

However, the relationships of MAFLD with humoral factors including adipokines and hepatokines have not been fully addressed. We investigated associations of MAFLD determined by FLI for hepatosteatosis with metabolic markers, including FABP4; adiponectin, an adipokine; and fibroblast growth factor 21 (FGF21), a hepatokine, in a Japanese general population.

METHODS

Study participants

In a population-based cohort, the Tanno-Sobetsu Study, a total of 627 Japanese individuals (men/women 292/335) were recruited from residents of Sobetsu Town in 2016. This study was approved by the Ethical Committee of Sapporo Medical University and was carried out in accordance with the Declaration of Helsinki. Written informed consent was obtained from all of the study participants.

Measurements

Medical checkups, including measurement of blood pressure, calculation of BMI and collection of blood samples after an overnight fast were carried out as previously described[22]. Concentrations of FABP4, adiponectin and FGF21 were measured

Table 2 | Correlation analyses for FLI, FABP4, adiponectin and FGF21

	Log FLI		Log FABP4		Log Adiponectin		Log FGF21	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Age	0.078	0.051	0.227	<0.001	0.234	<0.001	0.175	<0.001
Body mass index	0.803	<0.001	0.384	<0.001	–0.232	<0.001	0.088	0.028
Waist circumference	0.827	<0.001	0.339	<0.001	–0.234	<0.001	0.117	0.004
Systolic blood pressure	0.255	<0.001	0.226	<0.001	0.101	0.011	0.156	<0.001
Diastolic blood pressure	0.271	<0.001	0.079	0.049	–0.053	0.187	0.116	0.004
Biochemical data								
Log AST	0.251	<0.001	0.161	<0.001	0.002	0.968	0.155	<0.001
Log ALT	0.448	<0.001	0.100	0.012	–0.203	<0.001	0.070	0.079
Log GGT	0.502	<0.001	0.003	0.944	–0.206	<0.001	0.328	<0.001
Log FLI	–	–	0.331	<0.001	–0.312	<0.001	0.268	<0.001
Blood urea nitrogen	0.043	0.288	0.180	<0.001	0.063	0.117	0.022	0.580
Creatinine	0.136	0.001	0.151	<0.001	–0.012	0.762	0.211	<0.001
eGFR	–0.042	0.292	–0.289	<0.001	–0.208	<0.001	–0.144	<0.001
Uric acid	0.342	<0.001	0.137	0.001	–0.231	<0.001	0.228	<0.001
Total cholesterol	0.002	0.966	0.025	0.538	0.108	0.007	–0.070	0.081
LDL cholesterol	0.046	0.255	0.067	0.095	0.028	0.484	–0.105	0.009
HDL cholesterol	–0.463	<0.001	–0.198	<0.001	0.337	<0.001	–0.119	0.003
Log Triglycerides	0.627	<0.001	0.198	<0.001	–0.225	<0.001	0.281	<0.001
Log Fasting glucose	0.305	<0.001	0.173	<0.001	–0.124	0.002	0.069	0.086
Log Hemoglobin A1c	0.233	<0.001	0.189	<0.001	–0.089	0.026	0.017	0.674
Log Insulin	0.177	<0.001	0.148	<0.001	–0.104	0.011	0.066	0.109
Log HOMA-R	0.220	<0.001	0.170	<0.001	–0.120	0.003	0.076	0.063
Log FABP4	0.331	<0.001	–	–	–0.046	0.247	0.224	<0.001
Log Adiponectin	–0.312	<0.001	–0.046	0.247	–	–	–0.079	0.048
Log FGF21	0.268	<0.001	0.224	<0.001	–0.079	0.048	–	–

Total $n = 627$. AST, aspartate transaminase; ALT, alanine transaminase; eGFR, estimated glomerular filtration rate; FABP4, fatty acid-binding protein 4; FGF21, fibroblast growth factor 21; FLI, fatty liver index; GGT, γ-glutamyl transpeptidase; HDL, high-density lipoprotein; HOMA-R, homeostasis model assessment of insulin resistance; LDL, low-density lipoprotein.

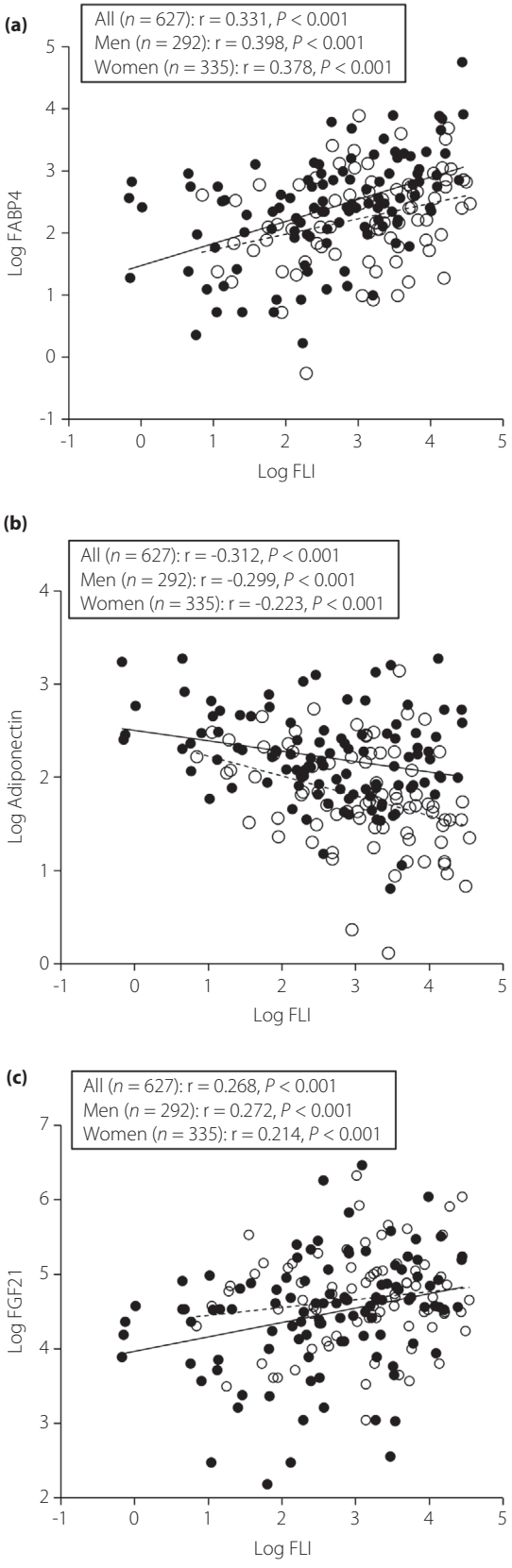

Figure 1 | Correlations of fatty liver index (FLI) with metabolic parameters. (a). Logarithmically transformed (Log) fatty acid-binding protein 4 (FABP4), (b) Log adiponectin and (c) Log fibroblast growth factor 21 (FGF21) were plotted against Log FLI in each participant (n = 627). Open circles and broken regression line: men (n = 292), closed circles and solid regression line: women (n = 335).

using enzyme-linked immunosorbent assay kits for FABP4 (BioVendor, Brno, Czech Republic), adiponectin (R&D Systems, Minneapolis, MN, USA) and FGF21 (R&D Systems), respectively. Estimated glomerular filtration rate was calculated by an equation for Japanese individuals: estimated glomerular filtration rate (mL/min/1.73 m^2) = 194 × creatinine $^{(-1.094)}$ × age $^{(-0.287)}$ × 0.739 (if female)[30]. Homeostasis model assessment of insulin resistance (HOMA-R) was calculated by the formula: insulin (μU/mL) × glucose (mg/dL) / 405. FLI was calculated by using WC, BMI, and levels of triglycerides and GGT[5]: FLI = [e(0.953 × ln (triglycerides) + 0.139 × BMI + 0.718 × ln (GGT) + 0.053 × WC − 15.745)] / [1 + e (0.953 × ln (triglycerides) + 0.139 × BMI + 0.718 × ln (GGT) + 0.053 × WC − 15.745)] × 100.

A self-administered questionnaire survey was carried out to obtain information on current smoking habit, alcohol drinking habit (≥3 times/week), and use of drugs for diabetes mellitus, hypertension and dyslipidemia. Hypertension was defined as self-reported use of drugs for hypertension, systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg. Diabetes mellitus was defined as self-reported use of drugs for diabetes mellitus, hemoglobin A1c (NGSP scale) ≥6.5% or fasting plasma glucose ≥7.0 mmol/L. Dyslipidemia was defined as self-reported use of drugs for dyslipidemia, triglycerides ≥ 1.7 mmol/L, high-density lipoprotein (HDL) cholesterol <1.0 mmol/L or low-density lipoprotein cholesterol ≥3.6 mmol/L.

Definition of MAFLD

MAFLD was defined by criteria based on evidence of hepatosteatosis with one of the following three criteria: type 2 diabetes mellitus, overweight/obesity (BMI ≥23 in Asian individuals) and evidence of metabolic dysregulation as previously reported[4]. Evidence of metabolic dysregulation was defined as the presence of at least two metabolic risk abnormalities, including waist circumference ≥90/80 cm in Asian men and women; blood pressure ≥130/85 mmHg or specific drug treatment; plasma triglycerides ≥1.7 mmol/L or specific drug treatment; plasma HDL cholesterol <1.0 mmol/L for men and <1.3 mmol/L for women or specific drug treatment; prediabetes (fasting glucose levels of 5.6–6.9 mmol/L, 2-h post-load glucose levels of 7.8–11.0 mmol/L or hemoglobin A1c of 5.7–6.4%); HOMA-R ≥ 2.5; and plasma high-sensitivity C-reactive protein level >2 mg/L (no measurement in the present study)[4]. Detection of hepatic steatosis has been recommended to be carried

Table 3 | Multivariable regression analyses for Log fatty liver index

	Log FABP4			Log adiponectin			Log FGF21		
	β	*P*	R^2	β	*P*	R^2	β	*P*	R^2
Model 1	0.377	<0.001	0.201	–0.297	<0.001	0.146	0.226	<0.001	0.119
Model 2	0.358	<0.001	0.220	–0.285	<0.001	0.174	0.222	<0.001	0.151
Model 3	0.242	<0.001	0.344	–0.163	<0.001	0.316	0.157	<0.001	0.318
Model 4	0.223	<0.001	0.369	–0.179	<0.001	0.353	0.143	<0.001	0.347
Model 5	0.189	<0.001	0.398	–0.160	<0.001	0.398	0.097	0.005	0.398

Standardized regression coefficient (β). Model 1, adjusted for age and sex. Model 2, adjusted for Model 1 + Log homeostasis model assessment of insulin resistance. Model 3, adjusted for model 2 + uric acid and high-density lipoprotein cholesterol. Model 4, adjusted for model 3 + systolic blood pressure. Model 5, adjusted for model 4 + Log adiponectin, Log fatty acid-binding protein 4 (FABP4) and Log fibroblast growth factor 21 (FGF21).

out either by blood biomarkers/scores, imaging techniques or liver histology[4]. In the present study, hepatic steatosis was determined by FLI as a recommended biomarker[4], and FLI ≥35 for men (area under the curve 0.82; sensitivity 76.7%; specificity 71.3%) and FLI ≥16 for women (area under the curve 0.91; sensitivity 85.2%; specificity 81.4%) were used for detection of fatty liver as previously reported using 14,471 Japanese individuals (men/women 9,240/5,231; mean age 48 ± 9 years)[6].

Statistical analysis

After checking normality of parameters by the Shapiro-Wilk *W*-test, variables are expressed as the mean ± standard deviation for normal distributions, or medians (interquartile ranges) for skewed variables. Differences in percentages of variables were analyzed by the χ^2-test. Comparisons between two groups were carried out by Student's *t*-test for parametric parameters, and the Mann–Whitney *U*-test for non-parametric parameters. For regression analyses, skewed variables were logarithmically transformed, and the correlation between two variables was carried out by Pearson's correlation analysis. Multivariable regression analyses were carried out to identify independent associations of FLI with FABP4, adiponectin and FGF21 after adjustment of age, sex, systolic blood pressure, and levels of HOMA-R, uric acid, HDL cholesterol, FABP4, adiponectin and FGF21 by several models, showing the standardized regression coefficient (β) and the percentage of variance for the selected independent predictors explained (R^2). Multivariable logistic regression analyses were carried out in several models to identify independent determinants of the risk for MAFLD using age, sex and variables with a significant difference between subgroups divided by the absence and presence of MAFLD as independent predictors after consideration of multicollinearity, showing the odds ratio (OR), 95% confidence interval (CI) and Akaike's information criterion (AIC). Since hepatosteatosis in MAFLD was defined by FLI, which was calculated by using indicators of obesity, BMI and WC were not incorporated into multivariable logistic regression analyses. Parameters with a lower AIC score constitute a better-fit model. A $P < 0.05$ was considered statistically significant. Statistical analyses were carried out using JMP15.2.1 for Macintosh (SAS Institute, Cary, NC, USA).

RESULTS

Basal characteristics of the studied participants

Basal characteristics of the 627 recruited participants (men/women 292/335) are shown in Table 1. The mean age of the participants was 65 years (range 19–98 years, median 68 years interquartile range 56–76 years). The numbers of participants with habits of current smoking and alcohol drinking were 105 (16.7%) and 261 (41.6%), respectively. Hypertension, diabetes mellitus, dyslipidemia and MAFLD were found in 358, 69, 336 and 268 participants, respectively.

Correlation analyses for FLI, FABP4, adiponectin and FGF21

As shown in Table 2, FLI was positively correlated with BMI, WC, systolic and diastolic blood pressures, aspartate transaminase, alanine transaminase, GGT, creatinine, uric acid, triglycerides, fasting glucose, hemoglobin A1c, insulin, HOMA-R and FABP4 (Figure 1a), and was negatively correlated with HDL cholesterol and adiponectin as an adipokine (Figure 1b). There was a positive correlation of FLI with a hepatokine, FGF21 (Figure 1c). When men and women were separately analyzed, similar correlations of FLI with FABP4 (Figure 1a), adiponectin (Figure 1b) and FGF21 (Figure 1c) were found. Correlations of FABP4, adiponectin and FGF21 with parameters are also shown in Table 2.

Multivariable regression analyses for FLI

Multivariable regression analyses showed that the level of FABP4, adiponectin or FGF21 was independently associated with FLI after adjustment of age and sex (model 1; Table 3). When HOMA-R was additionally incorporated, the level of FABP4, adiponectin or FGF21 was an independent determinant of FLI (model 2). When uric acid and HDL cholesterol (model 3) or uric acid, HDL cholesterol and systolic blood pressure (model 4) were incorporated into the adjustment in model 2, the level of FABP4, adiponectin or FGF21 was an

Table 4 | Characteristics of the studied subjects divided by metabolic dysfunction-associated fatty liver disease

	non-MAFLD (n = 359)	MAFLD (n = 268)	P
Age (years)	64 ± 16	66 ± 14	0.321
Sex (men/women)	176/183	116 (43.3)	0.154
Body mass index	21.3 ± 2.4	26.3 ± 3.4	<0.001
Waist circumference (cm)	79.4 ± 8.0	94.0 ± 8.5	<0.001
Systolic blood pressure (mmHg)	132 ± 22	139 ± 21	<0.001
Diastolic blood pressure (mmHg)	74 ± 11	78 ± 12	<0.001
Current smoking habit	62 (17.3)	43 (16.0)	0.703
Alcohol drinking habit	153 (42.6)	108 (40.3)	0.529
Comorbidity			
Hypertension	175 (48.7)	183 (68.3)	<0.001
Diabetes mellitus	30 (8.4)	39 (14.6)	0.015
Dyslipidemia	159 (44.3)	177 (66.0)	<0.001
Medication			
Antihypertensive drugs	106 (29.5)	119 (44.4)	<0.001
Antidiabetic drugs	26 (7.2)	33 (12.3)	0.031
Lipid-lowering drugs	52 (14.5)	71 (26.5)	<0.001
Biochemical data			
AST (IU/L)	22 (19–26)	24 (20–28)	<0.001
ALT (IU/L)	16 (13–22)	22 (16–29)	<0.001
GGT (IU/L)	19 (15–27)	28 (19–44)	<0.001
FLI	9.9 (5.3–17.4)	45.8 (31.2–63.9)	<0.001
Blood urea nitrogen (mmol/L)	5.8 ± 1.8	5.7 ± 1.6	0.469
Creatinine (μmol/L)	72 ± 21	71 ± 16	0.629
eGFR (mL/min/1.73 m^2)	67.7 ± 14.8	66.3 ± 15.2	0.238
Uric acid (μmol/L)	308 ± 74	337 ± 81	<0.001
Total cholesterol (mmol/L)	5.4 ± 0.9	5.4 ± 0.9	0.361
LDL cholesterol (mmol/L)	3.1 ± 0.8	3.2 ± 0.8	0.339
HDL cholesterol (mmol/L)	1.7 ± 0.4	1.4 ± 0.4	<0.001
Triglycerides (mmol/L)	0.8 (0.6–1.1)	1.3 (1.0–1.8)	<0.001
Fasting glucose (mmol/L)	5.1 (4.7–5.5)	5.3 (5.0–6.0)	<0.001
Hemoglobin A1c (%)	5.4 (5.2–5.7)	5.6 (5.3–5.9)	<0.001
Insulin (pmol/L)	52 (27–97)	76 (39–145)	<0.001
HOMA-R	1.55 (0.83–3.25)	2.74 (1.25–5.15)	<0.001
FABP4 (μg/L)	9.6 (5.9–15.1)	15.9 (10.4–21.8)	<0.001
Adiponectin (mg/L)	8.2 (5.4–11.4)	6.2 (4.1–10.0)	<0.001
FGF21 (ng/L)	96 (61–146)	127 (85–180)	<0.001

Variables are expressed as number (%), mean ± standard deviation or median (interquartile ranges). AST, aspartate transaminase; ALT, alanine transaminase; eGFR, estimated glomerular filtration rate; FABP4, fatty acid-binding protein 4; FGF21, fibroblast growth factor 21; FLI, fatty liver index; GGT, γ-glutamyl transpeptidase; HDL, high-density lipoprotein; HOMA-R, homeostasis model assessment of insulin resistance; LDL, low-density lipoprotein; MAFLD, metabolic dysfunction-associated fatty liver disease.

independent determinant of FLI. With further additional adjustment of FABP4, adiponectin and FGF21 into model 4, levels of FABP4 (β = 0.189, P < 0.001, R^2 = 0.201), adiponectin (β = –0.160, P < 0.001) and FGF21 (β = 0.097, P = 0.005) remained as independent determinants of FLI, explaining 39.8% of the variance (model 5, R^2 = 0.398).

Comparisons of metabolic parameters in participants with and those without MAFLD

Basal characteristics of the recruited participants divided by the absence and presence of MAFLD into a non-MAFLD group (n = 359, men/women 176/183) and MAFLD group (n = 268, men/women 116/152) are shown in Table 4. There was no significant difference in age or the proportion of sex. Prevalences of hypertension, diabetes mellitus and dyslipidemia were significantly higher in the MAFLD group than in the non-MAFLD group. Participants in the MAFLD group had significantly larger BMI and WC, significantly higher levels of systolic and diastolic blood pressures, aspartate transaminase, alanine transaminase, GGT, FLI, uric acid, triglycerides, fasting glucose, hemoglobin A1c, insulin, HOMA-R, and FABP4 (Figure 2a), and significantly lower levels of HDL cholesterol and adiponectin (Figure 2b) than did participants in the non-MAFLD group. FGF21 level was significantly higher in the MAFLD group than in the non-MAFLD group (Figure 2c).

Level of FABP4 as the risk of MAFLD

Multivariable logistic regression analysis showed that FABP4 (OR 1.080, 95% CI 1.057–1.103, per 1 μg/L, P < 0.001), adiponectin and FGF21 were independent determinants of the risk for MAFLD (model 1, AIC 773; Table 5). When age and sex were additionally incorporated into model 1, the risk of FABP4 for MAFLD was significant (OR 1.076, 95% CI 1.053–1.100, per 1 μg/L, P < 0.001; Model 2, AIC 774). When hypertension, diabetes mellitus and dyslipidemia were additionally incorporated into model 2, the risk of FABP4 for MAFLD was still significant (OR 1.070, 95% CI 1.047–1.095, per 1 μg/L, P < 0.001; model 3, AIC 749). When uric acid and HOMA-R were additionally incorporated into model 3, FABP4 was a significantly independent risk factor for MAFLD (OR 1.061, 95% CI 1.037–1.086, per 1 μg/L, P < 0.001) with the minimum AIC among the models (model 4, AIC 701). When medications for antihypertensive, antidiabetic and lipid-lowering drugs were used instead of the presence of hypertension, diabetes mellitus and dyslipidemia in analyses, the results using medications for diseases (Table S1) were similar to those using the presence of diseases (Table 5).

DISCUSSION

The present study showed for the first time that FABP4 concentration was independently associated with FLI and was an independent parameter of the risk for MAFLD, a new feature of chronic liver disease, in a Japanese general population of mainly middle-aged and elderly individuals. FABP4 is secreted from adipocytes in connection with lipolysis through a non-classical pathway[11,12]. Secretion of FABP4 from macrophages and injured endothelial cells has also been confirmed[13,14], although the main source of circulating FABP4 level is

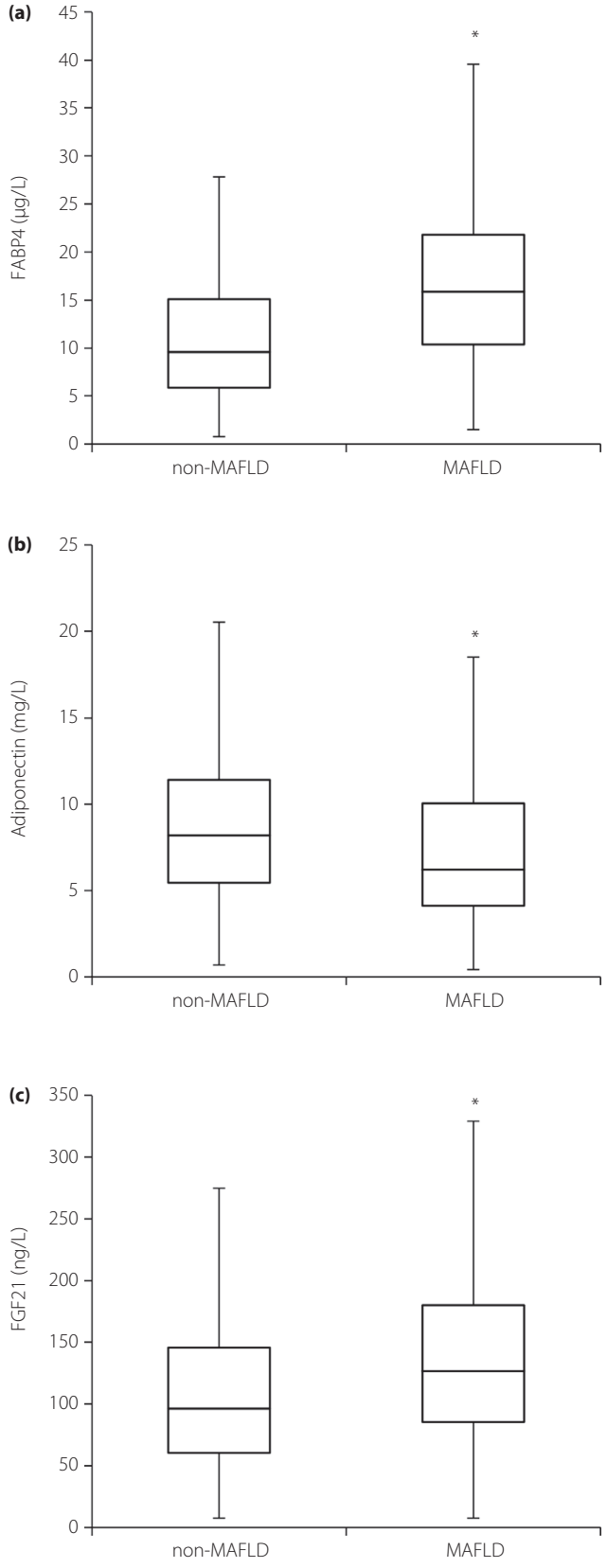

Figure 2 | Comparisons of metabolic parameters in participants with and those without metabolic dysfunction-associated fatty liver disease (MAFLD). (a) Comparisons of levels of fatty acid-binding protein 4 (FABP4), (b) adiponectin and (c) fibroblast growth factor 21 (FGF21) shown by box plots in participants divided by the absence and presence of MAFLD into a non-MAFLD group (n = 359, men/women 176/183) and MAFLD group (n = 268, men/women 116/152). *$P < 0.05$ versus non-MAFLD.

adipocytes[8,12]. Kupffer cells and hepatic stellate cells in the liver have been reported to play significant roles in the development of NAFLD[31,32]. FABP4 was also reported to be expressed in Kupffer cells[33] and hepatic stellate cells[34], as well as hepatocytes in hepatic cell carcinoma[35], although secretion of FABP4 from those hepatic cells has not yet been proved. Previous studies showed that secreted FABP4 as an adipokine can directly cause the development of insulin resistance and atherosclerosis *in vivo* and *in vitro*[12–14,36]. It has also been reported that treatment with FABP4 exogenously induces endoplasmic reticulum stress in HepG2 liver cells as a potential link between hepatic insulin resistance and obesity-associated metabolic dysfunction[37], suggesting that circulating FABP4 directly affects liver dysfunction, resulting in the development of MAFLD. Conversely, the condition of MAFLD might increase circulating FABP4 concentration through augmentation of catecholamine-induced lipolysis in adipose tissue, since chronic liver disease has been reported to increase sympathetic nervous system activation[38]. These findings indicate that circulating FABP4, derived from not only adipose tissue but also some hepatic cells, is one of the key modulators of MAFLD.

It has been reported that FABP4 concentration is associated with NAFLD in patients with type 2 diabetes mellites[39] and individuals in the general population[40–42]. Furthermore, FLI was reported to be positively associated with FABP4 level[42,43], which was confirmed in the present study. Transcriptome analyses showed that FABP4 in the liver is upregulated in patients with NAFLD[44] and patients with non-alcoholic steatohepatitis[45], and that FABP4 is a predictive factor for poor prognosis in patients with NAFLD[46]. Therefore, it is possible that modulations of FABP4 might contribute to the prognosis of MAFLD and its related metabolic and cardiovascular diseases in humans. It has been shown that the use of a small molecule-specific FABP4 inhibitor could be a novel therapeutic strategy for diabetes mellitus and atherosclerosis, as well as hepatic steatosis[9,15,47]. Neutralizing serum FABP4 by a monoclonal FABP4 antibody has recently been reported to be a new therapy for diabetes mellitus, atherosclerosis and vascular injury in experimental models[12,14,16]. It is necessary to prospectively evaluate whether a change in the FABP4 level by direct inhibition, neutralization and/or blockade of unidentified receptors indeed reflects conditions of MAFLD in the future.

Several hormones, including FABP4, adiponectin and FGF21, are secreted from adipose tissue as adipokines. Adiponectin is

Table 5 | Multivariable logistic regression analyses for the risk of metabolic dysfunction-associated fatty liver disease

	Model 1		Model 2		Model 3		Model 4	
	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
FABP4 (per 1 μg/L)	1.080 (1.057–1.103)	<0.001	1.076 (1.053–1.100)	<0.001	1.070 (1.047–1.095)	<0.001	1.061 (1.037–1.086)	<0.001
Adiponectin (per 1 mg/L)	0.943 (0.911–0.976)	0.001	0.934 (0.900–0.969)	<0.001	0.943 (0.908–0.980)	0.003	0.948 (0.911–0.987)	0.009
FGF21 (per 1 ng/L)	1.001 (1.000–1.002)	0.014	1.001 (1.000–1.002)	0.011	1.001 (1.000–1.002)	0.014	1.002 (1.000–1.003)	0.016
Age (per 1 year)	–	–	1.002 (0.990–1.014)	0.722	0.986 (0.972–0.999)	0.046	0.985 (0.971–1.000)	0.050
Sex (Men)	–	–	0.728 (0.507–1.045)	0.085	0.715 (0.491–1.042)	0.081	0.488 (0.312–0.764)	0.002
Hypertension	–	–	–	–	2.278 (1.495–3.473)	<0.001	2.064 (1.333–3.194)	0.001
Diabetes mellitus	–	–	–	–	1.127 (0.632–2.008)	0.686	1.018 (0.547–1.893)	0.956
Dyslipidemia	–	–	–	–	1.942 (1.356–2.780)	<0.001	1.862 (1.276–2.716)	0.001
Uric acid (per 1 mg/dL*)	–	–	–	–	–	–	1.284 (1.081–1.524)	0.004
HOMA-R (per 1)	–	–	–	–	–	–	1.087 (1.026–1.153)	0.005
	R^2 = 0.102, AIC: 773		R^2 = 0.106, AIC: 774		R^2 = 0.142, AIC: 749		R^2 = 0.168, AIC: 701	

*59.48 μmol/L. AIC, Akaike's information criterion; CI, confidence Interval; FABP4, fatty acid-binding protein 4; FGF21, fibroblast growth factor 21; HOMA-R, homeostasis model assessment of insulin resistance; OR, odds ratio.

abundantly expressed in adipocytes, and protects against the development of diabetes mellitus and atherosclerosis[48]. FGF21 is widely expressed in metabolic tissues, including adipose tissue and the liver, and secreted FGF21 beneficially acts as an adipokine and/or hepatokine with therapeutic relevance[49]. It has been reported that plasma activity of xanthine oxidoreductase, a potential enhancer of reactive oxidative stress[50], was strongly associated with liver dysfunction[51], and was independently associated with levels of FABP4, adiponectin and FGF21[25]. As a possible mechanism, activation of xanthine oxidoreductase might underlie the link of MAFLD with adipokines and hepatokines. In the present study, FABP4 level was associated with FLI and the risk for MAFLD independently of levels of adiponectin and FGF21.

It has been reported that the prevalences of MAFLD were 26.1% (men/women 35.4%/14.1%) in China (n = 139,170, mean age 47 years)[52] and 34.8% (men/women 38.5%/31.1%) in National Health and Nutrition Examination Survey data of the USA[53]. Prevalences of MAFLD in premenopausal, perimenopausal and postmenopausal Chinese women were 6.1, 16.8 and 30.2%, respectively[52]. Furthermore, the prevalence of MAFLD increased with advance of age from 23.2% in individuals aged 18–39 years to 43.8% in individuals aged ≥60 years in the USA[53]. In the present study using mainly middle-aged and elderly Japanese individuals (mean age 65 years), the prevalence of MAFLD was 42.7% (men/women 39.7%/45.7%), which is similar to results of previous studies using elderly individuals. MAFLD is prevalent and its prevalence varies depending on age and sex.

The present study had several limitations. First, causal relations of FLI and MAFLD with associated biomarkers, including FABP4, were not proven, since this was a cross-sectional study. It is necessary to show what underlies the associations in longitudinal and interventional studies using a large number of participants. Second, hepatic steatosis in the definition of MAFLD was determined by FLI, but not by abdominal ultrasonography or liver biopsy. Therefore, the pathological severity of hepatic steatosis was not taken into consideration. However, a non-invasive method is useful for epidemiological studies using a large number of participants. In the original report about MAFLD, the use of FLI was recommended for detection of hepatic steatosis as a reliable biomarker[4]. Furthermore, there have been several studies showing that FLI is associated with the development of hypertension[54], diabetes mellitus[55], chronic kidney disease[56] and heart failure[57]. Third, since the recruited participants were all Japanese, the results obtained in the present study might not be applicable to other races. In this study, FLI ≥35 for men and FLI ≥16 for women were used for detection of fatty liver, as previously reported using 14,471 Japanese individuals (men/women 9,240/5,231; mean age 48 ± 9 years)[6]. Optimal cut-off values of FLI would be required according to sex and races. Finally, several therapeutic drugs for hypertension, diabetes mellitus and dyslipidemia have been shown to affect FABP4 concentration[10]. Therefore, those drugs might have affected the results of concentrations and correlations of FABP4.

In conclusion, FABP4 concentration is independently associated with FLI and is an independent predictor of MAFLD in a general population of mainly middle-aged and elderly individuals. A further understanding of the relationships of FABP4 with FLI and MAFLD might lead to novel therapies for MAFLD and its related diseases.

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DISCLOSURE

The authors declare no conflict of interest.

The protocol for this research project has been approved by a suitably constituted Ethics Committee of the institution and it conforms to the provisions of the Declaration of Helsinki. Ethical Committee of Sapporo Medical University.

Approval of the research protocol: H24-7-30. All informed consent was obtained from the participants.
Approval date of registry and the registration no. of the study/-trial: N/A.
Animal studies: N/A.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 | Multivariable logistic regression analyses for the risk of metabolic dysfunction-associated fatty liver disease.