Original Article

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Prevalence and Independent Factors for Fatty Liver and Significant Hepatic Fibrosis Using B-Mode Ultrasound Imaging and Two Dimensional-Shear Wave Elastography in Health Check-up Examinees

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Summary: ***Background and Aim*:** Exercise is beneficial for metabolic syndrome. Fatty liver and significant hepatic fibrosis, hepatic manifestations of metabolic syndrome, are becoming an epidemic. We aimed to investigate the prevalence of fatty liver and significant fibrosis and examined the independent factors for these conditions.
***Subjects and Methods*:** We enrolled 1,361 health check-up examinees (median age, 53 years; female/male, 813/548). Fatty liver and fibrosis were evaluated by B-mode ultrasound imaging and shear wave elastography. Factors associated with fatty liver and significant fibrosis were analyzed by logistic regression analysis.
***Results*:** Fatty liver and significant fibrosis were observed in 50.5% and 42.7% of enrolled subjects, respectively. Independent factors associated with fatty liver were BMI (OR 1.46; 95%CI 1.397–1.537; P<0.0001) and no exercise habits (OR 1.47; 95% CI 1.101–1.984; P=0.0093). Independent factors associated with significant fibrosis were age, female, BMI (OR 1.37; 95%CI 1.311–1.436; P<0.0001), and no exercise habits (OR 1.49; 95% CI 1.102–2.031; P=0.0097).
***Conclusions*:** Fatty liver and significant fibrosis were frequently seen in health check-up examinees and the common independent factors were higher BMI and no exercise habits. Thus, weight loss and exercise may ameliorate fatty liver and significant hepatic fibrosis in the general population.

Keywords steatosis, liver stiffness, non-invasive test, obesity, physical activity

INTRODUCTION

Exercise is a fundamental intervention for the prevention and treatment of metabolic disorders [1]. Exercise-related energy consumption promotes proper energy balance and prevents or reverses obesity and metabolic syndrome [2,3]. Exercise is also known to increase glycolipid utilization and improve insulin resistance in patients with type 2 diabetes mellitus [4]. Moreover, exercise increases circulating nitric oxide levels and lowers blood pressure in patients with essential hypertension [5]. Thus, exercise is beneficial

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Abbreviations: BMI, body mass index; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; kPa, kilopascals; LDL, low-density lipoprotein; NAFLD, nonalcoholic fatty liver disease; 2D-SWE, two-dimensional share wave elastography

for individuals with various types of metabolic disorders.

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of liver disease worldwide [6,7]. NAFLD is associated with the features of metabolic syndrome, and lifestyle intervention is the first-line therapy for patients with NAFLD [8,9]. Exercise reduces intrahepatic fat content by increasing insulin sensitivity in patients with obesity [10]. We have performed a meta-analysis and demonstrated that both aerobic and resistance exercises reduce hepatic steatosis in patients with NAFLD [11]. In addition, exercise is known to suppress anti-inflammatory mediators and inhibit overproduction of reactive oxygen species, which promotes hepatic fibrosis [12,13]. Although exercise has been reported to improve hepatic fibrosis in mouse models of NAFLD [14,15], the effects of exercise on hepatic fibrosis remain unclear in patients with NAFLD, partly due to limitations in the assessment of hepatic fibrosis.

Liver biopsy, the gold standard for staging hepatic fibrosis, is not always available in clinical practice owing to its invasiveness [16]. In addition, the usefulness of liver biopsy is limited by intraobserver/interobserver variability and sampling errors [17]. Thus, the prevalence of subjects with significant hepatic fibrosis in the general population remains unclear. In recent years, non-invasive alternatives to liver biopsy have been developed for the assessment of hepatic fibrosis [18].

Elastography is a tissue elasticity imaging technique, which enables non-invasive evaluation of hepatic fibrosis [18]. Two-dimensional shear wave elastography (2D-SWE) is an imaging technique that has proven to be an efficient and reproducible non-invasive technique for the detection and staging of hepatic fibrosis [19]. Obesity and elevated waist circumference were associated with unreliable results owing to the poor transmission of the ultrasonic beams [20]. However, one of the advantages of this technique is that it can be performed during a routine ultrasound examination; therefore, 2D-SWE is the only non-invasive imaging modality that is practically available in daily clinical practice [17].

The aim of this study was to investigate the prevalence of fatty liver and significant hepatic fibrosis in the general population using B-mode ultrasound imaging and 2D-SWE, respectively. In addition, we examined the characteristics, including exercise habits, of individuals with fatty liver and significant hepatic fibrosis.

MATERIALS AND METHODS

Study design and ethics

This single-center, prospective, observational cohort study aimed to examine the prevalence and independent factors of fatty liver and significant hepatic fibrosis using B-mode ultrasound imaging and 2D-SWE, respectively, in health check-up examinees. This protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki, as reflected by the prior approval of the institutional review board of Kurume University School of Medicine. All experiments were performed according to relevant guidelines and regulations. We used an opt-out approach to obtain informed consent from the patients, and personal information was protected during data collection.

Study population

We enrolled 1,879 consecutive health check-up examinees who visited the Saga Health and Clinical Examination Center from May 2017 to June 2019. Exclusion criteria included (1) duplication of participants (n=158), (2) lack of data for body mass index (BMI) (n=5), (3) lack of data on alcohol consumption (n=27), (4) subjects with hepatitis B virus infection (n=17), hepatitis C virus infection (n=13), hepatitis B virus infection/hepatitis C virus infection co-infection (n=2), (5) subjects who consumed more than 60 g/day of alcohol (n=33), (6) and subjects with chronic liver disease under medication (except for NAFLD) (n=18). The remaining 1,606 non-overlapping participants underwent 2D-SWE. Of these, 245 participants were excluded because of unreliable SWE measurements (interquartile range >30%). Finally, a total of 1,361 subjects were analyzed in this study (Supplementary Fig. 1).

Data collection

The following information was recorded using a self-reporting questionnaire: age, sex, current alcohol consumption, exercise habits (exercise for more than 30 minutes at least twice/week), eating habits (skipping breakfast more than 3 times a week, eating snacks after supper more than 3 times a week). Smoking habit was evaluated using the Brinkman index ([number of cigarettes smoked per day/20] × number of years smoked) as previously described [21]. We also obtained the following data: BMI, systolic blood pressure, and diastolic blood pressure.

Classification of alcohol consumption

In this study, daily alcohol consumption was cate-

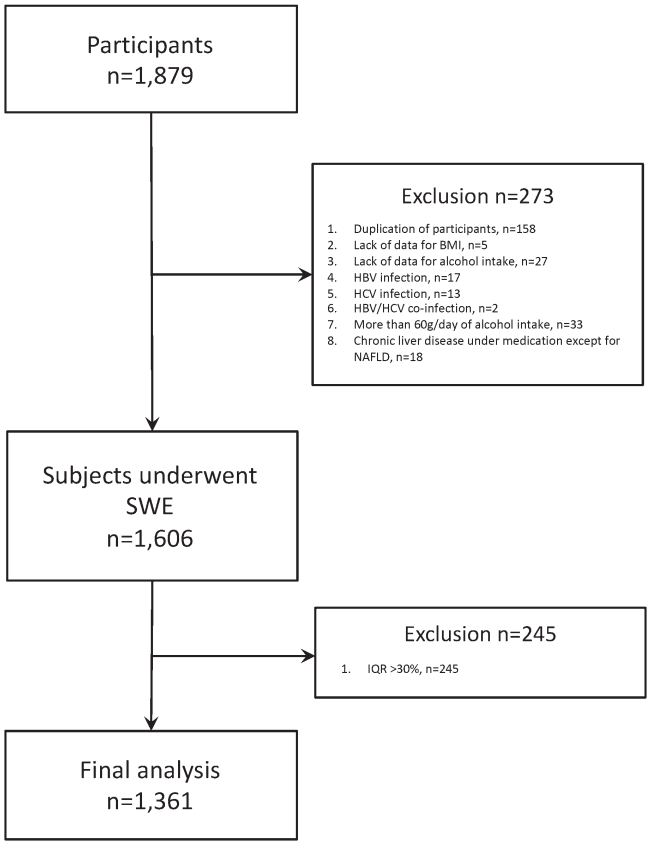
gorized as 1) none, 2) mild (less than 20 g/day), and 3) moderate (20–59 g/day), as previously described [22].

Definition of exercise habits

In this study, we defined an exercise habit as exercising (>30 minutes/session) at least twice a week for more than 1 year based on the self-reporting questionnaire.

Biochemistry

A blood sample was obtained after an overnight fast. The following laboratory tests were performed: hepatitis B surface antigen, hepatitis C virus antibody, red blood cell count, hemoglobin, hematocrit, white blood cell count, platelet count, aspartate aminotransferase, alanine aminotransferase, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, amylase, lactate dehydrogenase, alkaline phosphatase, γ-glutamyl transpeptidase, total protein, cholinesterase, albumin, total bilirubin, total cholesterol, triglycerides, blood urea nitrogen, creatinine, estimated glomerular filtration rate, C-reactive protein, uric acid, sodium, potassium, chloride, fasting glucose, and hemoglobin A1c (HbA1c).



Supplementary Fig 1. Study populations

Calculation of FIB-4 index

FIB-4 index was calculated as previously described [23]. The formula was as follows: FIB-4 index = ([age (years) × aspartate aminotransferase (U/L)]/platelet count [10^9/L]) × (alanine aminotransferase [U/L])$^{1/2}$ [23].

Diagnosis of fatty liver

Abdominal ultrasound examination was performed in all subjects using LOGIQ S8 (GE Healthcare, Wauwatosa, WI). Examination was performed by three sonographers (S.Y., Y.T., and Y.D.) who had >15 years, >8 years, and >7 years of sonography experience, respectively. Fatty liver was diagnosed based on any of the following ultrasonographic findings: the presence of increased hepatorenal contrast, the presence of bright liver, the presence of posterior beam attenuation of the right hepatic lobe, the presence of blurring of the hepatic vein trunk, and/or areas of focal sparing, as previously described [24,25]. Subjects were classified into a Fatty liver (n=687) or non-Fatty liver (n=674) group.

Measurement of shear wave elastography

2D-SWE was performed in a routine ultrasound examination using LOGIQ S8 with the C1-6-D abdominal convex probe (GE Healthcare) by the same sonographers. Collection of 2D-SWE data was started after a 1-month training period to achieve stable data. Sonographers were blinded to clinical data. SWE acquisitions were performed after an overnight fast and were performed in the right lobe with an intercostal approach, during breath hold. The region of interest (a circle of approximately 15.0 mm) was placed 1.0–2.0 cm beneath the liver capsule, avoiding artifacts and large vessels on a grayscale B-mode image. Elasticity values are displayed as a real-time color-coded 2D quantitative SWE map of tissue stiffness that was placed over a grayscale B-mode image. Three valid SWE measurements were performed on each subject, and the median value was determined as the representative measurement based on the European Federation of Societies for Ultrasound in Medicine and Biology Guidelines and Recommendations on the Clinical Use of Liver Ultrasound Elastography [26]. The SWE measurement was expressed in kilopascals (kPa). Invalid results were defined as an interquartile range/median value >30% as recommended by European Federation of Societies for Ultrasound in Medicine and Biology Guidelines and Recommendations on the Clinical Use of Liver Ultrasound Elastography [26] and in accordance with the manufacturer's recommen-

dations; therefore, we excluded these results.

Definition and classification of significant hepatic fibrosis

Liver stiffness 6.48–6.60 kPa, 6.60–8.07 kPa, 8.07–9.31 kPa, and >9.31 kPa were defined as F1, F2, F3, and F4, respectively, based on the manufacturer's technical data (GE Healthcare). Thus, in this study, subjects who showed liver stiffness $\geq$6.60 kPa were defined as having significant hepatic fibrosis. Subjects were classified into the Normal-Mild hepatic fibrosis (n=780) or Significant hepatic fibrosis (n=581) group in accordance with this definition.

Statistical analysis

Continuous variables are expressed as median and range or number. Categorical variables are expressed as frequencies and percentages. The differences between the two groups were analyzed using the Wilcoxon rank-sum test for continuous variables and using Fisher's exact test for categorical variables. A logistic regression model was used to identify independent variables associated with fatty liver or significant fibrosis in a multivariate analysis. Explanatory variables were selected from the variables listed in Table 1 in a stepwise manner minimizing the Bayesian information criterion as previously described [27]. Data were analyzed with JMP Pro 14 (SAS Institute Inc., Cary, NC). Values of $P<0.05$ were considered to indicate statistically significant differences as previously described [28].

RESULTS

Subjects' characteristics

The subject characteristics are summarized in Table 1. The median age was 53 years. The percentage of female subjects was 59.7%. The median BMI was 22.2 kg/m^2. The percentage of subjects with fatty liver was 50.5% of all subjects. Subjects of grade F2, F3, and F4 comprised 12.2%, 7.0%, and 23.5% of enrolled subjects, respectively, and significant hepatic fibrosis was seen in 42.7% of all subjects. The median FIB-4 index level was 1.00. The median levels of HDL cholesterol, LDL cholesterol, triglycerides, and HbA1c were within normal limits. Exercise habits were seen in 21.6% of all subjects. The percentage of subjects who skipped breakfast more than 3 times a week was 17.8% (Table 1).

Prevalence of subjects with fatty liver and significant hepatic fibrosis according to age

The prevalence of fatty liver was 55.4% and 50.7% in subjects aged 45–64 and 65–74 years, respectively (Figure 1A). A similar trend was seen in both male and female subjects (Figure 1B and 1C). The prevalence of subjects with significant hepatic fibrosis increased with aging (Figure 2A). A similar trend was seen in both male and female subjects (Figure 2B and C).

Prevalence of subjects with obesity according to severity of hepatic fibrosis

The prevalence of subjects with obesity increased along with the progression of fibrosis stage (Figure 3).

Differences in characteristics between the Fatty liver and non-Fatty liver groups

There was no significant difference in age between the Fatty liver and non-Fatty liver groups (Table 2). The prevalence of fatty liver was significantly higher in males than in females. BMI and systolic blood pressure were significantly higher in the Fatty liver group than in the non-Fatty liver group. FIB-4 index was significantly lower in the Fatty liver group than in the non-Fatty liver group. LDL cholesterol and HbA1c levels were significantly higher in the Fatty liver group than in the non-Fatty liver group. HDL cholesterol level was significantly lower in the Fatty liver group than in the non-Fatty liver group. The prevalence of fatty liver was significantly lower in subjects with exercise habits than in subjects without exercise habits (Table 2).

Differences in characteristics between the Normal-Mild and Significant hepatic fibrosis groups

The liver stiffness was significantly higher in the Significant hepatic fibrosis group than in the Normal-Mild hepatic fibrosis group. Age, BMI, and systolic blood pressure were significantly higher in the Significant hepatic fibrosis group than in the Normal-Mild hepatic fibrosis group. The prevalence of significant hepatic fibrosis was significantly higher in males than in females (Table 3). FIB-4 index was significantly higher in the Significant hepatic fibrosis group than in the Normal-Mild hepatic fibrosis group. LDL cholesterol and HbA1c levels were significantly higher in the Significant hepatic fibrosis group than in the Normal-Moderate hepatic fibrosis group. HDL cholesterol level was significantly lower in the Significant hepatic fibrosis group than in the Normal-Moderate hepatic fibrosis group. There was no significant difference in exercise habits between the two groups.

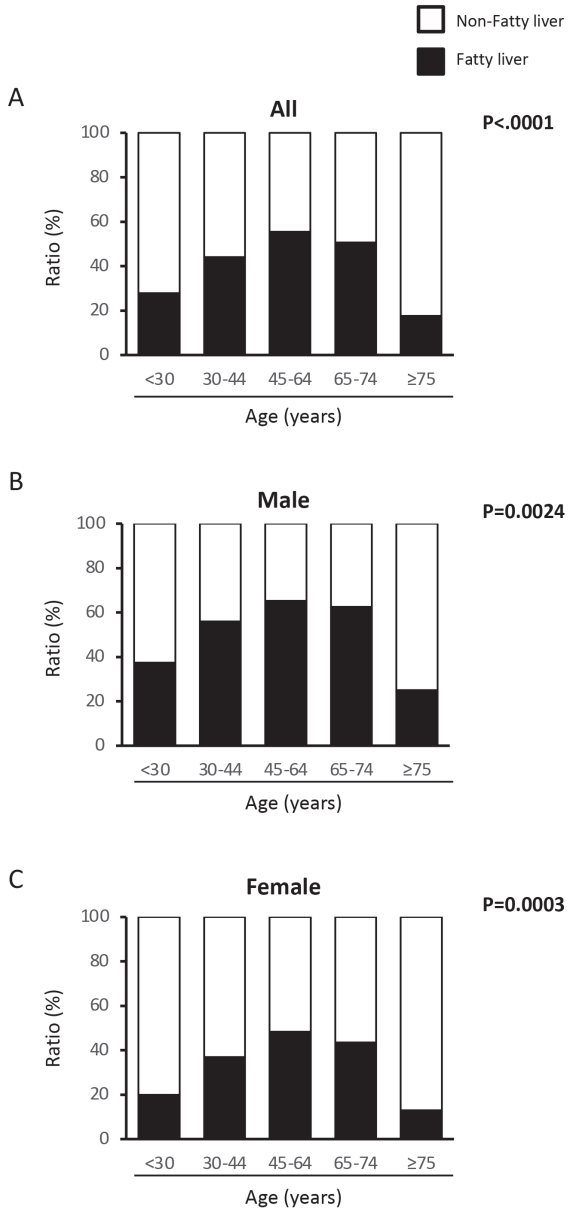


Fig. 1. Prevalence of fatty liver according to age
(A) All subjects, (B) male, (C) female. White indicates the non-Fatty liver group. Black indicates the Fatty liver group.

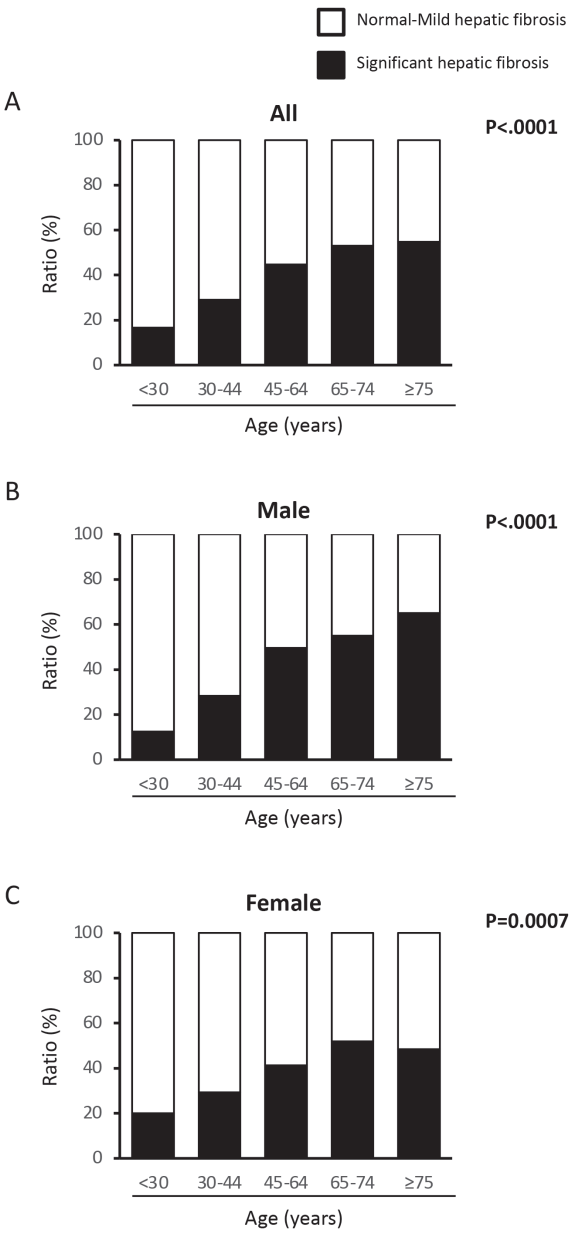


Fig. 2. Prevalence of significant fibrosis according to age
(A) All subjects, (B) male, (C) female. White indicates the Normal-Mild fibrosis group. Black indicates the Significant fibrosis group.

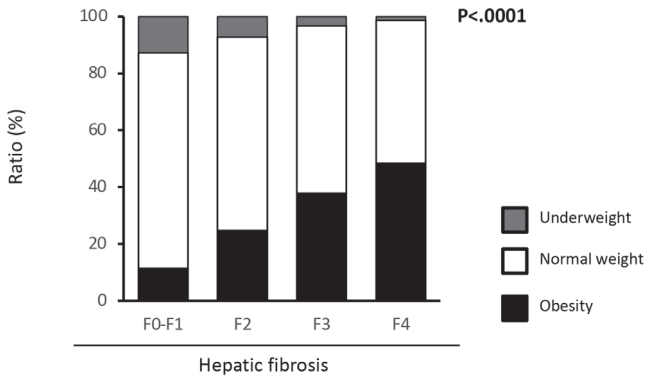


Fig. 3. Prevalence of obesity according to the severity of hepatic fibrosis
Gray indicates the underweight subjects. White indicates the subjects with normal weight. Black indicates the subjects with obesity.

TABLE 1.
Patient characteristics

	Reference Value	Median (IQR)	Range (min–max)
Number	N/A	1,155	N/A
Age (years)	N/A	53 (45–62)	23–89
Sex (female/male)	N/A	59.7%/40.3 (813/548)	N/A
BMI (kg/m^2)	18.5–24.9	22.2 (20.2–24.7)	14.5–42.1
Presence of fatty liver evaluated by ultrasonography (Yes/No)	N/A	50.5%/49.5% (687/674)	N/A
Shear wave elastography (median; kPa)	$<$ 6.57	5.88 (4.40–9.08)	2.60–47.80
Shear wave elastography (F0-F1/F2/F3/F4)	N/A	57.3%/12.2%/7.0%/23.5% (780/166/95/320)	N/A
Shear wave elastography (IQR)	$<$ 0.3	0.12 (0.07–0.19)	0–0.3
Liver stiffness evaluated by SWE ($<$6.60 kPa/ $\geq$6.60 kPa)	N/A	57.3%/42.7% (780/581)	N/A
Systolic blood pressure (mmHg)	100–129	114 (102–126)	72–174
Diastolic blood pressure (mmHg)	$<$ 84	72 (64–79)	43–121
Brinkmann index (Pack-years)	N/A	0 (0–11)	0–80
Alcohol consumption (g/day) (None/$<$20/20-59)	N/A	50.7%/21.5%/27.8% (690/292/379)	N/A
Exercise for more than 30 minutes at least twice/week (Yes/No)	N/A	21.6%/78.4% (294/1067)	N/A
Skipping breakfast more than 3 times a week (Yes/No)	N/A	13.7%/86.3% (186/1175)	N/A
Eating snacks after supper more than 3 times a week (Yes/No)	N/A	0.3%/96.7% (41/1320)	N/A
Biochemical examinations			
Red blood cell count ($\times 10^4/\mu$L)	410–530	454 (425–486)	251–592
Hemoglobin (g/dL)	13.1–17.9	13.7 (12.8–14.9)	7.1–18.7
Hematocrit (%)	36.0-45.9	41.0 (38.3–43.9)	26.0–56.2
White blood cell count (/μL)	3200–8900	5,000 (4,200–6,000)	1900–15300
Platelet count ($\times$ 10^3/mm^3)	15.2–36.1	24.2 (20.8–27.7)	5.5–75.0
AST (U/L)	10–30	19 (16–23)	7–113
ALT (U/L)	5–30	17 (13–24)	4–188
HDL cholesterol (mg/dL)	40–95	66 (55–79)	28–161
LDL cholesterol (mg/dL)	61–119	119 (101–138)	47–254
Amylase (U/L)	44–132	73 (58–89)	25–298
Lactate dehydrogenase (U/L)	120–230	170 (152–191)	65–317
ALP (U/L)	119–303	197 (161–241)	54–705
GGT (U/L)	10–50	21 (14–36)	7–681
Total protein (g/dL)	6.5–7.9	7.0 (6.8–7.3)	6.0–8.8
Cholinesterase (U/L)	240–486	323 (279–376)	110–839
Albumin (g/dL)	4.1–5.1	4.4 (4.2–4.5)	3.5–5.2

Total bilirubin (mg/dL)	0.4–1.6	0.7 (0.6–0.9)	0.3–4.6
FIB-4 index	< 1.30	1.00 (0.74–1.40)	0.27–5.01
Total cholesterol (mg/dL)	140–199	205 (185–229)	118–351
Triglycerides (mg/dL)	30–149	83 (60–119)	22–1000
BUN (mg/dL)	8.0–20.0	13.5 (11.3–15.9)	5.2–37.5
Creatinine (mg/dL)	0.60–1.00	0.66 (0.57–0.79)	0.37–2.00
eGFR (mL/min/1.73 m^2)	> 60.0	81.1 (72.2–91.3)	26.4–154.3
CRP (mg/dL)	< 0.04	0.04 (0.02–0.08)	0.01–4.52
Uric acid (mg/dL)	2.1–7.0	5.0 (4.1–6.0)	0.8–11.8
Sodium (mmol/L)	138–146	142 (141–143)	129–149
Potassium (mmol/L)	3.6–4.9	4.1 (4.0–4.4)	3.1–5.3
Chloride (mmol/L)	99–109	106 (104–107)	93–112
Fasting plasma glucose (mg/dL)	70–99	96 (90–102)	65–215
HbA1c (%)	4.3–5.8	5.6 (5.5–5.9)	4.7–9.9

Note. Data are expressed as median (interquartile range [IQR]), range, or number. Abbreviations: N/A, not applicable; BMI, Body mass index; SWE, shear wave elastography; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HDL cholesterol, high-density lipoprotein cholesterol; LDL cholesterol, low-density lipoprotein cholesterol; ALP, alkaline phosphatase; GGT, gamma-glutamyl transpeptidase; BUN, blood urea nitrogen; eGFR, estimated glomerular filtration rate; CRP, C-reactive protein; HbA1c, hemoglobin A1c.

Multivariate stepwise analysis of the factors associated with fatty liver

The independent factors associated with fatty liver are summarized in Table 4. In logistic regression analysis, BMI was identified as an independent factor for fatty liver. In addition, exercise habits were identified as an independent factor for fatty liver (Table 4).

Multivariate stepwise analysis of the factors associated with significant hepatic fibrosis

The independent factors associated with significant hepatic fibrosis are summarized in Table 5. In logistic regression analysis, age, male, and BMI were identified as independent factors for significant hepatic fibrosis. Furthermore, exercise habits were identified as an independent factor for significant hepatic fibrosis (Table 5).

DISCUSSION

This study found that fatty liver was present in 50.5% of all subjects. BMI and exercise habits were identified as independent factors for fatty liver. We also found that significant hepatic fibrosis evaluated by 2D-SWE was seen in 42.7% of all subjects. Age, male, and BMI as well as exercise habits were independently associated with significant hepatic fibrosis. Thus, obesity and physical inactivity were associated with both fatty liver and significant hepatic fibrosis.

The prevalence of NAFLD in Asia was reported to be 29–62% in a meta-analysis [29]. The prevalence of subjects with fatty liver was 50.5% of enrolled subjects, and our result was in good agreement with this previous report. However, in present study, fatty liver was evaluated by B-mode ultrasound imaging so patients with <30% hepatic steatosis may not be diagnosed as fatty liver [30]. Controlled attenuation parameter (CAP) is useful for the quantification of hepatic steatosis and could diagnose 5–29% hepatic steatosis as fatty liver. Thus, the prevalence of fatty liver evaluated by CAP could be higher than that by B-mode ultrasound imaging [30,31].

We also found significant hepatic fibrosis in 42.7% of the health check-up examinees. Meanwhile, previous studies have reported that the prevalence of significant fibrosis was 7.0–9.0% of enrolled subjects [32,33]. Although the reason for this discrepancy remains unclear, it may be due to imprecise measurement of SWE in obese patients. In this study, subjects of grade F2, F3, and F4 comprised 12.2%, 7.0%, and 23.5% of enrolled subjects, respectively. The prevalence of obesity was 48.4% in subjects of grade F4. Hence, the higher prevalence of significant hepatic fibrosis in this study may be overestimated owing to obesity [34,35]. Thus, SWE should be improved to precisely measure stiffness even in obese patients.

TABLE 2.
Comparison of characteristic between Fatty liver and Non-Fatty liver groups

	Non-Fatty liver		Fatty liver		
	Median (IQR)	Range (min–max)	Median (IQR)	Range (min–max)	P
Age (years)	52 (44–63)	23–89	54 (47–62)	23–88	0.1239
Sex (female/male)	68.4%/31.6% (461/213)	N/A	51.2%/48.8% (352/335)	N/A	<0.0001
BMI (kg/m^2)	20.8 (19.2–22.5)	14.5–30.4	24.1 (21.8–26.2)	15.0–42.1	<0.0001
Systolic blood pressure (mmHg)	110 (99–120)	72–174	118 (107–129)	79–168	<0.0001
Diastolic blood pressure (mmHg)	69 (62–77)	43–107	75 (66–83)	43–121	<0.0001
Brinkmann index (Pack-years)	0 (0–7.5)	0.0–70	0 (0–15)	0–80	<0.0001
Alcohol consumption (g/day) (None/<20/20-59)	53.7%/22.1%/24.2% (362/149/163)	N/A	47.8%/20.8%/31.4% (328/143/216)	N/A	0.0106
Exercise for more than 30 minutes at least twice/week (Yes/No)	24.5%/75.5% (165/509)	N/A	18.8%/81.2% (129/558)	N/A	0.0106
Skipping breakfast more than 3 times a week (Yes/No)	13.6%/86.4% (92/582)	N/A	13.7%/86.3% (94/593)	N/A	0.9859
Eating snacks after supper more than 3 times a week (Yes/No)	3.7%/96.3% (25/649)	N/A	2.3%/97.7% (16/671)	N/A	0.1364
Red blood cell count ($\times 10^4/\mu$L)	443 (417–474)	251–592	466 (434–496)	348–591	<0.0001
Hemoglobin (g/dL)	13.4 (12.6–14.4)	7.2–18.2	14.2 (13.1–15.2)	7.1–18.7	<0.0001
Hematocrit (%)	40.2 (37.9–42.8)	26.7–53.7	42.0 (39.0–44.6)	26.0–56.2	<0.0001
White blood cell count (/μL)	48 (41–58)	19–128	52 (43–63)	25–153	<0.0001
Platelet count ($\times$ 10^3/mm^3)	23.7 (20.7–27.1)	5.5–75.0	24.7 (20.8–28.4)	10.3–56.3	0.0055
AST (U/L)	18 (16–22)	9–57	20 (17–25)	7–113	<0.0001
ALT (U/L)	15 (12–20)	4–89	19 (14–30)	4–188	<0.0001
HDL cholesterol (mg/dL)	73 (61–84)	30–161	60 (50–71)	28–114	<0.0001
LDL cholesterol (mg/dL)	113 (98–131)	47–235	126 (105–145)	56–254	<0.0001
Amylase (U/L)	77 (62–95)	26–298	69 (55–84)	25–194	<0.0001
Lactate dehydrogenase (U/L)	169 (150–191)	65–290	170 (153–191)	65–317	0.3006
ALP (U/L)	187 (155–235)	54–705	204 (169–245)	87–445	<0.0001
GGT (U/L)	17 (13–27)	7–681	26 (17–45)	8–445	<0.0001
Total protein (g/dL)	7.0 (6.8–7.3)	6.0–8.6	7.1 (6.9–7.3)	6.2–8.8	0.0078
Cholinesterase (U/L)	303 (266–344)	110–839	350 (308–395)	203–671	<0.0001
Albumin (g/dL)	4.3 (4.2–4.5)	3.5–5.2	4.4 (4.2–4.5)	3.6–5.1	0.0561
Total bilirubin (mg/dL)	0.7 (0.6–0.9)	0.3–2.5	0.7 (0.6–0.9)	0.3–4.6	0.9432
FIB-4 index	1.02 (0.76–1.47)	0.30–5.01	0.96 (0.72–1.30)	0.27–3.42	0.0194
Total cholesterol (mg/dL)	203 (183–227)	118–337	206 (188–232)	130–351	0.0602
Triglycerides (mg/dL)	69 (53–94)	22–356	101 (75–146)	30–1000	<0.0001
BUN (mg/dL)	13.8 (11.5–16.2)	5.2–37.5	13.1 (11.2–15.4)	5.7–27.3	0.0155
Creatinine (mg/dL)	0.64 (0.55–0.76)	0.38–2.00	0.68 (0.58–0.81)	0.37–1.52	<0.0001

eGFR (mL/min/1.73 m^2)	81.1 (72.3–92.4)	26.4–154.3	81.1 (71.9–90.5)	37.5–131.9	0.4037
CRP (mg/dL)	0.03 (0.02–0.05)	0.01–3.71	0.05 (0.03–0.10)	0.01–4.52	<0.0001
Uric acid (mg/dL)	4.7 (3.9–5.5)	0.9–8.2	5.3 (4.5–6.3)	0.8–11.8	<0.0001
Sodium (mmol/L)	142 (141–143)	129–147	142 (141–143)	137–149	0.7580
Potassium (mmol/L)	4.1 (4.0–4.4)	3.1–5.2	4.2 (4.0–4.4)	3.4–5.3	0.3446
Chloride (mmol/L)	106 (104–107)	93–112	106 (104–107)	100–112	0.6068
Fasting plasma glucose (mg/dL)	94 (89–99)	65–215	97 (92–105)	69–207	<0.0001
HbA1c (%)	5.6 (5.4–5.8)	4.7–9.6	5.7 (5.5–5.9)	4.8–9.9	<0.0001

Note. Data are expressed as median (interquartile range [IQR]), range, or number. Abbreviations: N/A, not applicable; BMI, Body mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HDL cholesterol, high-density lipoprotein cholesterol; LDL cholesterol, low-density lipoprotein cholesterol; ALP, alkaline phosphatase; GGT, gamma-glutamyl transpeptidase; BUN, blood urea nitrogen; eGFR, estimated glomerular filtration rate; CRP, C-reactive protein ; HbA1c, hemoglobin A1c.

In this study, higher BMI and no exercise habits were identified as factors associated with fatty liver. Ok et al. reported that exercise alone without dietary change leads to improvement of NAFLD without weight loss in high-fat diet-induced obese mice [36]. Oh et al. reported that exercise attenuates the degree of hepatic steatosis independent of weight reduction in patients with NAFLD [37]. In a meta-analysis, we previously demonstrated that approximately 50% of patients showed an improvement of in NAFLD without weight reduction by exercise (40–45 min/session and 3 times/week) [11]. Although the duration and frequency of exercise for improvement of NAFLD remains unclear, these findings suggest that exercise may have specific mechanisms for lipid metabolism besides exercise-related weight reduction. Recently, exercise has been reported to improve NAFLD by up-regulating peroxisome proliferator-activated receptor-γ and genes involved in the beta-oxidation of fatty acids in a mouse model of NAFLD [38]. Moreover, exercise is reported to decrease hepatic fatty acid-binding protein 1 expression, leading to up-regulation of autophagic flux via restoring lysosomal function in a high-fat diet mouse model [39]. Thus, exercise may directly enhance beta-oxidation and autophagic clearance and subsequently alleviate hepatic steatosis.

We demonstrated that age, male sex, and BMI were independent factors for significant hepatic fibrosis, which is consistent with previous reports [40-42]. In addition, we revealed that exercise habit was an independent factor for significant hepatic fibrosis. The association between exercise and hepatic fibrosis remains controversial. Previous studies reported that exercise has no effect on improvement of hepatic fibrosis [43,44], which was not consistent with our results. A possible reason for this discrepancy is the duration of the exercise habit. Both studies evaluated the efficacy of a 12-week exercise program [43,44], which may be insufficient for analyzing the association. On the other hand, long-term lifestyle interventions including exercise are reported to reduce the NAFLD-fibrosis score [45]. In our study, an exercise habit was defined as more than 1 year of exercise. Thus, a long period of exercise may improve hepatic fibrosis in health check-up examinees.

Although mechanisms for exercise-caused improvement of hepatic fibrosis remain unclear, several possibilities exist. First, insulin resistance is known to promote hepatic fibrosis through induction of lysyl oxidase-like 2, which leads to matrix stabilization [46]. Meanwhile, exercise is known to reduce insulin resistance through up-regulation of hepatic peroxisome proliferator-activated receptors (PPAR)-γ in a mouse model of NAFLD [38]. Second, hepatic fibrosis can be caused by apoptosis of hepatic stellate cells, which is induced by cysteine-rich angiogenic protein 61-related endoplasmic reticulum stress [47]. On the other hand, exercise, but not food restriction, significantly decreased hepatic expression of cysteine-rich angiogenic protein 61 and improved hepatic fibrosis in a rat model of NAFLD [14]. Third, irisin, a myokine, has been recently reported to be associated with hepatic steatosis as well as fibrosis in patients with NAFLD [48,49]. Meanwhile, exercise increases circulating irisin levels in individuals with metabolic syndrome as well as in healthy individuals [50]. Thus, exercise may improve hepatic fibrosis through regulation of hepatic insulin resistance and decrease of endoplasmic reticulum stress as well as myokine secretion from muscle cells.

TABLE 3.

Comparison of characteristic between the Normal-Mild and the Significant hepatic fibrosis groups

	Normal-Mild hepatic fibrosis		Significant hepatic fibrosis		
	Median (IQR)	Range (min–max)	Median (IQR)	Range (min–max)	P
Liver stiffness (median; kPa)	4.61 (3.90–5.39)	2.59–6.48	9.83 (7.78–14.06)	6.57–47.76	<0.0001
SWE (IQR)	0.09 (0.05–0.15)	0–0.30	0.16 (0.11–0.23)	0.01–0.03	<0.0001
Age (years)	51 (43–60)	23–80	56 (49–64)	28–89	<0.0001
Sex (female/male)	62.2%/37.8% (485/295)	N/A	56.4%/43.6% (328/253)	N/A	0.0332
BMI (kg/m^2)	21.2 (19.4–23.2)	14.5–35.0	24.1 (21.6–26.3)	15.0–42.1	<0.0001
Presence of fatty liver evaluated by ultrasonography (Yes/No)	40.9%/59.1% (319/461)	N/A	63.3%/36.7% (368/213)	N/A	<0.0001
Systolic blood pressure (mmHg)	110 (100–121)	72–174	119 (110–130)	79–174	<0.0001
Diastolic blood pressure (mmHg)	69 (62–77)	43–110	75 (68–82)	47–121	<0.0001
Brinkmann index (Pack-years)	0 (0–10)	0.0–80	0 (0–15)	0–80	0.0199
Alcohol consumption (g/day) (None/<20/20-59)	51.4%/21.0%/27.6% (401/164/215)	N/A	49.7%/22.0%/28.2% (289/128/164)	N/A	0.8219
Exercise for more than 30 minutes at least twice/week (Yes/No)	22.7%/77.3% (177/603)	N/A	20.1%/79.9% (117/464)	N/A	0.2573
Skipping breakfast more than 3 times a week (Yes/No)	14.9%/85.1% (116/664)	N/A	12.1%/87.9% (70/511)	N/A	0.1336
Eating snacks after supper more than 3 times a week (Yes/No)	3.5%/96.5% (27/753)	N/A	2.4%/97.6% (14/567)	N/A	0.2615
Red blood cell count ($\times 10^4/\mu$L)	448 (422–483)	336–590	461 (431–492)	251–592	<0.0001
Hemoglobin (g/dL)	13.6 (12.7–14.7)	7.2–18.7	14.0 (13.0–15.1)	7.1–18.5	<0.0001
Hematocrit (%)	40.6 (38.1–43.4)	26.7–56.2	41.4 (38.9–44.3)	26.0–54.8	<0.0001
White blood cell count (/μL)	49 (41–58)	19–129	52 (44–64)	26–153	<0.0001
Platelet count ($\times$ 10^3/mm^3)	24.0 (20.8–27.8)	5.5–47.4	24.2 (20.8–27.6)	11.6–75.0	0.9066
AST (U/L)	19 (16–23)	7–68	19 (17–24)	10–113	0.0003
ALT (U/L)	16 (12–23)	4–104	18 (14–26)	4–188	<0.0001
HDL cholesterol (mg/dL)	69 (58–81)	29–132	63 (51–75)	28–161	<0.0001
LDL cholesterol (mg/dL)	117 (100–135)	56–240	122 (102–143)	47–254	0.0036
Amylase (U/L)	75 (60–91)	29–271	71 (56–88)	25–298	0.0113
Lactate dehydrogenase (U/L)	166 (148–187)	65–292	174 (156–195)	65–317	<0.0001
ALP (U/L)	189 (156–230)	54–705	208 (169–249)	87–445	<0.0001
GGT (U/L)	19 (14–31)	7–326	24 (16–44)	8–681	<0.0001
Total protein (g/dL)	7.0 (6.8–7.3)	6.0–8.6	7.1 (6.8–7.3)	6.0–8.8	0.5830
Cholinesterase (U/L)	314 (273–365)	110–672	338 (293–388)	194–839	<0.0001
Albumin (g/dL)	4.4 (4.2–4.5)	3.5–5.1	4.3 (4.2–4.5)	3.5–5.2	0.0110
Total bilirubin (mg/dL)	0.7 (0.6–0.9)	0.3–2.6	0.7 (0.6–0.9)	0.3–4.6	0.9411
FIB-4 index	0.98 (0.72–1.35)	0.3–3.6	1.05 (0.77–1.42)	0.27–5.01	0.0058
Total cholesterol (mg/dL)	205 (185–228)	118–339	205 (186–232)	131–351	0.3597
Triglycerides (mg/dL)	76 (57–106)	22–652	97 (68–135)	28–1000	<0.0001

BUN (mg/dL)	13.4 (11.1–15.7)	5.4–37.5	13.6 (11.6–16.0)	5.2–28.6	0.0545
Creatinine (mg/dL)	0.65 (0.56–0.77)	0.38–2.00	0.67 (0.57–0.79)	0.37–1.52	0.1266
eGFR (mL/min/1.73 m^2)	82.0 (73.4–92.5)	26.4–139.6	80.0 (70.4–89.6)	37.5–154.3	0.0057
CRP (mg/dL)	0.03 (0.02–0.06)	0.01–3.71	0.05 (0.02–0.10)	0.01–4.52	<0.0001
Uric acid (mg/dL)	4.8 (3.9–5.7)	1.5–9.2	5.3 (4.4–6.2)	0.8–11.8	<0.0001
Sodium (mmol/L)	142 (141–143)	129–149	142 (141–144)	137–147	0.1064
Potassium (mmol/L)	4.2 (4.0–4.4)	3.4–5.2	4.1 (4.0–4.4)	3.1–5.3	0.9309
Chloride (mmol/L)	106 (104–107)	93–112	106 (104–107)	100–112	0.1799
Fasting plasma glucose (mg/dL)	94 (89–100)	65–215	98 (92–105)	75–214	<0.0001
HbA1c (%)	5.6 (5.4–5.8)	4.7–8.5	5.7 (5.5–5.9)	4.8–9.9	<0.0001

Note. Data are expressed as median (interquartile range [IQR]), range, or number. Abbreviations: N/A, not applicable; SWE, shear wave elastography; BMI, Body mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HDL cholesterol, high-density lipoprotein cholesterol; LDL cholesterol, low-density lipoprotein cholesterol; ALP, alkaline phosphatase; GGT, gamma-glutamyl transpeptidase; BUN, blood urea nitrogen; eGFR, estimated glomerular filtration rate; CRP, C-reactive protein; HbA1c, hemoglobin A1c.

TABLE 4.
Independent factors associated with fatty liver

Factors	Unit	Odds ratio	95% Confidence interval	P value
BMI	1	1.466	1.397–1.537	<0.0001
Exercise habits (No)	N/A	1.478	1.101-1.984	0.0093

TABLE 5.
Independent factors associated with significant hepatic fibrosis

Factors	Unit	Odds ratio	95% Confidence interval	P value
Age	1	1.046	1.034–1.058	<0.0001
Sex (Male)	N/A	1.541	1.181–2.012	0.0014
BMI	1	1.370	1.311–1.436	<0.0001
Exercise habits (No)	N/A	1.496	1.102–2.031	0.0097

It remains unclear if amelioration of fatty liver and significant hepatic fibrosis improves prognosis in the general population. However, Adams et al. examined the natural history of NAFLD and reported that mortality among community-diagnosed patients with NAFLD is higher than in the general population [51]. Hagström et al. also investigated the long-term prognosis of a large cohort of patients with NAFLD and reported that significant hepatic fibrosis is associated with an increase in mortality [52]. These data suggest that amelioration of fatty liver and significant hepatic fibrosis may improve prognosis of patients with NAFLD.

This study has some limitations. First, this was a single center study, and it remains unclear if our findings are applicable in other areas. Second, hepatic fibrosis was not evaluated by liver histology because invasive examination was not always ethically justified in all subjects. Third, the prevalence of significant hepatic fibrosis depends on the threshold of SWE. Although we used a threshold based on the manufacture's technical data, SWE is a relatively new modality; therefore, the optimal threshold has not been established yet. Fourth, we have no data about kind of exercise in our study, although exercise habit was an independent factor associated with fatty liver and significant fibrosis. Thus, a multi-center prospective study is required to clarify in detail the causative factors for fatty liver and hepatic fibrosis.

In conclusion, we found that fatty liver and sig-

nificant fibrosis were frequently seen in health check-up examinees using B-mode ultrasound imaging and 2D-SWE, respectively, and that independent factors were a higher BMI and no exercise habits. Thus, weight loss and exercise may ameliorate fatty liver as well as significant hepatic fibrosis in the general population.

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