Impact of fatty liver disease and metabolic syndrome on incident type 2 diabetes; a population based cohort study

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Abstract. Fatty liver disease and metabolic syndrome (MetS) are both shown to increase the risk of type 2 diabetes. The aim of this study was to investigate the combined effect of fatty liver and MetS on incident diabetes. In this cohort study of 17,810 participants, fatty liver was diagnosed by abdominal ultrasonography and MetS was defined by a joint interim statement. We divided the participants into four groups according to the presence of fatty liver and/or MetS. Type 2 diabetes was defined as HbA1c ≥6.5%, fasting plasma glucose ≥7.0 mmol/L or treatment for diabetes. During the follow up examination (median 5.1 years), 804 participants developed diabetes. Compared with non-MetS without fatty liver, hazard ratios (HR) for incident diabetes after adjusting for age, body mass index, smoking status, exercise habit, alcohol consumption, family history of diabetes logarithm of alanine aminotransferase and fasting plasma glucose, were as follow: 2.35 (95% CI 1.91-2.89, p<0.001) in non-MetS with fatty liver, 1.70 (95% CI 1.30-2.20, p<0.001) in MetS without fatty liver, and 2.33 (95% CI 1.85-2.94, p<0.001) in MetS with fatty liver. In addition, adjusted HRs for incident diabetes compared with MetS without fatty liver were 1.39 (95% CI 1.07-1.80, p=0.012) in non-MetS with fatty liver and 1.38 (95% CI 1.07–1.79, p=0.013) in MetS with fatty liver. Fatty liver affects more on the risk of incident diabetes than MetS. To prevent the further risk of diabetes, we should pay more attention to fatty liver.

Key words: Obesity, Epidemiology, Metabolic syndrome, Nonalcoholic fatty liver disease, Diabetes
The purpose of a medical health checkup program and the detailed characteristics of participants were described previously [5]. We called this longitudinal analysis using with this database as NAGALA (NAfld in Gifu Area, Longitudinal Analysis) study. In this study, we included the result of the individuals who received the health checkup programs from 2004 to 2015. We excluded the participants who did not receive the repeated health-checkup programs. We also excluded the participants who had diabetes mellitus at baseline examination, known liver disease and missing data of covariates. Known liver disease was included tested positive for hepatitis B antigen or hepatitis C antibody and a history of known liver disease, including viral, genetic, autoimmune and drug-induced liver disease [13]. The study was approved by the ethics committee of Murakami Memorial Hospital and was conducted in accordance with the Declaration of Helsinki and informed consents was obtained from all participants [5].

**Date collection and measurements**

Body mass index (BMI) was calculated as body weight in kilograms divided by the square of the participant’s height in meters. The medical history and lifestyle factors of participant, including physical activity and habits pertaining to smoking and alcohol consumption, were surveyed by a standardized self-administered questionnaire. Hemoglobin A1c (HbA1c) was assayed using high-performance liquid chromatography and was expressed in National Glycohemoglobin Standardization Program (NGSP) units. Diagnosis of type 2 diabetes was made according to the American Diabetes Association (ADA) criteria of self-reported clinician-diagnosed diabetes, a fasting plasma glucose level of ≥7.0 mmol/l, or HbA1c ≥6.5% [14] or as the initiation of diabetes treatment.

**Standardized questionnaire for lifestyle factors**

A standardized questionnaire was administered to all participants by the same trained team of interviewers [5]. Alcohol consumption was evaluated by the amount and type of alcoholic beverages consumed per week during the past month, then estimating the mean ethanol intake per week. The total amount of alcohol consumed per week was calculated in grams and then we categorized participants into the four groups: non or minimal alcohol consumption, <40 g/wk; light alcohol consumption, 40–140 g/wk; moderate alcohol consumption, 140–280 g/wk; or heavy alcohol consumption, >280 g/wk [15,16]. Smoking status was also categorized into three groups (never smoker, ex-smoker and current smoker). On the questionnaire, participants reported the type, duration and frequency of their participation in sports or recreational activities [17]. When participants performed any kind of sports at least once a week regularly, we categorized them as regular exercisers [18].

**Definition of metabolic syndrome**

The diagnosis of MetS was determined by a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; the National Heart, Lung and Blood Institute; the American Heart Association; the World Heart Federation; the International Atherosclerosis Society; and the International Association for the Study of Obesity, using the criteria for Asians [19]. The participants were diagnosed with the presence of MetS when three or more of the following criteria were present: hypertension (systolic blood pressure ≥130 mmHg and diastolic blood pressure ≥85 mmHg in both sexes); hyperglycemia (fasting plasma glucose ≥5.6 mmol/L in both sexes); hypertriglyceridemia (serum triglycerides ≥1.70 mmol/L in both sexes); low HDL cholesterol levels (serum HDL cholesterol <1.03 mmol/L in men and <1.29 mmol/L in women); and abdominal obesity (waist circumference ≥90 cm in men and ≥80 cm in women).

**Definition of fatty liver**

Fatty liver was diagnosed by the results of abdominal ultrasonography, which was done by trained technicians. Gastroenterologists checked the images and made the diagnosis of fatty liver without reference to any of the participant’s other individual data. Of four known criteria (hepatorenal echo contrast, liver brightness, deep attenuation and vascular blurring), the participants were required to have hepatorenal contrast and liver brightness to be given a diagnosis of fatty liver [20].

**Statistical analysis**

The statistical analysis was performed using the JMP software version 10.0 software (SAS Institute Inc., Cary, North Carolina) and p value <0.05 was considered to represent a statistically significant difference. Continuous variables were expressed as mean (SD) and categorical variables were expressed as number
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as potential covariates: age, BMI, smoking status, exercise habit, family history of diabetes and fasting plasma glucose at baseline examination. Moreover, we also performed subgroup analyses in men and women, separately and in lean population (BMI < 22kg/m²).

Results

From Jan 1st in 2004 to Dec 31st in 2015, 27,941 participants were enrolled in this cohort (Fig. 1). Among them, 10,130 participants were excluded. Thus, 17,810 participants were eligible for this cohort study. Baseline characteristics of participants are shown in Table 1. Among 17,810 participants, 21.6% (3,846 participants) had fatty liver at baseline examination. On the other hand, 11.3% (2,016 participants) had MetS at baseline examination. Among 2,016 participants who had MetS at baseline examination, 65.4% (1,318 participants) had fatty liver.

During the follow up examination (median 5.1 years), 804 participants developed diabetes. The incident proportion of diabetes was 1.7% (case/N = 226/13,266) in non-MetS without fatty liver, 8.3% (211/2,528) in non-MetS with fatty liver, 12.5% (87/611) in MetS without fatty liver and 21.2% (280/1,318) in MetS with fatty liver.

Hazard ratio (HR) of the four groups for incident type 2 diabetes was calculated by cox regression analyses. The following variables were analyzed as potential covariates [21,22]: age, BMI, smoking status, exercise habit, alcohol consumption, family history of diabetes and log ALT at baseline examination in this cohort study. In addition, we also added and fasting plasma glucose level, because fasting plasma glucose level has a close association with incident diabetes [21].

Furthermore, we performed subgroup analysis in non to minimal drinker, light-moderate drinker and heavy drinker. The following variables were analyzed

Fig. 1 Inclusion and exclusion flow chart.

NAGALA, NAfld in Gifu Area, Longitudinal Analysis.
(log rank test, p<0.001 for all six comparisons) (Fig. 2).

Adjusted HRs for incident diabetes compared with non-MetS without fatty liver were as follow: 2.35 (95% CI 1.91–2.89, p<0.001) in non-MetS with fatty liver, 1.70 (95% CI 1.30–2.20, p<0.001) in MetS without fatty liver and 2.33 (95% CI 1.85–2.94, p<0.001) in MetS with fatty liver (Table 2). In addition, adjusted HRs for incident diabetes compared with MetS without fatty liver were as follow: 1.39 (95% CI 1.07–1.80, p=0.012) in non-MetS with fatty liver and 1.38 (95% CI 1.07–1.79, p=0.013) in MetS with fatty liver.

In addition, we also investigated the impact of the presence of fatty liver and/or MetS on incident diabetes according to the alcohol consumption (Table 3). Adjusted HRs for incident diabetes compared with non-MetS without fatty liver were as follow: 2.60 (95% CI 2.00–3.40, p<0.001) in non-MetS with fatty liver, 1.36 (95% CI 0.90–2.01, p=0.140) in MetS without fatty liver and 2.81 (95% CI 2.08–3.80, p<0.001) in MetS with fatty liver among non or minimal alcohol drinker. Adjusted HRs for incident diabetes compared with MetS without fatty liver were as follow: 1.92 (95% CI 1.24–2.94, p<0.001) in non-MetS with fatty liver, 1.60 (95% CI 0.96–2.56, p =0.068) in MetS without fatty liver and 1.63 (95% CI 1.01–2.59, p=0.044) in MetS with fatty liver among light-moderate alcohol drinker. Adjusted HRs for incident diabetes compared with non-MetS without fatty liver were as follow: 2.83 (95% CI 1.60–4.94, p<0.001) in non-MetS with fatty liver, 3.00 (95% CI 1.76–5.10, p<0.001) in MetS without fatty liver and 2.64 (95% CI 1.43–4.84, p<0.002) in MetS with fatty liver among heavy alcohol drinker.

We also investigated the men and women, separately. The proportions of incident diabetes were 6.4% (case/n = 668/10,445) in men and 1.9% (136/7,365) in women.

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**Table 1 Characteristics of study participants according to the presence of fatty liver and/or metabolic syndrome**

<table>
<thead>
<tr>
<th></th>
<th>Non-MetS without fatty liver</th>
<th>Non-MetS with fatty liver</th>
<th>MetS without fatty liver</th>
<th>MetS with fatty liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>13,266</td>
<td>2,528</td>
<td>698</td>
<td>1,318</td>
</tr>
<tr>
<td>Men §</td>
<td>50.8% (6,734)</td>
<td>83.2% (2,102)</td>
<td>72.6% (507)</td>
<td>83.6% (1,102)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>44.5 (9.4)</td>
<td>46.3 (8.8)</td>
<td>50.1 (9.3)*†</td>
<td>47.6 (8.7)**‡</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>21.4 (2.5)</td>
<td>24.8 (2.8)*</td>
<td>25.0 (2.8)*</td>
<td>27.2 (3.2)*†‡</td>
</tr>
<tr>
<td>Waist circumstance (cm)</td>
<td>74.6 (7.8)</td>
<td>84.5 (6.8)*</td>
<td>85.9 (7.2)*</td>
<td>91.3 (7.8)***‡‡</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>113.5 (14.8)</td>
<td>121.8 (13.6)*</td>
<td>132.7 (14.8)**‡</td>
<td>134.3 (14.9)**‡</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>71.0 (10.3)</td>
<td>77.0 (9.4)*</td>
<td>84.0 (10.0)**‡</td>
<td>85.0 (9.8)**‡</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/L)</td>
<td>5.1 (0.5)</td>
<td>5.4 (0.5)*</td>
<td>5.7 (0.5)*†</td>
<td>5.8 (0.5)**‡</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.1 (0.3)</td>
<td>5.3 (0.4)*</td>
<td>5.3 (0.4)*†</td>
<td>5.5 (0.4)**‡</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.8 (0.5)</td>
<td>1.3 (0.7)*</td>
<td>1.9 (1.2)*†</td>
<td>2.0 (1.2)*‡</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.5 (0.4)</td>
<td>1.3 (0.3)</td>
<td>1.1 (0.3)*†</td>
<td>1.1 (0.2)*‡</td>
</tr>
<tr>
<td>Aspartate transaminase (IU/L)</td>
<td>17.8 (8.9)</td>
<td>22.1 (9.5)*</td>
<td>20.7 (11.0)**‡</td>
<td>25.4 (12.0)**‡‡</td>
</tr>
<tr>
<td>Alanine aminotransferase (IU/L)</td>
<td>17.5 (11.4)</td>
<td>30.6 (17.6)*‡</td>
<td>23.2 (12.7)***†</td>
<td>37.2 (22.0)**‡‡</td>
</tr>
<tr>
<td>gamma-glutamyl transferase (IU/L)</td>
<td>19.3 (19.0)</td>
<td>30.3 (29.4)*‡</td>
<td>34.6 (40.0)***‡</td>
<td>37.7 (33.4)**‡‡</td>
</tr>
</tbody>
</table>

**Smoking status §**

| Ex-smoker              | 19.2% (2,553)               | 30.2% (764)              | 27.8% (194)              | 31.0% (408)           |
| Current-smoker         | 22.3% (2,955)               | 27.5% (694)              | 32.1% (224)              | 30.6% (403)           |
| Exerciser §            | 18.7% (2,483)               | 16.1% (408)              | 18.6 (130)               | 13.5% (178)           |

**Alcohol consumption (none to minimal/light/moderate/heavy) §**

| Light                  | 11.5% (1,523)               | 9.7% (246)               | 14.3% (100)              | 12.2% (161)           |
| Moderate               | 9.2% (1,218)                | 9.9% (250)               | 11.9% (83)               | 10.6% (139)           |
| Heavy                  | 6.6% (880)                  | 8.4% (212)               | 18.2% (127)              | 10.8% (142)           |
| Family history of diabetes | 10.9% (1,451)             | 9.0% (228)               | 16.9% (118)              | 10.6% (140)           |

MetS, Metabolic syndrome; HDL, High-density lipoprotein. Data are % (number) or mean (standard deviation). The analyses of continuous variables among four groups were performed by Tukey HSD test.

*, vs. Non-MetS without fatty liver; †, vs. Non-MetS with fatty liver; ‡, vs. MetS without fatty liver. The analyses of categorical variables among four groups were performed by χ² test. § p<0.05.
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Fig. 2 Kaplan-Meier Analysis of incident diabetes according to the presence of fatty liver and/or metabolic syndrome. The vertical axis is cumulative incidence of diabetes and the horizontal axis is time as days. The black line represents the non-MetS without fatty liver. The red line represents non-MetS without fatty liver. The blue line represents the MetS without fatty liver. The green line represents MetS with fatty liver. The cumulative incidence of diabetes was statistically different among groups (log rank test, \( p < 0.001 \) for all six comparisons).

Table 2 Hazard ratio for incident type 2 diabetes according to the presence of fatty liver and/or metabolic syndrome

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th>( p ) value</th>
<th>Model 2</th>
<th>( p ) value</th>
<th>Model 3</th>
<th>( p ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-MetS without fatty liver</td>
<td>1 (Reference)</td>
<td>–</td>
<td>1 (Reference)</td>
<td>–</td>
<td>1 (Reference)</td>
<td>–</td>
</tr>
<tr>
<td>Non-MetS with fatty liver</td>
<td>4.10 (3.38-4.97)</td>
<td>(&lt;0.001)</td>
<td>3.19 (2.60-3.92)</td>
<td>(&lt;0.001)</td>
<td>2.35 (1.91-2.89)</td>
<td>(&lt;0.001)</td>
</tr>
<tr>
<td>MetS without fatty liver</td>
<td>5.81 (4.50-7.44)</td>
<td>(&lt;0.001)</td>
<td>4.14 (3.17-5.37)</td>
<td>(&lt;0.001)</td>
<td>1.70 (1.30-2.20)</td>
<td>(&lt;0.001)</td>
</tr>
<tr>
<td>MetS with fatty liver</td>
<td>11.0 (9.16-13.1)</td>
<td>(&lt;0.001)</td>
<td>6.80 (5.45-8.49)</td>
<td>(&lt;0.001)</td>
<td>2.33 (1.85-2.94)</td>
<td>(&lt;0.001)</td>
</tr>
<tr>
<td>Men</td>
<td>1.79 (1.48-2.17)</td>
<td>(&lt;0.001)</td>
<td>1.63 (1.31-2.04)</td>
<td>(&lt;0.001)</td>
<td>0.80 (0.64-1.01)</td>
<td>0.063</td>
</tr>
<tr>
<td>Age (per one year)</td>
<td>1.05 (1.04-1.06)</td>
<td>(&lt;0.001)</td>
<td>1.06 (1.05-1.07)</td>
<td>(&lt;0.001)</td>
<td>1.04 (1.03-1.05)</td>
<td>(&lt;0.001)</td>
</tr>
<tr>
<td>Body mass index (kg/m(^2))</td>
<td>–</td>
<td>–</td>
<td>1.09 (1.07-1.12)</td>
<td>(&lt;0.001)</td>
<td>1.06 (1.03-1.08)</td>
<td>(&lt;0.001)</td>
</tr>
<tr>
<td>Habit of exercise</td>
<td>–</td>
<td>–</td>
<td>0.74 (0.60-0.91)</td>
<td>0.004</td>
<td>0.72 (0.58-0.88)</td>
<td>0.001</td>
</tr>
<tr>
<td>Ex-smoker</td>
<td>–</td>
<td>–</td>
<td>0.97 (0.74-1.26)</td>
<td>0.802</td>
<td>0.94 (0.77-1.14)</td>
<td>0.540</td>
</tr>
<tr>
<td>Current smoker</td>
<td>–</td>
<td>–</td>
<td>1.46 (1.02-2.09)</td>
<td>0.036</td>
<td>1.55 (1.29-1.87)</td>
<td>(&lt;0.001)</td>
</tr>
<tr>
<td>Light alcohol consumption</td>
<td>–</td>
<td>–</td>
<td>0.89 (0.71-1.10)</td>
<td>0.284</td>
<td>0.83 (0.66-1.03)</td>
<td>0.094</td>
</tr>
<tr>
<td>Moderate alcohol consumption</td>
<td>–</td>
<td>–</td>
<td>0.95 (0.75-1.20)</td>
<td>0.686</td>
<td>0.74 (0.59-0.93)</td>
<td>0.011</td>
</tr>
<tr>
<td>Heavy alcohol consumption</td>
<td>–</td>
<td>–</td>
<td>1.39 (1.12-1.72)</td>
<td>0.003</td>
<td>1.00 (0.81-1.24)</td>
<td>0.976</td>
</tr>
<tr>
<td>Log aminotransferase (per 1 SD increase)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1.23 (1.05-1.43)</td>
<td>0.009</td>
</tr>
<tr>
<td>FPG (per 0.1 mmol/L)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1.27 (1.26-1.29)</td>
<td>(&lt;0.001)</td>
</tr>
<tr>
<td>Family history of diabetes</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.96 (0.77-1.20)</td>
<td>0.724</td>
</tr>
</tbody>
</table>

MetS, Metabolic syndrome; FPG, Fasting plasma glucose. Model 1 adjusted for age and body mass index. Model 2 adjusted for Model 1 and habit of exercise, smoking status and alcohol consumption. Model 3 adjusted for Model 2 and FPG at baseline examination. Ex- and current smoker were used with never smoker as a reference group. Light, moderate and heavy alcohol consumption were used none to minimal alcohol consumption as a reference group.
On the other hand, the proportions of incident diabetes were 2.6% (case/n = 175/6,734) in men and 0.8% (case/n = 51/6,532) in women in non-MetS without fatty liver, 8.4% (176/2,102) in men and 8.2% (35/426) in women in non-MetS with fatty liver, 15.0% (76/507) in men and 5.8% (11/191) in women in MetS without fatty liver and 21.9% (241/1,102) in men and 28.7% (39/216) in women in MetS with fatty liver. Compared with non-MetS without fatty liver, adjusted hazard ratios (HR) for incident diabetes in men were as follow: 2.10 (95% CI 1.68–2.63, \(p<0.001\)) in non-MetS with fatty liver, 1.77 (95% CI 1.33–2.34, \(p<0.001\)) in MetS without fatty liver and 2.33 (95% CI 1.82–3.00, \(p<0.001\)) in MetS with fatty liver and adjusted hazard ratios (HR) for incident diabetes in women were as follows: 4.34 (95% CI 2.67–7.01, \(p<0.001\)) in non-MetS with fatty liver, 1.34 (95% CI 0.63–2.64, \(p=0.426\)) in MetS without fatty liver, and 2.00 (95% CI 1.10–3.62, \(p=0.024\)) in MetS with fatty liver in women.

In addition, among the lean population (BMI < 22kg/m²), the proportion of incident diabetes were 1.3% (case/n = 104/8,059) in non-MetS without fatty liver, 5.2% (17/327) in non-MetS with fatty liver, 19.0% (18/95) in MetS without fatty liver and 22.2% (10/45) in MetS with fatty liver. Compared with non-MetS without fatty liver, adjusted hazard ratios (HR) for incident diabetes were as follow: 1.67 (95% CI 0.93–2.85, \(p=0.085\)) in non-MetS with fatty liver, 1.71 (95% CI 0.92–3.01, \(p=0.086\)) in MetS without fatty liver, and 2.07 (95% CI 0.98–3.97, \(p=0.056\)) in MetS with fatty liver.

### Discussion

In this study, we showed that the group of MetS with fatty liver and non-MetS with fatty liver had the higher incidence rate of type 2 diabetes. To our surprise, the non-MetS with fatty liver group had a greater risk of incident type 2 diabetes than MetS without fatty liver group, after adjusting for covariates. These results suggested that fatty liver had a greater impact on incident type 2 diabetes than MetS.

A previous study reported that the individuals with insulin resistance, overweight/obesity and fatty liver, increase the risk of incident type 2 diabetes [23] and that combination of insulin resistance and fatty liver has a close association with incident type 2 diabetes [23]. In addition, we previously reported that non-overweight with NAFLD has higher risk of incident diabetes than overweight/obesity without NAFLD [24]. Moreover, recent studies revealed that metabolic syndrome individuals with NAFLD have higher risk of incident diabetes than those without NAFLD [25,26]. These studies showed that NAFLD would be a major risk factor for incident type 2 diabetes than overweight/obesity.

It is well known that NAFLD is a hepatic component of MetS [5]. In fact, 65% of individuals with MetS accompanied with fatty liver in this study population. Both NAFLD [27] and MetS [28] are associated with insulin resistance. Visceral fat secretes inflammatory adipokines, such as interleukin-6, tumor necrosis factor-α and macrophage chemoat-
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tractant protein-1, which leads to insulin resistance [29]. It has been reported that accumulating a high amount of visceral fat leads to type 2 diabetes [30]. On the other hand, NAFLD has a close association with ectopic fat [31]. Ectopic fat, which has close association with organ-specific insulin resistance via a process termed ‘lipotoxicity’ [32], is one of the major causes of type 2 diabetes [27]. The impaired subcutaneous fat storage capacity leads to accumulate fat in ectopic tissues, including liver [33]. Therefore, NAFLD represents fat overflow into secondary deep subcutaneous and visceral fat compartments [34]. In addition, a previous study showed that fat accumulation in liver is more associated with insulin resistance than fat accumulation in adipose tissue [31], because liver is the main insulin sensitive organ and glucose production is charged in liver [35]. Moreover, it was reported that hepatokines, which are released from the liver, are directly associated with incident type 2 diabetes [35]. Hepatokines, such as selenoprotein P and hepatocyte-derived fibrinogen-related protein 1, exacerbate hepatic glucose metabolism and insulin signaling, through increasing of hepatic fat accumulation and activation of inflammatory signaling [36,37]. Thus, NAFLD is more associated with incident type 2 diabetes than MetS. On the other hand, alcoholic fatty liver disease is also associates with diabetes. Previous studies revealed that fatty liver occurs in almost all individuals with excessive alcohol consumption [38]. The pathogenesis of fatty liver in alcohol consumption is that ethanol metabolism reduced nicotinamide adenine dinucleotide for use in clinical settings. Moreover, ultrasonography has reasonable noninvasive surrogate measure for use in clinical settings. Furthermore, alcohol consumption is a risk of incident type 2 diabetes [39]. These might be the reason why the non-MetS with fatty liver group had a greater risk of incident type 2 diabetes than MetS without fatty liver group.

Because liver function test is more common than ultrasonography, we defined fatty liver as ALT >35 IU/L or AST >40 IU/L [40]. The proportion of fatty liver by liver function test was 9.5% (1,692/17,810), and 69.3% of fatty liver, which was diagnosed by ultrasonography, were overlooked by serum liver function test. In fact, previous studies revealed that although transaminases levels are raised in most cases, some of fatty liver may exist without elevation of transaminases [41,42]. Thus, using ultrasonography is important to not overlook fatty liver.

In this study, although liver fibrosis and cirrhosis are strongly affect the glucose metabolism, we did not perform liver biopsy, thus we cannot diagnose liver fibrosis or cirrhosis. We used fib-4 index [43] as a marker for liver fibrosis. Among 3,846 participants with fatty liver disease, 9.2% (355 participants) were indeterminate range (FIB-4 index 1.30-2.67) and 0.3% (13 participants) were high-risk (FIB-4 index >2.67). Thus, few participants may be liver fibrosis and cirrhosis.

Some limitations of our study should be noted. First, we did not perform liver biopsy, which is the gold standard for accurate diagnosis of fatty liver. However, it is impossible to perform liver biopsy in such a large number of apparently healthy participants, and ultrasonography has reasonable noninvasive surrogate measure for use in clinical settings. Moreover, ultrasonography had a high sensitivity and specificity in diagnosing fatty liver [20,44]. Second, we did not measure serum insulin levels, so we could not assess the relationship between new onset of type 2 diabetes and baseline insulin resistance in this study. Third, the retrospective design of our study did not negate the possibility of influences by unknown confounding factors on our observations. Fourth, we lacked data on oral glucose tolerance testing for the diagnosis of diabetes. This might underestimate the incidence of diabetes. Fifth, we did not have a data of presence or absence of four criteria (hapato-renal contrast, liver brightness, deep attenuation, and vascular blurring) which was used for the definition of fatty liver. Thus, we cannot investigated the association between four criteria and incident diabetes. Sixth, the abdominal ultrasonography machines were changed during the follow-up duration. Thus, we cannot deny the possibility that change of abdominal ultrasonography machine may affect the sensitivity and specificity of the diagnosis of fatty liver. However, although several abdominal ultrasonography machines were used for diagnose fatty liver, diagnosis of fatty liver was performed by the supervision of a liver specialist, using standardization of ultrasonic imaging method and diagnostic criteria. In addition, a previous study also showed that the impact of change of abdominal ultrasonography machine might be small [45]. Seventh, there is a possibility of selection bias, because we excluded the 7,403 participants who did not receive the repeated health check-up in this study. We checked the background difference between excluded group and included group. Age (45.0 (11.3)
vs. 45.2 (9.4), \( p=0.217 \), fasting plasma glucose (5.3 (0.5) vs. 5.3 (0.5), \( p=0.798 \)) and proportion of MetS (8.1% vs. 8.5%, \( p=0.399 \)) were not difference between excluded group and included group, although proportion of men (55.1% vs. 58.7%, \( p<0.001 \)), BMI (22.3 (3.3) vs. 22.5 (3.3), \( p<0.001 \)), proportion of fatty liver (20.0% vs. 21.6%, \( p=0.004 \)) were difference between groups, which might be a selection bias. Finally, the study population consisted of Japanese men and women. Therefore, it is uncertain whether these findings are generalized in other ethnic groups. Despite these limitations, the large sample size and comprehensive database enabled us to accurately examine the impact of fatty liver and MetS on the risk of incident type 2 diabetes in men.

In conclusion, this large-scale cohort study demonstrated that fatty liver affects more on the risk of incident type 2 diabetes than MetS. Thus, to prevent the further risk of incident type 2 diabetes, we should pay more attention to fatty liver and it may be advisable to follow the health conditions of such individuals after the detection of fatty liver.

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**Conflicts of Interest**

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**Author’s contributions**

K.M. originated and designed the study, researched data and wrote manuscript. Y.H., T.K., A.O. and T.F. researched data and reviewed the manuscript. M.H. and M.F. researched data and reviewed and edited the manuscript. M.H is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All author were involved in the writing of the manuscript and approved the final version in this article.

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Fatty liver, MetS and diabetes

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