Sex differences in the association between fatty liver and type 2 diabetes incidence in non-obese Japanese: A retrospective cohort study

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ABSTRACT

Aims/Introduction: Asians develop type 2 diabetes at a lower body mass index (BMI) compared with other races, which is partly because of Asian-specific fat depots. Sex plays a role in fat deposition, regardless of race. This retrospective cohort study aimed to investigate the association among fatty liver, sex and type 2 diabetes in non-obese Japanese.

Materials and Methods: The participants in this study (13,596 men and 6,037 women) were aged 30–64 years, and had undergone health checkups between 2013 and 2015, in Aichi, Japan. Baseline BMI was categorized as follows: <18.5, 18.5–19.9, 20–22.9, 23–24.9, 25–27.4 and ≥27.5 kg/m^2. Fatty liver was diagnosed by abdominal ultrasonography. The joint effect of BMI and fatty liver on the incidence of type 2 diabetes was assessed, stratified by sex.

Results: During follow up, 738 men and 138 women developed type 2 diabetes. Compared with the BMI of 20–22.9 kg/m^2 without fatty liver group, the BMI of 20–22.9 kg/m^2 with fatty liver was associated with a higher risk of type 2 diabetes in men, but not in women. Furthermore, men with a BMI of 23–24.9 and 25–27.4 kg/m^2 without fatty liver had no significant type 2 diabetes risk, whereas women with a BMI of 23–24.9 and 25–27.4 kg/m^2, regardless of fatty liver, had an increased risk.

Conclusions: These results suggest the association between fatty liver and type 2 diabetes in non-obese Asians is different by sex; fatty liver increases diabetes risk among male, not female, non-obese Asians.

INTRODUCTION

Prevention and control of type 2 diabetes among Asians has become an important public health issue due to its increasing prevalence worldwide[1,2]. Type 2 diabetes prevalence among Asian Americans is approximately twice that among non-Hispanic White Americans in the USA[3]. The International Diabetes Federation estimated that there were 231 million cases of diabetes in 2017 in Asian countries, and projected that Asia would have 334 million cases, approximately 53% of all cases worldwide, by 2045[4].

Asians develop type 2 diabetes at a lower body mass index (BMI) partly due to racial differences in body fat distribution; Asians easily develop visceral and ectopic fat obesity, and have a higher percentage of the trunk, visceral or ectopic fat than

white people with a similar BMI or waist circumference[5,6]. Ectopic fat is defined as extra lipids stored in skeletal muscle, the liver and other (e.g., cardiomyocytes) organs, which are locations not originally associated with adipose tissue storage, and causes insulin resistance[7]. An epidemiological study showed that Japanese people with ectopic fat obesity, defined as fatty liver, were at a far higher risk of type 2 diabetes compared with those who had other obesity phenotypes – obesity (BMI ≥25 kg/m^2) and visceral fat (abdominal) obesity[8]. This suggests that fatty liver might be the important factor for type 2 diabetes prevention in non-obese Asians.

Sex plays a role in fat deposition. Sex hormones (i.e., estrogen) regulate fat deposition and, thereby, also cardiometabolic risk, throughout the entire lifespan, and predominantly during puberty and early adulthood[9]. Men typically have more ectopic fat (fatty liver), as well as visceral fat, than women of the same

age and BMI, a feature associated with an increased risk of type 2 diabetes among men[10,11]. However, several cross-sectional studies[12,13], some of which were carried out in Asian countries, suggested that the association of visceral and ectopic fat with diabetes risk among non-obesity was stronger in women than man. For effective type 2 diabetes prevention in non-obese Asian people, longitudinal design research is require to assess these interrelationships. Thus, the aim of the present cohort study was to investigate the associations among fatty liver with the incidence of type 2 diabetes stratified by sex in non-obese Japanese.

MATERIALS AND METHODS

Study population

We used data from the 2013 to 2019 health checkups of a working-age cohort enrolled in the Aichi Health Promotion Study. The Aichi Health Promotion Study is a retrospective cohort study involving participants enrolled in a medical health checkup program at the Aichi Health Promotion Foundation, Aichi, Japan. Participants included full-time and part-time employees, self-employed individuals, and home-based and retired workers who receive health checkups to promote health through the early detection of disease or are required by Japanese law (Industry Safe and Health Act[14]) to receive an annual health examination. The study enrolled 41,460 participants of mean age 47.8 ± 8.5 years (range 30–64 years), consisting of 29.8% women, between April 2013 and March 2015 at baseline, and followed them up until March 2019. The study design was explained to participants through an intranet homepage. Participants were able to choose whether to participate in the study or not through a written consent form. The study protocol was approved by the ethics committee of the Aichi Medical University School of Medicine, and the study was carried out in accordance with the Declaration of Helsinki and the Japanese Government's Ethical Guidelines for Medical and Health Research Involving Human Subjects[15].

Procedures

All participants received annual health checkups, including questionnaires, anthropometric measurements, physiological and radiological examinations, and a blood test. The questionnaire was self-reported and included items about receiving antidiabetic treatment ('Are you taking medication to reduce blood glucose or insulin injection?'[14]), family history, lifestyle habits, and current or past medical history of heart disease and stroke. All blood tests involved measurements of plasma glucose and glycated hemoglobin levels. Fasting plasma glucose (FPG) was defined as plasma glucose levels after at least 10 h of fasting and random plasma glucose as those taken at all other times. Some participants received an abdominal ultrasonography examination, either paying for it out of pocket or the cost was covered by insurance through their employer; a cross-section of workers underwent this examination.

Exposure

BMI was calculated as weight in kilograms divided by squared height in meters. Based on the World Health Organization BMI criteria for Asians[16], we classified participants into six categories according to their baseline BMI: <18.5, 18.5–19.9, 20–22.9, 23–24.9, 25–27.4 and ≥27.5 kg/m^2.

Fatty liver, the proxy index of ectopic fat, was detected using abdominal ultrasonography carried out by trained technicians[8,17]. Participants whose ultrasonograms showed liver contrast and liver brightness from among the four known criteria (hepatorenal echo contrast, liver brightness, deep attenuation and vascular blurring, which has been reported to be 96.4% sensitive and 97.8% specific for detecting hepatic fat 20%[18,19]) were diagnosed as having fatty liver[8,17].

To investigate the role of fatty liver among participants with lower BMI, participants with BMIs of 20–22.9, 23–24.9 and 25–27.5 kg/m^2 were grouped into two subcategories: those with and without fatty liver.

In additional analyses, the role of visceral fat obesity was investigated instead of ectopic fat (Table S2). Visceral fat obesity was defined as waist circumference ≥85 cm in men or ≥90 cm in women[20].

Outcome

Using health checkup data, we identified incident type 2 diabetes. We defined type 2 diabetes as either glycated hemoglobin levels ≥6.5%, random plasma glucose ≥200 mg/dL, FPG ≥126 mg/dL or self-reported receipt of antidiabetic treatment. Participants that met any of the aforementioned conditions during follow up were considered incident type2 diabetes cases[21].

Covariates

Past and current medical history, family history of diabetes, smoking habits, alcohol consumption and physical activity were recorded using a self-report questionnaire[14]. A family history of diabetes was defined as a positive history reported in first-degree relatives. Participants were classified by smoking status into current smokers, past smokers or never smokers. Alcohol drinking was defined as consuming ≥150 g of alcohol per week. Physical activity was defined as exercising >30 min twice or more per week.

Hypertension was defined as either systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg or self-reported receipt of antihypertensive treatment. Dyslipidemia was defined as either high-density lipoprotein cholesterol <40 mg/dL, triglycerides ≥140 mg/dL or self-reported receipt of treatment for dyslipidemia[22].

Statistical analysis

All analyses were carried out separately for male and female participants. Differences in the baseline characteristics of participants by sex were analyzed using a χ^2-test for categorical variables and Student's t-test or Mann–Whitney U-test for

continuous variables. The joint effect of BMI and fatty liver on the incidence of type 2 diabetes was assessed using Cox proportional hazards models; that is, a specific BMI category (BMI of 20–22.9, 23–24.9 and 25–27.4 kg/m^2) with or without fatty liver. Adjusted hazard ratios (HRs) were calculated using the BMI category of 20–22.9 kg/m^2 without fatty liver as the reference group. A three-step analysis was carried out in addition to the crude model (model 0): model 1 was adjusted for age. Model 2 was additionally adjusted for physical activity, smoking status and alcohol consumption to exclude the possibility that BMI was associated with type 2 diabetes because of basic lifestyle habits. Model 3 additionally adjusted for family history of diabetes and medication for hypertension and dyslipidemia, which were considered as potential genetic and medical confounders. Finally, model 4 additionally adjusted for FPG value at baseline to adjust for the effects of baseline diabetes status[19].

We also evaluated the effect of BMI with type 2 diabetes. We calculated three-step (as mentioned above) and multivariable-adjusted HRs, using the BMI category of 20–22.9 kg/m^2. In addition, the joint effect of BMI and visceral fat, instead of fatty liver (ectopic fat), with type 2 diabetes using similar Cox proportional hazards models; that is, a specific BMI category with or without visceral fat obesity. Finally, we evaluated the joint effect of BMI and fatty liver on type 2 diabetes independently of visceral fat using the Cox model additionally adjusted for visceral fat obesity.

Furthermore, we carried out several additional analyses. First, we investigated whether the association of fatty liver with type 2 diabetes in women changed before and after menopause. Menopause was defined as women aged >50 years (the median age of menopause in Japanese[23]), because our data did not include it. We carried out subanalyses in women stratified by age ≤50 years and age >50 years. Second, instead of Japanese criteria (high-density lipoprotein cholesterol <40 mg/dL), we carried out analyses in women using the definition of dyslipidemia in 'metabolic health' proposed by Smiths et al.[24]; that is, high-density lipoprotein cholesterol <50 mg/dL for women.

RESULTS

Of 41,460 participants who received health checkups during the baseline period, we excluded the following participants: 47 participants (32 men and 15 women) whose glucose measurements were not taken at baseline; 1,023 participants (733 men and 290 women) without data on FPG; 2,808 participants (2,484 men and 324 women) who had diabetes at baseline; 228 participants (141 men and 87 women) without waist circumference data; 14,413 participants (10,173 men and 4,246 women) who did not take an abdominal ultrasonography examination; 483 participants (233 men and 250 women) who were undergoing treatment for cancer at baseline; 1,397 participants (1,158 men and 239 women) who had a current or past history of vascular diseases; and 243 participants (149 men and 94 women) who had missing information on covariates including alcohol consumption, exercise and smoking status.

We also excluded 170 female participants that reported being pregnant during follow up and participants who did not attend any subsequent health checkups (2,396 men and 1,194 women). Thus, 19,633 participants (13,596 men and 6,037 women; mean age 47.9 ± 7.9 years; mean BMI 22.9 ± 3.4 kg/m^2) were included in the analyses (Figure S1). Of these, 1,402 (7.1%) participants had BMI <18.5 kg/m^2 (underweight), 2,316 participants (11.8%) had BMI 18.5–19.9 kg/m^2, 7,113 (36.2%) participants had BMI 20–22.9 kg/m^2, 4,238 participants (21.6%) had BMI 23–24.9 kg/m^2, 2,789 (14.2%) participants had 25–27.4 kg/m^2 and 1,775 participants (9.0%) had BMI ≥ 27.5 kg/m^2.

Table 1 shows the characteristics of participants according to sex. The proportions of BMI <18.5, 18.5–19.9 and 20–22.9 kg/m^2 categories were smaller in men than in women, whereas those of BMI 23–24.9, 25–27.4 and ≥27.5 kg/m^2 were larger in men than in women. Fatty liver was diagnosed in 4,131 (30.4%) male and 560 (9.3%) female participants. In all BMI categories, the prevalence of fatty liver was higher in men than in women (Table S1).

During the median 4.8-year follow-up duration for men (interquartile range 3.0–5.0 years) and 4.7-year follow-up duration for women (interquartile range 3.1–5.0 years), 876 participants (738 men and 138 women) were diagnosed with newly incident type 2 diabetes (incidence rates were 13.6 and 5.7 per 1,000 person-years in men and women, respectively). In the analyses, which did not take into account fatty liver (Table S2), participants with a BMI 23–24.9 kg/m^2, as well as BMI 25–27.4 kg/m^2 or more, had an increased risk of type 2 diabetes compared with those with a BMI 20–22.9 kg/m^2 among both men (HR 1.32, 95% confidence interval [CI] 1.05–1.67) and women (HR 2.49, 95% CI 1.53–4.06).

Table 2 shows the combined association of BMI category and fatty liver with type 2 diabetes. The associations between fatty liver and type 2 diabetes in non-obesity differed by sex (Figure 1). Compared with a BMI of 20–22.9 kg/m^2 without fatty liver, men with a BMI of 20–22.9 kg/m^2 with fatty liver had an increased risk of type 2 diabetes (HR 1.57, 95% CI 1.04–2.36), but women with a BMI of 20–22.9 kg/m^2 with fatty liver did not (HR 1.52, 95% CI 0.53–4.40).

Men in the 23–24.9 kg/m^2 BMI categories with fatty liver were associated with a higher risk of type 2 diabetes (HR 2.03, 95% CI 1.52–2.73), although men with a BMI of 23–24.9 kg/m^2 without fatty liver were not (HR 1.11, 95% CI 0.83–1.50). In contrast, women with a BMI of 23–24.9 kg/m^2 both with and without fatty liver had an increased risk of type 2 diabetes (HR 5.07, 95% CI 2.69–9.57 and HR 1.85, 95% CI 1.03–3.35, respectively).

Furthermore, men with a BMI of 25–27.4 kg/m^2 with fatty liver had an increased risk of type 2 diabetes (HR 2.51, 95% CI 1.92–3.27), but men without fatty liver did not (HR 1.19, 95% CI 0.84–1.70). By contrast, a BMI of 25–27.4 kg/m^2, regardless of the presence of ectopic fat, was associated with a higher risk of diabetes in female participants (HR 2.30, 95% CI 1.13–4.66 and HR 2.13, 95% CI 1.10–4.12, respectively).

Table 1 | Characteristics of participants according to sex

	Men	Women	P
No. participants	13,596	6,037	
	Mean (SD)	Mean (SD)	
Age (years)	47.8 (8.0)	48.2 (7.7)	<0.001
Average BMI (kg/m^2)	23.5 (3.2)	21.4 (3.3)	<0.001
Waist circumference (cm)	83.3 (8.6)	76.3 (8.6)	<0.001
Fasting plasma glucose (mg/dL)	98.5 (8.8)	93.0 (8.4)	<0.001
AST (IU/L)	23.1 (10.6)	20.1 (21.0)	<0.001
ALT (IU/L)	27.2 (19.7)	17.0 (12.9)	<0.001
γGTP (IU/L)	46.6 (49.6)	22.4 (24.2)	<0.001
	n (%)	n (%)	
Age categories			
30–39 years	1,853 (13.6%)	791 (13.1%)	0.319
40–49 years	6,141 (45.2%)	2,589 (42.9%)	<0.001
50–59 years	4,255 (31.3%)	2,114 (35.0%)	<0.001
60–64 years	1,347 (9.9%)	543 (9.0%)	0.045
BMI categories			
BMI <18.5 kg/m^2	459 (3.4%)	943 (15.6%)	<0.001
BMI of 18.5–19.9 kg/m^2	1,052 (7.7%)	1,264 (20.9%)	<0.001
BMI of 20–22.9 kg/m^2	4,832 (35.5%)	2,281 (37.8%)	0.003
BMI of 23–24.9 kg/m^2	3,459 (25.4%)	779 (12.9%)	<0.001
BMI of 25–27.4 kg/m^2	2,349 (17.3%)	440 (7.3%)	<0.001
BMI ≥ 27.5 kg/m^2	1,445 (10.6%)	330 (5.5%)	<0.001
Visceral fat obesity	5,646 (41.5%)	422 (7.0%)	<0.001
Fatty liver	4,131 (30.4%)	560 (9.3%)	<0.001
Current smoking	4,923 (36.2%)	546 (9.0%)	<0.001
Past smoking	3,245 (23.9%)	426 (7.1%)	<0.001
Alcohol drinking	4,912 (36.1%)	693 (11.5%)	<0.001
Physical activity	2,507 (18.4%)	933 (15.5%)	<0.001
Medication for hypertension	1,272 (9.4%)	322 (5.3%)	<0.001
Hypertension	3,074 (22.6%)	738 (12.2%)	<0.001
Medication for dyslipidemia	680 (5.0%)	305 (5.1%)	<0.001
Dyslipidemia	3,932 (28.9%)	601 (10.0%)	<0.001
Family history of diabetes mellitus	1,509 (11.1%)	834 (13.8%)	<0.001

γGTP, γ-glutamyl transpeptidase; ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index.

In addition to fatty liver, we further investigated the combined association of BMI category and visceral fat (assessed by measuring waist circumstance)[20] with type 2 diabetes (Table S3; Figure S2). The results showed, almost similar to fatty liver, that BMI 23–24.9 kg/m^2 without visceral fat did not increase the risk of diabetes among men (HR 1.11, 95% CI 0.81–1.53), but did increase risk among women (HR 2.42,; 95% CI 1.46–4.02). Finally, we assessed whether fatty liver was independently associated with type 2 diabetes in non-obese Asians (Table S4). We found almost similar results: a BMI of 23–24.9 kg/m^2 without fatty liver tended to have an increased risk of type 2 diabetes in women (HR 1.77, 95% CI 0.98–3.20), but not in men (HR 1.03, 95% CI 0.75–1.41), although those with fatty liver had increased risks in both men and women (HR 1.83, 95% CI 1.53–2.33 and HR 4.77, 95% CI 2.52–9.00, respectively).

Subgroup analyses for women stratified by age of 50 years, the surrogate index for menopause, are shown in Table S5. The

results showed that the association among fatty liver and type 2 diabetes in non-obesity women were different between those aged ≤50 and >50 years. A BMI of 23–24.9 kg/m^2 without fatty liver did increase the risk of type 2 diabetes in women aged ≤50 years (HR 3.97, 95% CI 1.15–13.77), but not women aged >50 years (HR 1.42, 95% CI 0.70–2.88).

Sensitivity analyses using different cut-off for dyslipidemia in women showed consistent findings (Table S6).

DISCUSSION
In the present cohort study, we investigated the combined effects of fatty liver and several BMI categories with the development of type 2 diabetes stratified by sex to assess how ectopic fat (fatty liver) affects the incidence of type 2 diabetes according to sex among non-obese Asians. The results showed that fatty liver plays different roles between men and women (Table 2; Figure 1). In men, a BMI of 23–24.9 and 25–27.4 kg/m^2

Table 2 | Association of the risk of type 2 diabetes with three body mass index categories (20–22.9, 23–24.9 and 25–27.4 kg/m^2) combined with fatty liver among male and female Japanese participants

BMI in kg/m^2 and fatty liver status	n	Case	Incidence rate (per 1,000 person-years)	Model 0 HR	Model 0 95% CI	Model 1 HR	Model 1 95% CI	Model 2 HR	Model 2 95% CI	Model 3 HR	Model 3 95% CI	Model 4 HR	Model 4 95% CI
Male (n = 13,596)													
BMI 20–22.9	4,832	130	6.67										
Without fatty liver	4,268	99	5.73	1	Reference	1	Reference	1	Reference	1	Reference	1	Reference
With fatty liver	564	31	13.95	2.42	1.62–3.62	2.25	1.50–3.37	2.25	1.50–3.37	2.03	1.35–3.04	1.57	1.04–2.36
BMI 23–24.9	3,459	165	12.00										
Without fatty liver	2,404	76	7.93	1.38	1.02–1.86	1.32	0.98–1.78	1.32	0.98–1.77	1.25	0.93–1.69	1.11	0.83–1.50
With fatty liver	1,055	89	21.35	3.70	2.78–4.93	3.44	2.58–4.58	3.44	2.58–4.59	2.94	2.20–3.94	2.03	1.52–2.73
BMI 25–27.4	2,349	183	19.79										
Without fatty liver	1,043	47	11.16	1.94	1.37–2.74	1.83	1.29–2.59	1.81	1.28–2.56	1.59	1.12–2.25	1.19	0.84–1.70
With fatty liver	1,306	136	27.01	4.70	3.63–6.09	4.51	3.48–5.84	4.48	3.46–5.81	3.62	2.77–4.73	2.51	1.92–3.27
Female (n = 6,037)													
BMI 20–22.9	2,281	31	3.36										
Without fatty liver	2,194	24	3.04	1	Reference	1	Reference	1	Reference	1	Reference	1	Reference
With fatty liver	87	4	11.56	3.92	1.37–11.20	2.96	1.03–8.51	2.95	1.02–8.48	2.81	0.97–8.09	1.52	0.53–4.40
BMI 23–24.9	779	36	11.55										
Without fatty liver	661	19	7.08	2.29	1.27–4.11	2.18	1.21–3.92	2.22	1.23–4.00	2.17	1.20–3.90	1.85	1.03–3.35
With fatty liver	118	17	39.08	12.80	6.98–23.50	11.10	6.03–20.42	11.06	6.00–20.40	9.80	5.26–18.27	5.07	2.69–9.57
BMI 25–27.4	440	27	15.35										
Without fatty liver	301	11	9.13	3.07	1.52–6.20	3.00	1.49–6.04	3.00	1.49–6.05	2.88	1.42–5.81	2.30	1.13–4.66
With fatty liver	139	16	28.89	9.16	5.27–14.59	8.07	4.34–15.01	8.02	4.28–15.01	6.80	3.56–13.00	2.13	1.10–4.12

Model 0: adjusted for body mass index (BMI) category. Model 1: adjusted for BMI category and age. Model 2: adjusted for BMI category, age, smoking status, alcohol consumption and physical activity. Model 3: adjusted for BMI category, age, smoking status, alcohol consumption, physical activity, family history of diabetes, hypertension and dyslipidemia. Model 4: adjusted for BMI category, age, smoking status, alcohol consumption, physical activity, family history of diabetes, hypertension, dyslipidemia and fasting plasma glucose. 95% CI, 95% confidence interval; BMI, body mass index; HR, hazard ratio.
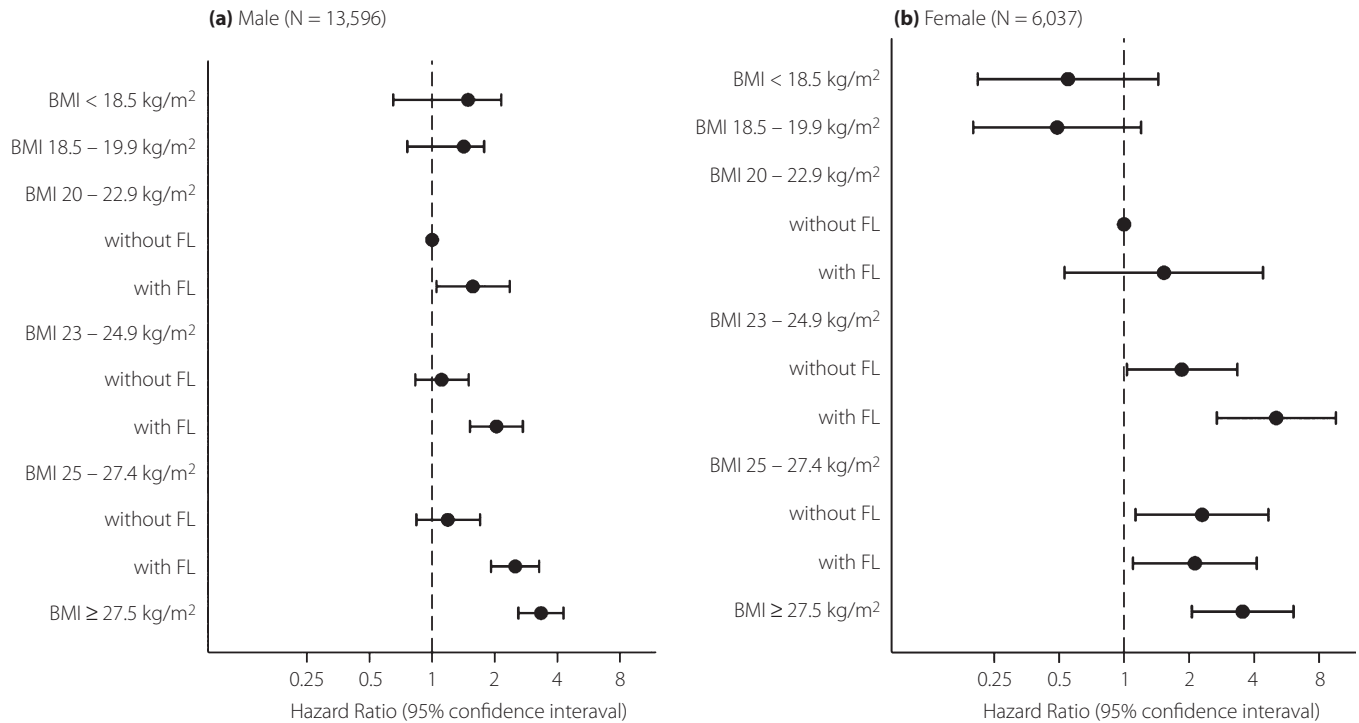
Figure 1 | Association of type 2 diabetes with three body mass index categories (20–22.9, 23–24.9 and 25–27.4 kg/m^2) combined with fatty liver (FL) among male and female Japanese participants. (a) Male (*n* = 13,596); (b) female (*n* = 6,037).

with fatty liver had increased risk of type 2 diabetes, whereas those without fatty liver did not. In women, in contrast, a BMI of 23–24.9 and 25–27.4 kg/m^2 had higher risk of type 2 diabetes regardless of fatty liver. In addition, male participants with a BMI of 20–22.9 kg/m^2 and fatty liver were associated with a higher risk of type 2 diabetes, whereas female participants with a BMI of 20–22.9 kg/m^2 and fatty liver were not. These results suggest that fatty liver (ectopic fat) plays a more important role in type 2 diabetes among non-obese male Asians than among female Asians; in other words, the weight itself plays an important role among female Asians, but not male Asians.

Asians have an increased risk of diabetes, as well as other lifestyle-related diseases, at a lower BMI than do non-Asians. The present results, based on a specific Japanese population sample, as well as those from other Japanese studies[25,26], are in line with this evidence; even people with a BMI of 23–24.9 kg/m^2 have an increased risk of type 2 diabetes (Table S2).

Increased risk of type 2 diabetes at a lower BMI among Asians is thought to be partly due to race-specific characteristics of body fat distribution; there is a propensity for Asians to develop visceral versus peripheral adiposity, which is more closely associated with insulin resistance and type 2 diabetes than overall adiposity[5,6,27–30]. There has been evidence to suggest that ectopic fat accumulation (such as that observed in the non-alcoholic fatty liver) presents a risk of type 2 diabetes[31], and that

non-obese people can also have fatty liver[32], although it is closely associated with obesity. Okamura *et al.* reported in a recent cohort study that ectopic fat obesity (obesity with fatty liver) presented the greatest risk of incident type 2 diabetes among Japanese people[8].

In contrast, fat deposit differs by sex regardless of race; men feature more visceral and ectopic fat (fatty liver) compared with women of the same age and BMI, which might be associated with an increased risk of type 2 diabetes among men[10,11]. Similarly, the present study found a higher prevalence of fatty liver among men compared to women in all BMI categories, also in the case of lower BMI (i.e., BMI 23–24.9 kg/m^2: 30.6 vs 15.3%, *P* < 0.001; Table S1).

To our knowledge, this is the first cohort study to investigate whether fatty liver (ectopic fat) plays a different role in the development of type 2 diabetes among non-obese Asians depending on sex, and the results are indicative of this. Having a BMI of 23–24.9 and 25–27.4 kg/m^2 without fatty liver did not increase the risk of type 2 diabetes among men, although it did increase risk among women. In contrast, having a BMI of 20–22.9 kg/m^2 with fatty liver increased the risk of diabetes among men, but not women (Table 2; Figure 1). These results suggest that ectopic fat might play an important role in the development of diabetes at a lower BMI among male, but not female, Asians. For type 2 diabetes prevention among Asians with a lower BMI, who have more type 2 diabetes risk than

other race, the presence of fatty liver might be a useful indicator, especially in men.

Several studies showed that visceral fat was correlated with more diabetes risk factors among non-obese women than among men[12,13]. Zhang et al. also reported in a recent Chinese population-based study that, despite involving obesity, visceral fat was more sensitive in predicting hyperglycemic risk in women than in men[33]. The present study suggested the opposite: visceral fat was correlated with a higher type 2 diabetes risk in non-obese men than in women. This inconsistency might be due to several differences between the present study and previous studies. First, the present study used a longitudinal design, whereas previous studies used a cross-sectional design. Thus, the present study examined the combined effects of fat and sex on type 2 diabetes more causally than previous studies. Second, the outcome in the present study was a direct incidence of type 2 diabetes, whereas previous studies used indirect proxy indices (risk factors) associated with type 2 diabetes, such as metabolic syndrome[12] and homeostatic model assessment as an index of insulin resistance[13]. Third, the present study focused on those with a BMI of 23–24.9 kg/m^2, whereas previous studies assessed outcomes in a broader range of non-obese participants. Although ectopic fat was correlated with increased type 2 diabetes risk among men, but not among women, even in the BMI category of 20–22.9 kg/m^2, the present sample did not include participants with BMI <20 kg/m^2. Finally, in our study, fat was measured indirectly according to abdominal ultrasonography findings and waist circumstance, whereas previous studies measured fat directly with computed tomography assessment of the adipose tissue area.

Another possible reason might be a preference for leaner appearance among Japanese women. A Japanese national survey suggested that women might be more likely to avoid obesity or obesity-related diseases in Japan[34]. A recent Japanese study also reported that remission of fatty liver is associated with remission of diabetes[35]. Taken together, women diagnosed as fatty liver might have modified their lifestyle more aggressively, resulting in the low incidence of type 2 diabetes. The interrelationships between sex, fat and type 2 diabetes might be multidimensional[10]. There might be not only biological, but also behavioral, psychological- and social pathway-related (i.e., lifestyle-related[36], occupational[37–39], psychological stress[37,38] or socioeconomic status-related factors[40–42]) among them. Further studies accounting for these factors are required. These factors also might be useful for type 2 diabetes prevention among Asians.

There is increasing attention on 'metabolic health'[9,24] and growing evidence that 'metabolically unhealthy normal weight' is associated with cardiometabolic risk, including type 2 diabetes[43]. In a review, Stefan reported that sex hormones played an important role in 'metabolically unhealthy normal weight' and its related unhealthy fat distribution[9]. The present results suggesting that associations among fatty liver, non-obesity and type 2 diabetes might be different by menopause are consistent with this review (Table S5). Although it is important to investigate the relationship between fatty liver and type 2 diabetes in lean participants, we could not do due to the small sample size. Further studies are required.

A strength of the present study was the relatively large sample size and cross-section of several occupations, allowing us to assess the impacts of the specific BMI categories, ectopic fat (or visceral fat) and sex.

The present study had several limitations. First, the participants were workers. Therefore, our results might be explained in part by the 'healthy worker effect.' Second, fatty liver in this study was defined according to abdominal ultrasonography findings. Liver biopsy is the gold standard for diagnosis; however, ultrasonography has been shown to have high sensitivity and specificity for diagnosing fatty liver[17]. Third, some of our participants might have been receiving lifestyle interventions from occupational health professionals. In particular, participants aged >40 years with metabolic syndrome might receive specific health-related guidelines from their employer, as required by the Japanese Act[14,44]. The present study did not include data about lifestyle interventions. Therefore, the reported risks for type 2 diabetes might have been underestimated. Fourth, the participants who did not take an abdominal ultrasonography examination and who did not attend any subsequent health checkups were excluded from analyses. These participants might have less health awareness than analytic samples, despite the fact there were no differences in baseline characteristics between them. Thus, the reported risks might have been underestimated. Fifth, in women, the CIs were larger owing to a smaller sample size. This might have contributed to the sex difference in the association of fatty liver with type 2 diabetes, especially in participants with a BMI of 20–22.9 kg/m^2. Finally, we could not discriminate between type 1 and type 2 diabetes. Adult-onset type 1 diabetes is rare in Japan[45], and we expect that virtually all new cases in this cohort were type 2 diabetes.

In conclusion, fatty liver (ectopic fat) played different roles in the development of incident type 2 diabetes among non-obese Japanese people depending on sex; ectopic fat played a role in men, but not in women. To prevent type 2 diabetes in non-obese Asians, we should focus on those with ectopic fat in, especially men, but not in women.

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DISCLOSURE

The authors declare no conflict of interest.

REFERENCES

1. Chan JCN, Malik V, Jia W, et al. Diabetes in Asia epidemiology, risk factors, and pathophysiology. JAMA 2009; 301: 2129–2140.
2. Ramachandran A, Snehalatha C, Shetty AS, et al. Trends in prevalence of diabetes in Asian countries. World J Diabetes 2012; 15: 110–117.
3. Menke A, Casagrande S, Geiss L, et al. Prevalence of and trends in diabetes among adults in the United States, 1988–2012. JAMA 2015; 314: 1021–1029.
4. Cho NH, Shaw JE, Karuranga S, et al. IDF diabetes atlas: global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Res Clin Pract 2018; 138: 271–281.
5. Nazare JA, Smith JD, Borel AL, et al. Ethnic influences on the relations between abdominal subcutaneous and visceral adiposity, liver fat, and cardiometabolic risk profile: the international study of prediction of intra-abdominal adiposity and its relationship with cardiometabolic risk/intra-abdominal adiposity. Am J Clin Nutr 2012; 96: 714–726.
6. Azuma K, Kadowaki T, Cetinel C, et al. Higher liver fat content among Japanese in Japan compared with non-Hispanic whites in the United States. Metabolism 2009; 58: 1200–1207.
7. Shulman GI. Ectopic fat in insulin resistance, dyslipidemia, and cardiometabolic disease. N Engl J Med 2014; 371: 1131–1141.
8. Okamura T, Hashimoto Y, Hamaguchi M, et al. Ectopic fat obesity presents the greatest risk for incident type 2 diabetes: a population-based longitudinal study. Int J Obes 2019; 43: 139–148.
9. Stefan N. Causes, consequences, and treatment of metabolically unhealthy fat distribution. Lancet Diabetes Endocrinol 2020; 8: 616–627.
10. Kautzky-Willer A, Harreiter J, Pacini G. Sex and gender differences in risk, pathophysiology and complications of type 2 diabetes mellitus. Endocrine Rev 2016; 37: 278–318.
11. Tramunt B, Smati S, Grandgeorge N, et al. Sex differences in metabolic regulation and diabetes susceptibility. Diabetologia 2020; 63: 453–461.
12. Fox CS, Massaro JM, Hoffmann U, et al. Abdominal visceral and subcutaneous adipose tissue compartments association with metabolic risk factors in the Framingham Heart Study. Circulation 2007; 116: 39–48.
13. He H, Ni Y, Chen J, et al. Sex difference in cardiometabolic risk profile and adiponectin expression in subjects with visceral fat obesity. Transl Res 2010; 155: 71–77.
14. Japanese Government. Industry Safety and Health Act. Available from https://www.mhlw.go.jp/web/t_doc?dataId=74001000&dataType=0&pageNo=1 Accessed June 22, 2020 (Japanese).
15. The Ministry of Health, Labour and Welfare, Ministry of Education, Culture, Sports, Science and Technology. Ethical Guidelines for Medical and Health Research Involving Human Subjects. Available from https://www.mhlw.go.jp/file/06-Seisakujouhou-10600000-Daijinkanboukouseikagakuka/0000080278.pdf Accessed June 1, 2020.
16. WHO expert consultation. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. Lancet 2004; 363: 157–163.
17. Hernaez R, Lazo M, Bonekamp S, et al. Diagnostic accuracy and reliability of ultrasonography for the detection of fatty liver: a meta-analysis. Hepatology 2011; 54: 1082–1090.
18. Dasarathy S, Dasarathy J, Khiyami A, et al. Validity of real time ultrasound in the diagnosis of hepatic steatosis: a prospective study. J Hepatol 2009; 51: 1061–1067.
19. Saverymuttu SH, Joseph AE, Maxwell JD. Ultrasound scanning in the detection of hepatic fibrosis and steatosis. Br Med J 1986; 292: 13–15.
20. Examination Committee of Criteria for Metabolic Syndrome. Definition and criteria for metabolic syndrome in Japan. J Jpn Soc Intern Med 2005; 94: 794–809.
21. Araki E, Goto A, Kondo T, et al. Japanese clinical practice guideline for diabetes 2019. J Diabetes Investig 2020; 11: 1020–1076.
22. Yatsuya H, Li Y, Hirakawa Y, et al. A point system for predicting 10-year risk of developing type 2 diabetes mellitus in Japanese Men: Aichi Workers' Cohort Study. J Epidemiol 2018; 28: 347–352.
23. Japanese Society of Obstetrics and Gynecology. Menopause. Available from http://www.jsog.or.jp/modules/diseases/index.php?content_id=14 Accessed December 1, 2020 (Japanese).
24. Smith GI, Mittendorfer B, Klein S. Metabolically healthy obesity: facts and fantasies. J Clin Investig 2019; 129: 3978–3989.
25. Okura T, Nakamura R, Fujioka Y, et al. Body mass index≥23 is a risk factor for insulin resistance and diabetes in Japanese people: a brief report. PLoS One 2018; 13: e0201052.
26. Takeno K, Tamura Y, Kawaguchi M, et al. Relation between insulin sensitivity and metabolic abnormalities in Japanese men with BMI of 23–25 kg/m^2. J Clin Endocrinol Metab 2016; 101: 3676–3684.
27. Wang J, Thornton JC, Russell M, et al. Asians have lower body mass index (BMI) but higher percent body fat than do whites: comparisons of anthropometric measurements. Am J Clin Nutr 1994; 60: 23–28.
28. Kagawa M, Kerr D, Uchida H, et al. Differences in the relationship between BMI and percentage body fat

between Japanese and Australian-Caucasian young men. *Br J Nutr* 2006; 95: 1002–1007.

29. Tanaka S, Horimai C, Katsukawa F. Ethnic differences in abdominal visceral fat accumulation between Japanese, African-Americans, and Caucasians: a meta-analysis. *Acta Diabetol* 2003; 40(Suppl. 1): S302–S304.

30. Kadowaki T, Sekikawa A, Murata K, *et al.* Japanese men have larger areas of visceral adipose tissue than Caucasian men in the same levels of waist circumference in a population-based study. *Int J Obes* 2006; 30: 1163–1165.

31. Ballestri S, Zona S, Targher G, *et al.* Nonalcoholic fatty liver disease is associated with an almost twofold increased risk of incident type 2 diabetes and metabolic syndrome. Evidence from a systematic review and meta-analysis. *J Gastroenterol Hepatol* 2016; 31: 936–944.

32. Yoshitaka H, Hamaguchi M, Kojima T, *et al.* Nonoverweight nonalcoholic fatty liver disease and incident cardiovascular disease: a post hoc analysis of a cohort study. *Medicine* 2017; 96: e6712.

33. Zhang F, Li Y, Zhao Y, *et al.* Is visceral abdominal fat area a better indicator for hyperglycemic risk? Results from the Pinggu Metabolic Disease Study. *J Diabetes Investig* 2020; 11: 888–895.

34. Ministry of Health, Labour and Welfare, Japan. The National Health and Nutrition Survey in Japan, 2008. Available from https://www.mhlw.go.jp/bunya/kenkou/eiyou/h20-houkoku.html Accessed December 1, 2020 (Japanese).

35. Yamazaki H, Wang J, Tauchi S, *et al.* Inverse association between fatty liver at baseline ultrasonography and remission of type 2 diabetes over a 2-year follow-up period. *Clin Gastroenterol Hepatol* 2020. https://doi.org/10.1016/j.cgh.2020.06.032

36. Meisinger C, Thorand B, Schneider A, *et al.* Sex differences in risk factors for incident type 2 diabetes mellitus: the MONICA Augsburg cohort study. *Arch Intern Med* 2002; 162: 82–89.

37. Heraclides M, Chandola T, Witte DR, *et al.* Work stress, obesity and the risk of type 2 diabetes: gender-specific bidirectional effect in the Whitehall II study. *Obesity* 2012; 20: 428–433.

38. Eriksson AK, van den Donk M, Hilding A, *et al.* Work stress, sense of coherence, and risk of type 2 diabetes in a prospective study of middle-aged Swedish men and women. *Diabetes Care* 2013; 36: 2683–2689.

39. Silva-Costa A, Rotenberg L, Nobre AA, *et al.* Gender-specific association between nightwork exposure and type-2 diabetes: results from longitudinal study of adult health, ELSA-Brasil. *Scand J Work Environ Health* 2015; 41: 568–578.

40. Lee DS, Kim YJ, Han HR. Sex differences in the association between socioeconomic status and type 2 diabetes: data from the 2005 Korean National Health and Nutritional Examination Survey (KNHANES). *Public Health* 2013; 127: 554–560.

41. Rivera LA, Lebenbaum M, Rosella LC. The influence of socioeconomic status on future risk for developing Type 2 diabetes in the Canadian population between 2011 and 2022: differential associations by sex. *Int J Equity Health* 2015; 14: 101.

42. Nagamine Y, Kondo N, Yokobayashi K, *et al.* Socioeconomic disparity in the prevalence of objectively evaluated diabetes among older Japanese adults: JAGES Cross-Sectional Data in 2010. *J Epidemiol* 2019; 29: 295–301.

43. Stefan N, Schick F, Haring HU. Causes, characteristics, and consequences of metabolically unhealthy normal weight in humans. *Cell Metab* 2017; 26: 292–300.

44. Matsuzawa Y. Specific health guidance, the nationwide lifestyle intervention program targeting metabolic syndrome, seems to be successful in Japan. *J Atheroscler Thromb* 2018; 25: 304–305.

45. Morimoto A, Nishimura R, Tajima N. Trends in the epidemiology of patients with diabetes in Japan. *JMAJ* 2010; 53: 36–40.

SUPPORTING INFORMATION

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

Table S1 | Characteristics of male and female participants according to Body Mass Index (BMI) category.

Table S2 | Association of Body Mass Index (BMI) category with the risk of type 2 diabetes among male and female Japanese.

Table S3 | Association of the risk of type 2 diabetes (T2D) with three body mass index (BMI) categories (20–22.9, 23–24.9, and 25–27.4 kg/m^2) combined with visceral fat obesity (VFO) among male and female Japanese.

Table S4 | Association of the risk of type 2 diabetes with three body mass index (BMI) categories (20–22.9, 23–24.9, and 25–27.4 kg/m^2) combined with fatty liver additionally adjusted for visceral fat obesity among male and female Japanese.

Table S5 | Association of the risk of type 2 diabetes with three body mass index (BMI) categories (20–22.9, 23–24.9, and 25–27.4 kg/m^2) combined with fatty liver among female Japanese stratified by age ≤ 50 years and age > 50 years.

Table S6 | Association of the risk of type 2 diabetes with three body mass index (BMI) categories (20–22.9, 23–24.9, and 25–27.4 kg/m^2) combined with fatty liver using dyslipidemia different cut-offs (high-density lipoprotein [HDL] cholesterol <50 mg/dl) among female Japanese (n = 6,037).

Figure S1 | Flow diagram of study participants in this study.

Figure S2 | Association of the risk of type 2 (T2D) diabetes with three body mass index (BMI) categories (20–22.9, 23–24.9 and 25–27.4 kg/m^2) combined with visceral fat obesity (VFO) among male and female Japanese participants. (a) Males (n = 13,596). (b) Female (n = 6,037).