Nonalcoholic Fatty Liver With a Hepatic Arterial Buffer Response Strongly Associated With Future Metabolic Disease

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A change in hepatic blood flow caused by the hepatic arterial buffer response (HABR) occurs as fatty liver disease progress. The aim of this longitudinal cohort study was to investigate whether fatty liver with the HABR induces metabolic disorders. In 2009 and 2010, 494 (89.5%) participants were enrolled. The median follow-up duration was 5.0 (interquartile range, 3.9-6.0) years. The hazard ratios of fatty liver with the HABR for incident metabolic disorders were assessed by Cox proportional hazard models. A non–fatty liver group (non-FL group, hepatorenal echo intensity ratio <1.12), a fatty liver without portal hypertension (FL group, hepatorenal echo intensity ratio ≥1.12 and ratio of the maximal blood velocity in the right hepatic artery to maximal blood velocity in the right portal vein <3.1) group, and a fatty liver with portal hypertension (FL-HABR group, hepatorenal echo intensity ratio ≥1.12 and ratio of the maximal blood velocity in the right hepatic artery to maximal blood velocity in the right portal vein ≥3.1) group were defined based on echo intensity and Doppler ultrasonography. Fatty liver with and without the HABR was significantly associated with the incidence of diabetes on multivariate analysis (non-FL versus FL group, hazard ratio, 3.36; 95% confidence interval, 1.05-12.85; FL versus FL with the HABR group, HR, 2.68; 95% confidence interval, 1.28-6.04). With respect to the incidence of hypertension and dyslipidemia, only FL with the HABR was a significant factor (hypertension, non-FL versus FL, $P = 0.874$, FL versus FL-HABR, $P = 0.016$, non-FL versus FL-HABR, $P = 0.023$; dyslipidemia, non-FL versus FL, $P = 0.311$, FL versus FL-HABR, $P = 0.194$, non-FL versus FL-HABR, $P = 0.038$). Conclusion: Fatty liver with the HABR is a high-risk condition for metabolic diseases. (Hepatology Communications 2017;1:623-633)

There is an increasing prevalence of obesity worldwide, and this has become a major public health problem. The incidence of related conditions, such as diabetes mellitus, cardiovascular disease, and cancer, is likely to continue to rise.[1-3] Obesity leads to ectopic fat accumulation in several organs, such as the liver and pancreas.[4] Several recent studies have suggested that nonalcoholic fatty liver disease (NAFLD) is associated with increased rates of the metabolic syndrome, such as type 2 diabetes mellitus, atherosclerosis, and cardiovascular events.[5-9] NAFLD is a spectrum of liver diseases that ranges from simple

steatosis to a progressive form of liver disease called nonalcoholic steatohepatitis (NASH). Patients with NASH have higher rates of metabolic disorders and cardiovascular events than those without NASH.[10] Therefore, early detection of NASH could have benefit in clinical practice.

The hepatic arterial buffer response (HABR) is an important compensatory mechanism of the liver to maintain total hepatic blood flow by hepatic arterial vasodilation where there is reduction of portal venous perfusion. The HABR has been shown in patients with advanced cirrhosis.[11] Our previous study suggested that changes in hepatic blood flow by the HABR occurred during the earliest stage of hepatic fibrosis in patients with NAFLD.[12] Thus, because most cases of NASH and NAFLD with advanced fibrosis were included in the group of fatty liver with the HABR, it can be presumed that fatty liver with the HABR is an appropriate predictive marker for metabolic disorders.

The aim of this longitudinal cohort study was to investigate whether fatty liver with the HABR induced metabolic disorders.

Participants and Methods

PARTICIPANTS

This was a prospective cohort study in which all participants in a health checkup were evaluated to determine whether NAFLD with the HABR increased metabolic diseases, such as type 2 diabetes mellitus, hypertension, or dyslipidemia, from June 2009 to August 2016. All participants provided their written, informed consent before enrollment; and the study protocols were approved by the institutional ethics committee. To screen subjects for inclusion, public health nurses asked all subjects about alcohol intake and evidence of liver disease (such as autoimmune hepatitis, primary biliary cholangitis, or drug-induced hepatitis) before the health checkup. The inclusion criteria were (1) age <80 years; (2) no previous evidence of liver disease; and (3) no evidence of treatment with hypertensive agents, lipid-lowering agents, or antidiabetic agents. The study flow diagram is shown in Supporting Fig. S1. A total of 582 health check participants underwent abdominal ultrasound (US); all subjects underwent US because it was not performed for suspicion of NAFLD. Of the 582 patients, 552 were evaluated in this cross-sectional analysis after 30 were excluded by the following exclusion criteria: (1) alcohol consumption >20 g/day for men and >10 g for women, (2) positive for both hepatitis B surface antigen and antibody against hepatitis C virus, or (3) pancreatic atrophy (width of the body of the pancreas <5 mm). Of the 552 participants, 494 had repeat health checks after the baseline health check. The follow-up rate was 89.5%. The median follow-up duration was 5.0 years (interquartile range 3.9-6.0).

DATA COLLECTION

After an overnight 12-hour fast, all participants underwent blood tests. Waist circumference was measured at the end of normal expiration. Body mass index (BMI) was calculated as weight (kilograms) divided by height (meters) squared. Hypertension was defined as systolic blood pressure ≥ 140 mm Hg or diastolic blood pressure ≥ 90 mm Hg. Diabetes was defined as fasting plasma glucose ≥ 126 mg/dL or hemoglobin A1c $\geq 6.5\%$. Dyslipidemia was diagnosed as a high triglyceride level (≥ 150 mg/dL), decreased high-density lipoprotein cholesterol level (<40 mg/dL for men or <50 mg/dL for women), or elevated low-density lipoprotein cholesterol level (≥ 140 mg/dL). Hepatic fibrosis prediction scores were calculated. The aspartate aminotransferase to platelet ratio index was calculated as follows: {[aspartate aminotransferase level (IU/L)/aspartate aminotransferase (upper limit of normal) (IU/L)] $\times$ 100}/platelet count (10^9/L).[13] FIB-4 was calculated as follows: [age (years) $\times$ aspartate aminotransferase (IU/L)]/[platelet count ($\times$ 10^9/L) $\times$ $\sqrt{}$alanine aminotransferase (IU/L)].[14]

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ULTRASOUND

All participants fasted overnight and refrained from smoking cigarettes. All US examinations were performed using a Logiq 7 (GE Healthcare Japan, Tokyo, Japan) with a convex probe (3.5C Transducer; central frequency, 2.0-5.0 MHz; GE Healthcare Japan). These measurements were obtained by one gastroenterologist (M.H.) who had performed US for 11 years before the start of this study.

Echo intensity was measured by the US machine. As in the previous report, a region of interest in the liver parenchyma was placed so that no blood vessels or other focal lesions were crossed, to obtain a sample of liver parenchyma alone.[15] Another region of interest was placed at the right renal cortex along the focusing area of the image at the same distance from the probe and near the center line of the image to avoid distortion of ultrasonic wave patterns. The height of the region of interest was set to obtain an area of 0.5×0.5 cm (441 pixels) for both hepatic parenchyma and renal cortex. The hepatorenal echo intensity ratio (H/R ratio) was calculated from the results of hepatic intensity divided by renal intensity. After measurement of echo intensity 5 times in each patient, the median values were selected. Echo intensity of the body of the pancreas was also measured. Both long and short axes of the spleen were measured. Splenomegaly was defined as a spleen index (short axis $\times$ long axis) >30. Doppler US measurements were obtained 5 times, as reported.[12,16,17] Maximal blood velocities in the right portal vein and right hepatic artery were measured. The ratio of the maximal blood velocity in the right hepatic artery to maximal blood velocity in the right portal vein (A/P ratio) was calculated.

To screen for patients with fatty liver, a cutoff value of the hepatorenal ratio was defined. Receiver operating characteristic (ROC) analyses of the hepatorenal ratio in the prediction of US findings, diabetes, hypertension, hyperuricemia, or dyslipidemia are shown in Supporting Table S1. The cutoff values for US findings, diabetes, hypertension, hyperuricemia, and dyslipidemia were 1.13, 1.12, 1.11, 1.12, and 1.12, respectively. Thus, the cutoff value to diagnose fatty liver was defined as 1.12. To screen for patients with portal hypertension, the cutoff value for the A/P ratio was 3.1 using ROC analysis for the spleen index (>30). The three groups were defined as follows: (1) non–fatty liver group (non-FL group), H/R ratio <1.12; (2) fatty liver without portal hypertension (FL group), H/R ratio ≥1.12 and A/P ratio <3.1; and (3) fatty liver with portal hypertension (FL-HABR group), H/R ratio ≥1.12 and A/P ratio ≥3.1.

Peak systolic velocity, end diastolic velocity, and mean velocity were measured; and the hepatic resistive index and the hepatic pulsatility index were determined according to the following formulae:

$$resistive\ index = (peak\ systolic\ velocity$$
$$- end\ diastolic\ velocity)/peak\ systolic\ velocity$$
$$pulsatility\ index = (peak\ systolic\ velocity$$
$$- end\ diastolic\ velocity)/mean\ velocity$$

The splenic resistive index and pulsatility index were measured using the same formulae by placing the sampling cursor in the main branches of the intrasplenic artery near the splenic hilum at the left intercostal space.[18,19]

STATISTICAL ANALYSIS

Data are presented as medians and quartiles. Some normally distributed continuous variables are expressed as means ± standard deviation. To predict metabolic disorders, ROC curve analysis was performed. The area under the ROC curve was calculated by the trapezoidal rule. Optimal cutoff values for the prediction of metabolic disorders were selected to maximize sensitivity, specificity, and accuracy. The Kruskal-Wallis test was used in cases where the data were nonparametric. Cox proportional hazard models were used to estimate the crude hazard ratios (HRs), multivariate adjusted HRs, and 95% confidence intervals (CIs) for the association between fatty liver with or without the HABR at baseline and the incidence of metabolic diseases. On multivariate analysis, adjustments for age, sex, BMI, splenomegaly, the wave form of the hepatic vein, and the echo intensity of the pancreas were made. All statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC).

Results

BASELINE CHARACTERISTICS

The baseline characteristics of the participants are shown in Supporting Table S2. Most (71.2%) participants were male, and the median age was 49 years (interquartile range 41.3-55). The median BMI was 23.0 kg/m^2 (interquartile range 21.2-25.1). The prevalences of diabetes, hypertension, and dyslipidemia at

TABLE 1. COMPARISON OF PARTICIPANTS' CHARACTERISTICS BY THE PRESENCE OF FATTY LIVER

Factor	Non-FL	FL	FL-HABR
Follow-up period (years)	5.0 (4.0-6.0)	5.0 (3.0-6.1)	5.1 (4.0-6.0)
Age (years)[†,‡]	47 (40-54)	49 (42-56)	53 (46-58)
Female:male	115:190	30:127	14:76
BMI (kg/m^2)[*,†]	22.0 (20.4-23.6)	24.2 (22.5-26.4)	25.1 (22.9-27.2)
Waist circumference (cm)[*,‡]	80.0 (73.5-84.0)	85.0 (80.0-91.0)	89.0 (84.1-94.1)
FPG (mg/dL)[*,†]	96 (92-103)	101 (96-108)	106 (96-116)
HbA1c (%)[*,†]	5.0 (4.8-5.3)	5.1 (4.9-5.4)	5.2 (4.9-5.5)
Systolic blood pressure (mm Hg)[*,†]	112 (102-122)	118 (108-128)	121 (110-134)
Diastolic blood pressure (mm Hg)[*,†]	68 (60-76)	74 (64-80)	74(66-80)
AST (U/L)[*,†]	20 (18-24)	23 (19-29)	25 (19-30)
ALT (U/L)[*,†]	18 (14-24)	26 (18-38)	26 (18-40)
APRI[†,‡]	0.85 (0.69-1.10)	0.91 (0.70-1.14)	1.25 (0.94-1.54)
FIB-4[†,‡]	0.91 (0.72-1.23)	0.84(0.70-1.04)	1.24 (1.03-1.52)
GGT (U/L)[*,†]	25 (16-39)	38 (22-59)	38 (26-78)
TG (mg/dL)[*,†]	84 (61-111)	115 (84-148)	120 (84-184)
HDL-C (mg/dL)[*,†]	62 (52-73)	52 (46-63)	51 (43-59)
LDL-C (mg/dL)[*,†]	111 (96-125)	116 (103-142)	118 (105- 139)
Platelets ($\times 10^3/\mu$L)[*,‡]	244 (208-282)	255 (229-289)	198 (177-214)
Uric acid (mg/dL)[*,†]	5.4 (4.3-6.2)	6.0 (5.2-7.0)	6.2 (5.4-7.2)

*$P < 0.05$, non-FL group versus FL group.
†$P < 0.05$, non-FL group versus FL-HABR.
‡$P < 0.05$, FL group versus FL-HABR.
Abbreviations: ALT, alanine aminotransferase; APRI, aspartate aminotransferase to platelet ratio index; AST, aspartate aminotransferase; FPG, fasting plasma glucose; GGT, gamma-glutamyl transpeptidase; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; TG, triglycerides.

baseline were 30 (5.4%), 56 (10.1%), and 110 (19.9%), respectively. To assess the incidence of diabetes, hypertension, and dyslipidemia, 522, 496, and 442 subjects with no evidence of diabetes, hypertension, and dyslipidemia, respectively, were followed longitudinally. Because 494 subjects had repeated health checkups, 494 subjects were followed to analyze for diabetes (494/522, 94.6%) and hypertension (494/496, 99.6%), while 442 subjects were followed to assess dyslipidemia. The blood flow parameters measured by US are shown in Supporting Table S3. The median A/P ratio was 1.12 (interquartile range 1.00-1.29). The median echo intensities of the body of the pancreas, liver parenchyma, and right kidney cortex were 39.5 ± 6.4, 33.1 (30.5-37.0), and 29.9 (28.3-31.6), respectively. The median hepatorenal ratio was 1.1 (1.0-1.2).

COMPARISON OF BASELINE CHARACTERISTICS AMONG THE NON-FL, FL, AND FL-HABR GROUPS

The baseline characteristics of the three groups are shown in Table 1. The median age was significantly higher in the FL-HABR group than in the other groups. Men constituted the majority of participants in both the FL and FL-HABR groups. Fatty liver and HABR were positively associated with BMI (non-FL versus FL, $P < 0.001$; non-FL versus FL- HABR, $P < 0.001$) and waist circumference (non-FL versus FL, $P < 0.001$; non-FL versus FL- HABR, $P < 0.001$). Fasting plasma glucose, hemoglobin A1c, transaminase, gamma-glutamyl transpeptidase, triglycerides, high-density lipoprotein-cholesterol, low-density lipoprotein-cholesterol, and uric acid were significantly higher in the FL and FL-HABR groups than in the non-FL group, while there was no significant difference between the FL and FL-HABR groups. Hepatic fibrosis scores (aspartate aminotransferase to platelet ratio index and FIB-4) were significantly higher in the FL-HABR group, while there was no significant difference between the FL and non-FL groups (Fig. 1).

COMPARISON OF ULTRASOUND FACTORS AMONG THE NON-FL, FL, AND FL-HABR GROUPS

Imaging parameters are shown in Table 2. The H/R ratio was significantly higher in the FL and FL-HABR groups than in the non-FL group (non-FL versus FL, $P < 0.001$; non-FL versus FL-HABR, $P < 0.001$), while there was no significant difference
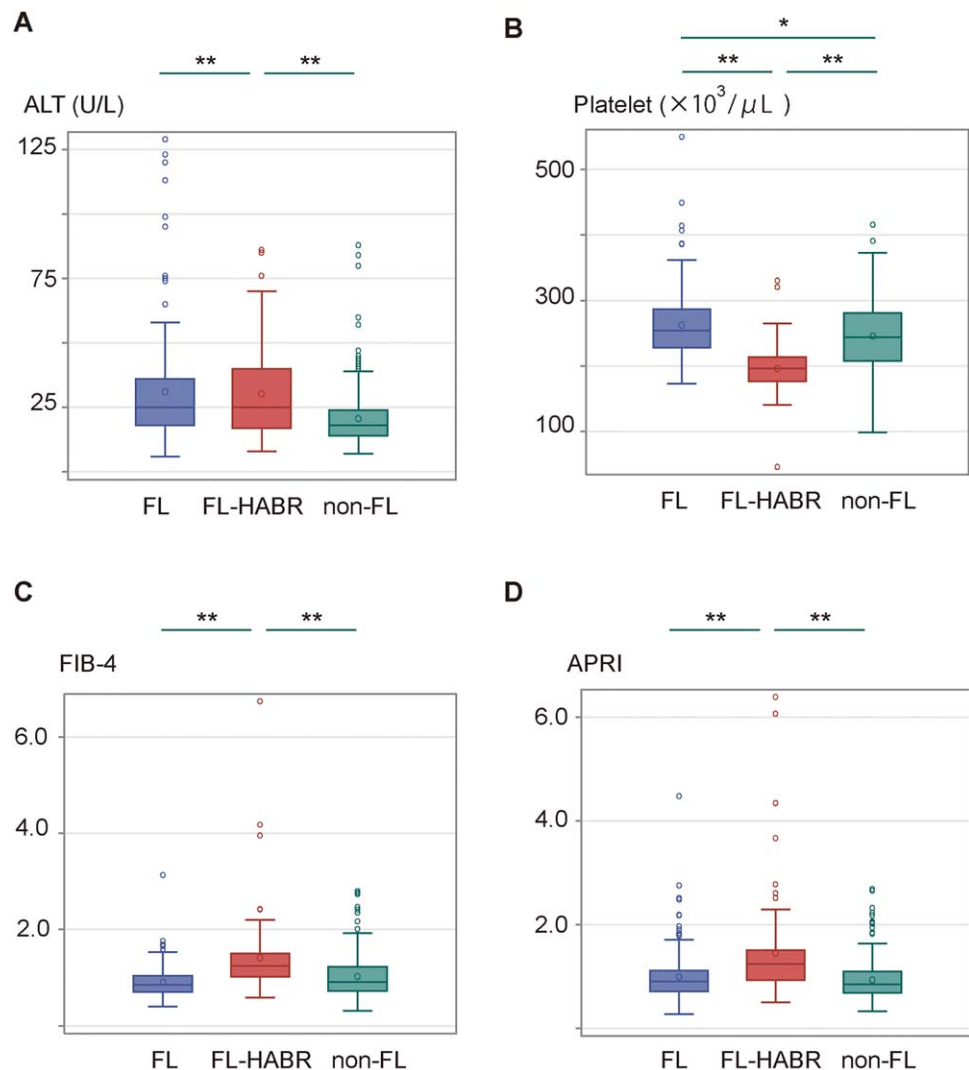
FIG. 1. Comparisons of the fibrosis score and platelets (n = 552). Alanine aminotransferase (A), platelets (B), FIB-4 (C), and the aspartate aminotransferase to platelet ratio index (D) are significantly different between non-FL and FL or FL-HABR. Only platelets are different between FL and FL-HABR. Abbreviations: ALT, alanine aminotransferase; APRI, aspartate aminotransferase to platelet ratio index.

** p < 0.01, * p < 0.05
FL: fatty liver
HABR: hepatic arterial buffer response

between the FL and FL-HABR groups ($P = 0.993$). The A/P ratio was significantly higher in the FL-HABR group (non-FL versus FL-HABR, $P < 0.001$; FL versus FL- HABR, $P < 0.001$), while there was no significant difference between the FL and FL-HABR groups ($P = 0.465$). The echo intensity of the pancreas was significantly higher in the FL and FL-HABR groups than in the non-FL group (non-FL versus FL, $P < 0.001$; non-FL versus FL-HABR, $P < 0.001$; FL versus FL- HABR, $P < 0.001$; Fig. 2).

COMPARISON OF BASELINE CHARACTERISTICS WITH AND WITHOUT FOLLOW-UP

To assess selection bias, the baseline characteristics of the participants with follow-up were compared to those of participants without follow-up (Supporting Table S4). The median age of the follow-up group was significantly greater ($P = 0.0350$). There were no significant differences in any other parameters between the two groups.

TABLE 2. COMPARISON OF IMAGING FACTORS BY THE PRESENCE OF FATTY LIVER

Factor	Non-FL	FL	FL-HABR
Maximal velocity of RPV (cm/s)[†,‡]	22.1 (18.7-26.0)	22.6 (20.0-25.9)	17.7 (15.8-19.6)
Maximal velocity of RHA (cm/s)[†,‡]	49.8 (42.4-57.2)	47.9 (40.7-56.7)	63.5 (56.1-70.4)
RHA-PI[‡]	1.12 (1.00-1.30)	1.08 (0.98-1.23)	1.21 (1.03-1.43)
RHA-RI[*,‡]	0.62 (0.54-0.67)	0.59 (0.52-0.65)	0.63 (0.56-0.68)
A/P ratio[†,‡]	2.19 (1.81-2.74)	2.18 (1.82- 2.47)	3.50 (3.29-3.87)
MPV (cm/s)[*,†]	33.4 (27.2- 43.8)	30.3 (24.2-37.2)	28.0 (22.3-36.5)
MPV diameter (mm)[*]	10.9 (9.4-12.0)	11.2 (10.0-12.4)	11.3 (10.3-12.2)
Maximal velocity of the splenic vein (cm/s)	16.0 (13.2-19.7)	16.0 (12.5-19.4)	15.1 (13.0-18.8)
Maximal velocity of the splenic artery (cm/s)	50.2 (42.4-60.2)	45.6 (38.9-59.2)	46.1 (40.8-59.4)
Splenic artery-PI	0.91 (0.77-1.05)	0.90 (0.77-1.10)	0.91 (0.72-1.10)
Splenic artery-RI[‡]	0.53 (0.44-0.60)	0.51 (0.43-0.59)	0.54 (0.48-0.62)
Spleen index[†,‡]	25.0 (21.0-30.0)	25.8 (21.3- 32.2)	31.7 (22.6-43.0)
Wave form of the RHV (triphasic:biphasic:monophasic)	275:28:2	119:36:2	57:32:1
Echo intensity of the pancreas[*,‡]	38.6 (33.9-41.1)	39.9 (35.0- 44.2)	44.4 (39.0-48.9)
Echo intensity of the liver[*,‡]	31.2 (29.8-32.9)	36.7 (34.2-38.9)	37.8 (36.0-39.2)
Echo intensity of the kidney[*,‡]	30.1 (28.9-31.7)	29.0 (26.5-31.1)	30.3 (28.6-32.0)
H/R ratio[*,†]	1.04 (1.01-1.07)	1.23 (1.18-1.34)	1.25 (1.18 -1.31)

[*]$P < 0.05$, non-FL group versus FL group.
[†]$P < 0.05$, non-FL group versus FL-HABR.
[‡]$P < 0.05$, FL group versus FL-HABR.
Abbreviations: MPV, major portal vein; PI, pulsatility index; RHA, right hepatic artery; RHV, right hepatic vein; RI, resistive index; RPV, right portal vein.

LONGITUDINAL STUDY

During the follow-up period, the numbers of subjects with incident diabetes, hypertension, and dyslipidemia were 40 (8.1%), 74 (15.0%), and 156 (35.3%), respectively. To clarify which factors were associated the incidences of diabetes (Table 3), hypertension (Table 4), and dyslipidemia (Table 5), HRs were determined by both univariate and multivariate analyses. Fatty liver with or without the HABR at baseline was associated with the incidence of diabetes on univariate analysis (FL versus non-FL, crude HR, 4.76; 95% CI, 1.59-17.39; FL-HABR versus FL, crude HR, 4.10; 95% CI, 2.03 - 8.95, respectively). Fatty liver with or without the HABR was associated with the incidence of diabetes after adjusting for age, sex, BMI, splenomegaly, the wave form of the hepatic vein, and the echo intensity of the pancreas (FL versus non-FL, adjusted HR, 3.36; 95% CI, 1.05-12.85; FL-HABR versus FL, adjusted HR, 2.68; 95% CI, 1.28 - 6.04, respectively). Therefore, fatty liver with or without the HABR was positively associated with the incidence of diabetes (Table 3).

Bright pancreas (echo intensity of the pancreas >40.0) at baseline was associated with the incidence of diabetes on univariate analysis (crude HR, 4.22; 95% CI, 2.10-9.43). However, bright pancreas was not associated with the incidence of diabetes after adjusting

for age, sex, BMI, splenomegaly, the wave form of the hepatic vein, and echo intensity of the pancreas (adjusted HR, 2.04; 95% CI, 0.97-4.70; Supporting Table S5). The association between bright pancreas and the incidence of diabetes was explained by the confounders. To clarify the confounders, further analyses were conducted in an age-adjusted and sex-adjusted model (Supporting Table S6). Each covariate was added to the age-adjusted and sex-adjusted model, and the results showed that fatty liver was the confounder. The multivariate adjusted HR was 1.92 (0.91-4.45) after adjusting for age, sex, and fatty liver. The positive association between bright liver and diabetes incidence was substantially explained by fatty liver. Fatty liver with or without the HABR at baseline was associated with the incidence of hypertension on univariate analysis (FL-HABR versus non-FL, crude HR, 4.51; 95% CI, 2.62-7.93; FL-HABR versus FL, crude HR, 2.58; 95% CI, 1.48-4.59). However, there was no association between non-FL and FL (crude HR, 1.75; 95% CI, 0.93-3.26). On multivariate analysis, the condition of FL with hemodynamic change was positively associated with the incidence of hypertension (Table 4). Table 5 shows the incidence of dyslipidemia. Fatty liver with or without the HABR at baseline was associated with the incidence of dyslipidemia on univariate analysis (FL-HABR versus non-FL, crude HR, 2.77; 95% CI, 1.84-4.17; FL versus non-
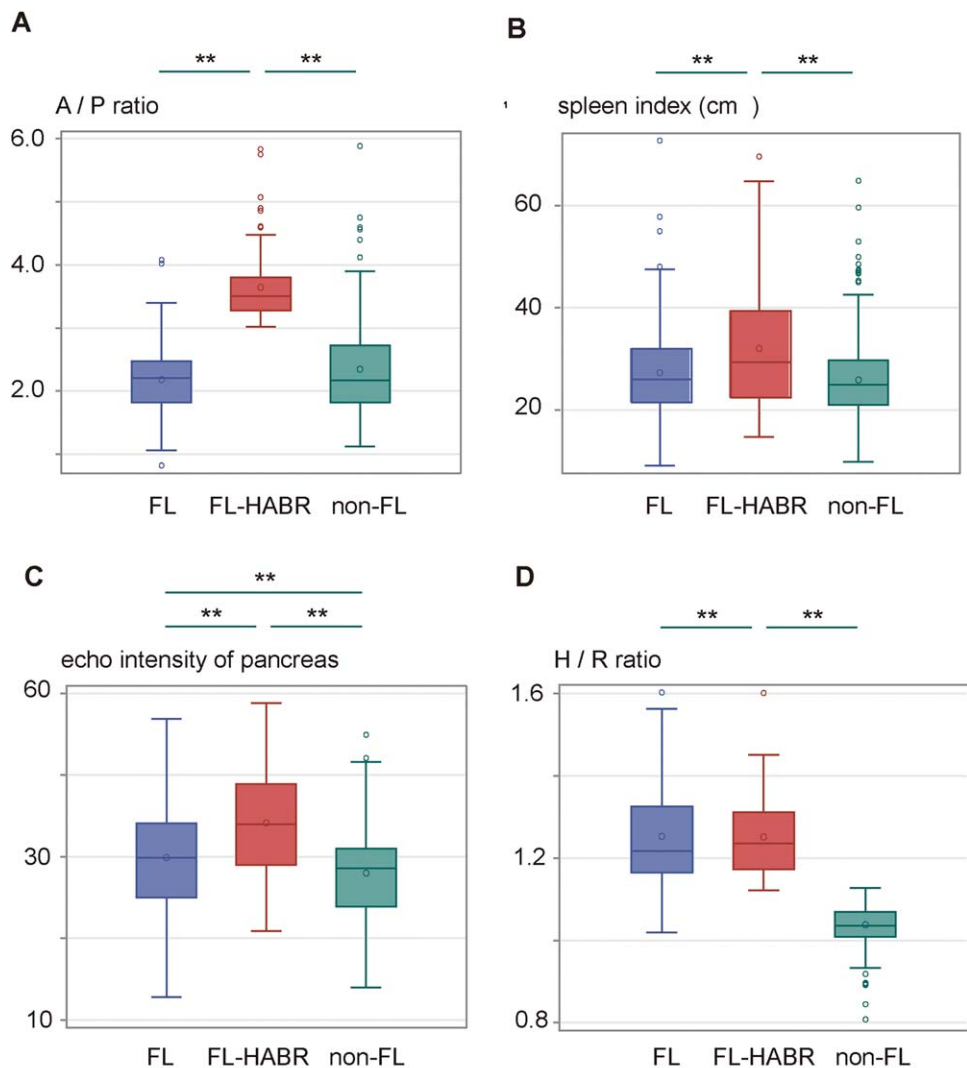
FIG. 2. Comparison of the echo imaging index (n = 552). The A/P ratio, spleen index, echo intensity of the pancreas, and the H/R ratio are significantly different between non-FL and FL or FL-HABR. Only the echo intensity of the pancreas is different between FL and FL-HABR.

** p < 0.01, * p < 0.05
FL: fatty liver
HABR: hepatic arterial buffer response

TABLE 3. ADJUSTED HR FOR THE ASSOCIATION BETWEEN FATTY LIVER WITH THE HABR AND THE INCIDENCE OF DIABETES

	Crude		Age and sex-adjusted		Model 2	
Model	HR (95% CI)	P	Adjusted HR (95% CI)	P	Adjusted HR (95% CI)	P
Non-FL versus FL	4.76 (1.59-17.39)	*0.005	4.68 (1.54-17.27)	*0.006	3.36 (1.05-12.85)	*0.041
FL versus FL-HABR	4.10 (2.03-8.95)	*<0.001	3.57 (1.74-7.90)	*<0.001	2.68 (1.28-6.04)	*0.008
Non-FL versus FL-HABR	19.50 (7.60-66.08)	*<0.001	16.74 (6.37-57.64)	*<0.001	8.99 (3.04-33.53)	*<0.001
Age (>65 years)			2.04 (0.87-4.29)	0.075	2.33 (0.96-5.05)	0.059
Male			1.74 (0.67-5.93)	0.302	1.69 (0.64-5.81)	0.312
BMI (>25 kg/m^2)					1.01 (0.52-1.95)	0.982
Splenomegaly (>30)					1.93 (1.01-3.78)	*0.048
Wave form of hepatic vein (triphasic)					1.81 (0.93-3.43)	0.078
Echo intensity of pancreas (>40.0)					2.04 (0.97-4.70)	0.060

*P < 0.05, non-FL group versus FL group.

TABLE 4. ADJUSTED HR FOR THE ASSOCIATION BETWEEN FATTY LIVER WITH THE HABR AND THE INCIDENCE OF HYPERTENSION

Model	Crude		Age and sex-adjusted		Model 2	
	HR (95% CI)	P	Adjusted HR (95% CI)	P	Adjusted HR (95% CI)	P
Non-FL versus FL	1.75 (0.93-3.26)	0.079	1.65 (0.88-3.09)	0.119	1.05 (0.53-2.10)	0.874
FL versus FL-HABR	2.58 (1.48-4.59)	*0.001	2.58 (1.47-4.62)	*<0.001	2.03 (1.14-3.69)	*0.016
Non-FL versus FL-HABR	4.51 (2.62-7.93)	*<0.001	4.25 (2.43-7.61)	*<0.001	2.14 (1.11-4.22)	*0.023
Age (>65 years)			1.27 (0.59-3.30)	0.566	1.34 (0.61-3.57)	0.489
Male			1.34 (.001)	0.355	1.24 (0.67-2.48)	0.506
BMI (>25 kg/m^2)					1.81 (1.09-3.03)	*0.022
Splenomegaly (>30)					2.39 (1.48-3.91)	*<0.001
Wave form of hepatic vein (triphasic)					1.05 (0.62-1.83)	0.870
Echo intensity of pancreas (>40.0)					1.56 (0.91-2.78)	0.108

*$P < 0.05$, non-FL group versus FL group.

FL, crude HR, 1.86; 95% CI, 1.24-2.78). However, there was no association between FL-HABR and FL (crude HR, 1.49; 95% CI, 0.99-2.24). On multivariate analysis, the condition of FL with hemodynamic change was positively associated with the incidence of dyslipidemia compared to the non-FL group.

Discussion

We previously reported that a change of hepatic blood flow in patients with fatty liver occurred even in earlier fibrosis.[12] In the present study, the hemodynamic change in fatty liver that is related to future metabolic disease was identified in a Japanese cohort.

Untreated NAFLD encompasses a wide spectrum of pathologic conditions, from simple steatosis to NASH, which may progress to liver cirrhosis.[20,21] Pericellular fibrosis around the central vein is present in the earliest fibrosis stage of NAFLD, with gradual progression to fibrosis connecting the central veins in neighboring lobules. Outflow block due to pericellular fibrosis causes the change in hepatic blood flow. Based on the above, the participants in the present study were divided into three groups.

Recently, efforts have been made to develop noninvasive methods for hepatic steatosis and fibrosis using US elastography,[12,16,22-27] magnetic resonance elastography,[28-33] and proton density fat fraction measurement.[33-36] These modalities, especially magnetic resonance imaging, have higher diagnostic accuracy for detecting liver fibrosis and steatosis,[33] and they are still better at predicting hepatic fibrosis. However, such techniques need special modalities, and the evaluation of liver fibrosis and steatosis cannot be routinely performed by these methods at most hospitals. On the other hand, B mode and Doppler US have been performed routinely worldwide with standard US machines, although experts trained in such techniques are needed. Thus, B mode and Doppler US are useful methods that are able to generally screen to predict hepatic steatosis and the change of hepatic blood flow due to portal hypertension caused by hepatic fibrosis. In the present study, the participants were allocated by B mode and Doppler US findings. Moreover, Angulo et al. reported that hepatic fibrosis, but no other

TABLE 5. ADJUSTED HR FOR THE ASSOCIATION BETWEEN FATTY LIVER WITH THE HABR AND THE INCIDENCE OF DYSLIPIDEMIA

Model	Crude		Age and sex-adjusted		Model 2	
	HR (95% CI)	P	Adjusted HR (95% CI)	P	Adjusted HR (95% CI)	P
Non-FL versus FL	1.86 (1.24-2.78)	*0.003	1.39 (0.95-2.05)	0.119	1.24 (0.81-1.90)	0.311
FL versus FL-HABR	1.49 (0.99-2.24)	0.0553	1.43 (0.94-2.15)	*<0.001	1.32 (0.87-2.01)	0.194
Non-FL versus FL-HABR	2.77 (1.84-4.17)	*<0.0001	1.99 (1.34-2.96)	*<0.001	1.65 (1.03-2.63)	*0.038
Age (>65 years)			1.00 (0.60-1.83)	0.566	1.34 (0.61-3.57)	0.489
Male			1.51 (1.00-2.37)	0.355	1.24 (0.67-2.48)	0.506
BMI (>25 kg/m^2)					1.81 (1.09-3.03)	*0.022
Splenomegaly (>30)					2.39 (1.48-3.91)	*<0.001
Wave form of hepatic vein (triphasic)					1.05 (0.62-1.83)	0.870
Echo intensity of pancreas (>40.0)					1.26 (0.89-1.81)	0.194

*$P < 0.05$, non-FL group versus FL group.

histologic feature, was independently associated with long-term outcomes of patients with NAFLD.[37] As we have reported, FL-HABR is positively associated with hepatic fibrosis and portal hypertension.[16,38] Thus, the NAFLD group with higher mortality can be identified by a simple and widespread US Doppler method.

The laboratory data associated with metabolic diseases and blood pressure were significantly worse with fatty liver. However, there were no significant differences between fatty liver with and without the HABR in these parameters at baseline. Therefore, it appears that fatty liver with a change of hepatic blood flow is an important condition related to the incidence of metabolic diseases in all patients with fatty liver because fatty liver with the HABR was identified as a significant parameter in the present cohort study. Because there were no differences between the participants with and without follow-up, except for age, selection bias was very small by excluding 58 participants. The presence of hepatic FL with or without the HABR was associated with the incidence of diabetes. In particular, the condition of hepatic fat accumulation and the HABR was strongly associated with the incidence of diabetes. FL is a risk factor for the onset of diabetes in a number of reports. Underlying insulin resistance in obese subjects is not only associated with the onset of diabetes but also related to FL. As we reported, several potential mechanisms could explain the association between FL and the onset of diabetes.[39] Levels of hepatokines, such as fetuin-A and selenoprotein P, are elevated in patients with FL. Fetuin-A is associated with insulin resistance through the inhibition of insulin-induced tyrosine phosphorylation of the insulin receptor and insulin receptor substrate-1, while selenoprotein P is thought to be associated with insulin resistance thorough inactivation of adenosine monophosphate–activated protein kinase. In addition, FL could lead to hepatic insulin resistance through stimulation of gluconeogenesis and activation of protein kinase C-ϵ and c-Jun N-terminal kinase 1, which may interfere with tyrosine phosphorylation of insulin receptor substrate-1 and insulin receptor substrate-2 and, thereby, impair the ability of insulin to activate glycogen synthase. Hypertension may occur not only by intrahepatic fat deposition because FL-HABR is a significant factor related to the incidence of hypertension, while FL is not an independent factor. Uno et al. reported that hepatic steatosis itself did not induce hypertension in a murine model.[40] The HABR is a condition with portal hypertension. Plasma renin activity and aldosterone levels were both elevated in patients with portal hypertension. In addition to insulin resistance or other metabolic disorders, FL-HABR is a high risk for hypertension. Armstrong et al. reported that NAFLD is a proinflammatory condition characterized by a milieu of proinflammatory mediators, oxidative stress, insulin resistance, and lipotoxicity.[41] Lipotoxicity, in turn, activates a cascade of inflammatory changes (activation of macrophages/immune cells), cellular dysfunction, and necroapoptosis in the liver and other organs (including the pancreas, muscle, and vascular beds). A cycle of hepatic and adipose tissue dysfunction occurs and leads to development of a pathogenic milieu containing excessive levels of insulin, glucose, lipids (nonesterified fatty acids, low-density lipoprotein, very low-density lipoprotein), carcinogenic growth factors (insulin growth factor 1, vascular endothelial growth factor), procoagulants (fibrinogen, plasminogen activator inhibitor 1), and proinflammatory cytokines.

Similar to the condition in the liver, excessive lipid deposition in the pancreas is referred to as "nonalcoholic fatty pancreas disease."[4,42] It has been reported that fatty pancreas is associated with metabolic disorders in cross-sectional studies.[43,44] However, Yamazaki et al. reported that fatty pancreas was not independently associated with future diabetes after adjusting for various parameters. The association was substantially explained by the confounders.[4] The present data also supported the finding that fatty pancreas was not independently associated with future diabetes, although fatty pancreas tended to be associated with diabetes.

In the present study, FL was found to be a confounder, as in a previous report.[4] Fatty pancreas was not independently associated with the incidence of diabetes because FL was a confounder. For hypertension and dyslipidemia, the condition with the HABR is a significant predictive marker, while the presence of fatty accumulation was not associated with the incidence of hypertension or dyslipidemia. Söderberg et al. reported that patients with NASH are at increased risk of death compared with the general population or patients with NAFLD after adjustment for sex, age, and calendar period.[10] The mortality rate was not analyzed in the present study. Although the present study cannot be simply compared with Söderberg et al.'s study, there seems to be homogeneity between the two studies. In their study, cardiovascular disease was the most common cause of death. Most of the participants with the HABR defined by Doppler US with

diabetes, hypertension, and dyslipidemia had NASH or a condition analogous to NASH. Thus, participants with the HABR are a group at high risk for metabolic disease. To prevent cardiovascular events, screening for FL with the HABR is very important. There is no need whatever to introduce high-level systems, such as magnetic resonance elastography, magnetic resonance spectroscopy, or US elastography.

This study has several limitations. First, the exact relationship between fatty liver with HABR and the presence of metabolic disease is not entirely clear based on the findings in this study. Furthermore, the biological mechanism has not been clarified by basic research. Thus, it needs to be further explored in future studies. Second, this was not a large-scale study and was performed only in Japan. A validation trial is needed in countries other than Japan. Third, the follow-up period of 5 years may be insufficient to analyze associations between FL with or without the HABR; a longer follow-up period is needed. Mortality should also be assessed over a longer period. Fourth, 58 participants (10.5%) could not be enrolled in the longitudinal study. Because the age of participants with follow-up was significantly higher, analysis was done after adjusting for age. However, there may have been substantial selection bias. Fifth, the median BMI was lower in this cohort. Because the relationship between body fat and BMI is ethnic group–specific, the BMI cutoff point is lower for the Asian population than for the Caucasian population. This is why Asian people have a lower BMI but a higher percentage of body fat. The World Health Organization has recently revised the BMI cutoff points to >23.0 kg/m^2 to indicate overweight status in Asian-specific populations, compared with the standard cutoff of >25.0 kg/m^2 for Caucasian populations.[45] It is better to perform a validation study for Caucasian populations. Sixth, the H/R ratio was used to define FL, and the A/P ratio was used to define portal hypertension. Because health checkup subjects were enrolled in this study, an easy method should be selected. The H/R ratio was used broadly for the diagnosis of hepatic steatosis; an H/R ratio of 1.12 was associated with a liver fat content of 20%-40% on ^{1}H-magnetic resonance spectroscopy in a previous study.[46] The median A/P ratios in NAFLD patients with fibrosis stages F0, F1, F2, F3, and F4 were 1.8, 2.0, 2.5, 3.2, and 3.7, respectively.[38] Thus, an A/P ratio of 3.1 is thought to correspond to F3 or F4.

In conclusion, fatty liver with the HABR diagnosed by US is a high-risk condition for metabolic diseases.

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