Non alcoholic fatty liver disease and risk of incident diabetes in subjects who are not obese

Ki-Chul Sung[a,*], Dae-Chul Seo[a], Seung-Jae Lee[a], Mi-Yeon Lee[b], Sarah H Wild[c], Christopher D Byrne[d,*]

[a]*Division of Cardiology, Department of Internal Medicine, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea*

[b]*Division of Biostatistics, Department of R&D Management, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea*

[c]*Centre for Population Health Sciences, University of Edinburgh, Edinburgh, UK*

[d]*Nutrition and Metabolism Unit, IDS Building, and Southampton National Institute for Health Research Biomedical Research Centre, Southampton General Hospital, University of Southampton, Southampton, UK*

[*]**Corresponding authors:**

Ki-Chul Sung, MD, PhD, Division of Cardiology, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, 29 Saemunan-ro, Jongno-gu, Seoul 03181, Republic of Korea

Tel: +82-2-2001-2001; Fax: +82-2-2001-2400; E-mail: kcmd.sung@samsung.com

Or

Christopher D Byrne, MB, BCh, PhD, Nutrition and Metabolism Unit, IDS Building, Southampton General Hospital, MP 887, Tremona Road, Southampton, UK. SO166YD

; E-mail: c.d.byrne@soton.ac.uk

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ABSTRACT

Background and Aims: It is not known whether non alcoholic fatty liver disease (NAFLD) is a risk factor for diabetes in non obese, non centrally-obese subjects. Our aim was to investigate relationships between fatty liver, insulin resistance and a biomarker score for liver fibrosis with incident diabetes at follow up, in subjects who were neither obese nor centrally-obese.

Methods: 70,303 subjects with a body mass index (BMI) <25kg/m^2 and without diabetes were followed up for a maximum of 7.9 years. At baseline, fatty liver was identified by liver ultrasound, insulin resistance (IR) by homeostatic model assessment of insulin resistance (HOMA-IR) ≥2.0, and central obesity by waist circumference (waist circumference ≥90cm (men) and ≥85cm (women). The Fibrosis-4 (FIB-4 score) was used to estimate extent of liver fibrosis. Cox proportional hazards models adjusted for confounders were used to estimate hazard ratios (aHRs) for incident diabetes.

Results: 852 incident cases of diabetes occurred during follow up (median [IQR] 3.71 [2.03] years). Mean±SD BMI was 22.8±1.8 and 21.7±2.0 kg/m^2 in subjects with and without diabetes at follow up. In subjects without central obesity and with fatty liver, aHRs (95% CI) for incident diabetes at follow up were 2.17 (1.56,3.03) for men, and 2.86 (1.50,5.46) for women. Similar aHRs for incident diabetes occurred with fatty liver, IR and the highest quartile of FIB-4 combined, in men; and there was a non significant trend toward increased risk in women.

Conclusions: In normal weight, non-centrally obese subjects NAFLD is an independent risk factor for incident diabetes.

KEYWORDS: Central obesity; Diabetes; Insulin resistance; Lean non alcoholic fatty liver disease; NAFLD fibrosis score; Obesity

Introduction

Although it is very well established that non alcoholic fatty liver disease (NAFLD) occurs frequently in obese subjects, it is now becoming clear that NAFLD also occurs in a substantial proportion of non-obese individuals [1-8]. The prevalence of NAFLD among subjects who are not obese varies between ~3 and 30% in different populations [1,2] and NAFLD has been previously identified in 12.6% of non-obese subjects in a study of ~30,000 subjects in Korea [9]. The mechanisms by which non-obese individuals develop NAFLD are not entirely clear but differential distribution of visceral adipose tissue, recent increases in body weight, intake of a high cholesterol diet, and genetic background are all thought to contribute to the pathogenesis of NAFLD in this group [10].

Subjects with NAFLD tend to be insulin resistant. A recent study showed that normal weight subjects with NAFLD are more insulin resistant than overweight subjects without NAFLD and that liver fat accumulation may be an important cause of insulin resistance in non-obese subjects with NAFLD [11]. Studies based on liver biopsies in lean subjects with NAFLD also suggest that the prevalence of more severe forms of NAFLD (such as nonalcoholic steatohepatitis (NASH) and fibrosis) is high in this group [2], with up to 20% of lean patients with NAFLD having NASH [12,13].

A recent meta-analysis suggests that NAFLD is independently associated with a ~2.2 fold increase in risk of developing type 2 diabetes [14]. Only large observational studies (n = 19) with a follow-up duration of at least 1 year were included in this meta-analysis. Although almost 300,000 individuals (30.1% with NAFLD) and nearly 16,000 cases of incident diabetes were included, none of the included studies specifically focussed on the effect of NAFLD in subjects who are not obese. To our knowledge only one small study of 669 consecutive patients with biopsy-proven NAFLD has investigated whether NAFLD is associated with increased risk of incident diabetes in subjects who are not obese [13]. 143 patients with a body mass index (BMI) <25 kg/m^2 and NAFLD were included, and despite a substantial proportion of lean patients with NAFLD having moderately severe liver disease [13], the results of this

study were inconclusive and it remains uncertain whether NAFLD adversely affects risk of developing diabetes in normal weight, non centrally obese subjects.

Our aim was to investigate relationships between fatty liver, insulin resistance (IR) and a biomarker score for liver fibrosis (the Fibrosis-4 Score (FIB-4) [15-17], with incident diabetes at follow up, in a large cohort of non-obese, non-centrally obese subjects. In this large cohort it was not possible to stage liver disease severity with liver histology. Therefore, we identified subjects with fatty liver and IR who were also in the highest quartile of FIB-4, since we reasoned that these subjects with NAFLD were also more like to have a more severe form of liver disease than those subjects with fatty liver alone.

Material and Methods

The study population consisted of individuals who participated in a comprehensive health screening program, at least twice, at Kangbuk Samsung Hospital, Seoul and Suwon, Korea from 2007 to 2014 (*n* = 219,417). Obesity was defined by BMI ≥25 kg/m^2 and central obesity by waist circumference ≥90 cms for men and ≥85 cms for women [18]. We excluded subjects with BMI≥25 kg/m^2 (*n* = 61,939) or age <20 years (*n* = 54). Subjects were also excluded with prevalent diabetes identified from self-report of diagnoses, or treatment, or screen detected diabetes based on HbA1c ≥6.5% (48 mmol/L) or fasting glucose ≥ 126mg/L (7mmol/L) (*n* = 7,505); subjects who consumed >30 g alcohol /day (men) or >20g alcohol /day (women) (*n* = 36,192); subjects with hepatitis C antibodies (*n* = 251), or who were hepatitis B surface antigen positive (*n* = 5,220). In addition subjects with missing data were excluded (*n* = 75,090 for HOMA IR); *n* = 38,777 for hs-CRP; *n* = 7 for BMI; *n* = 528 for assessment of fatty liver status; *n* = 83 for other key anthopometric or biochemical data. Some of the excluded subjects had more than one of the above exclusion criteria.

The cohort included 70,303 subjects with BMI <25kg/m^2, without diabetes and with complete data on co-variates, who were included in this analysis. Of these subjects, there were 32,802 subjects (47%) with waist circumference within the normal range (men and women, <90 cm and <85 cm respectively) for this ethnic group [9]. The median (IQR) follow up was 3.71±2.03 years.

The study was approved by the Institutional Review Board of Kangbuk Samsung Hospital and requirement for informed consent was waived by the Board because de-identified information was used for the analyses.

FIB-4 was calculated by the formula FIB 4 =(age [yr]x AST [U/L]) / ((PLT [109/L]) x (√ALT [U/L])).

Measurements

Participants completed self-administered questionnaires regarding their medical and social history and drug treatment. Individuals were asked about duration of education (years), regular exercise, smoking history (never, former, or current) and alcohol consumption (grams, g/week). Trained staff also undertook anthropometric measurements. Body weight was measured in light clothing with no shoes to the nearest 0.1 kilogram using a digital scale. Height was measured to the nearest 0.1 centimeter. BMI was calculated as weight in kilograms divided by height in meters squared.

Blood samples were collected after at least 10-hours of fasting and analyzed in the same core clinical laboratory, the Laboratory Medicine Department at the Kangbuk Samsung Hospital. The core clinical laboratory has been accredited and participates annually in inspections and surveys by the Korean Association of Quality Assurance for Clinical Laboratories. Abdominal ultrasonography (Logic Q700 MR; GE, Milwaukee, WI, USA) was undertaken by clinical radiologists using a 3.5MHz probe for all subjects at baseline. The following images were undertaken; i) sagittal view of the right lobe of the liver and right kidney, ii) transverse view of the left lateral segment of the liver and spleen and iii) transverse view of the liver for altered echo texture. Fatty infiltration of the liver (fatty liver) was identified if there was an increase in echogenicity of the liver compared with the echogenicity of the renal cortex where the diaphragm and intrahepatic vessels appeared normal [19]. Diabetes at follow up (to identify incident diabetes) was also defined as a self-reported history of diabetes, the use of glucose-lowering medications and/or HbA1c ≥6.5% (48 mmol/mol) or fasting glucose ≥ 126mg/dL (7 mmol/L).

Statistical analyses

The statistical analysis was performed using STATA version 15.0 (StataCorp LP, College Station, TX, USA). Reported p values were two-tailed, and <0.05 values were considered statistically significant. The distribution of continuous variables was evaluated and transformations were conducted for nonparametric variables. Cox proportional hazards models were used to estimate Hazard Ratios (HRs and 95% confidence intervals [CIs]). Fully adjusted HRs (aHRs and 95CIs) were estimated for the associations between the key exposures i.e. fatty liver diagnosed by ultrasonography, IR defined by

HOMA-IR≥2.0, and highest quartile of FIB-4 score (defined by quartile 4 = FIB-4 levels 0.83 to 21.27) and our outcome of interest i.e. incident diabetes at follow up.

The proportional hazards model assumption was tested with a graphical analysis of the hazard of incident diabetes over time. Models were adjusted for age, sex, center (Seoul or Suwon), year of screening examination, smoking status, alcohol intake, exercise, family history of diabetes, education level and baseline BMI (or baseline waist circumference) according to the individual models.

Results

During a median (IQR) 3.71 (2.03) year follow up (mean 3.32 years), there were 852 incident cases of diabetes. Baseline characteristics of the cohort stratified by follow up diabetes status are described in **Table 1** and the distribution of all characteristics were statistically significantly different between both groups. **Table 2** shows the baseline characteristics stratified by fatty liver status and BMI and waist categories. HOMA-IR (and the proportion with IR defined as HOMA-IR≥2.0) and FIB-4 were higher in subjects with fatty liver compared to subjects without fatty liver.

Supplementary Table 1 shows aHRs (95% CIs) for incident diabetes at follow up stratified by baseline HOMA-IR quartiles in men and women. With increasing quartiles of IR, there was an increase in aHR for incident diabetes in both men and women ($p<0.001$ for both sexes). In the highest quartile of HOMA-IR there was a similar ~4 fold increase in risk in men and in women compared to the risk of incident diabetes in the lowest IR quartile. **Supplementary Table 2** shows the unadjusted and age-adjusted HRs (95%CIs) for incident diabetes according to quartiles of FIB-4. The unadjusted data shows an increase in the HR for incident diabetes in the highest FIB-4 quartile (HR 95%CIs 1.36 (1.13,1.64)). However, there was a marked effect of age, and after adjustment for age there was a reversal of this effect and a decrease in risk of incident diabetes (HR 95%CIs 0.47 (0.38, 0.59) for the highest FIB-4 quartile). **Supplementary Table 3** describes the anthropometric and biochemical parameters at baseline and also at follow up in subjects with and without incident diabetes at follow up.

Table 3 (men) and Table 4 (women) show the aHRs (95% CIs) for incident diabetes at follow up, in normal weight men or women without central obesity, with different combinations of fatty liver, IR and the highest quartile of FIB-4, adjusted for potential confounders. The aHR (95% CIs) for incident diabetes with fatty liver alone, compared to the group of subjects without fatty liver or IR, and who were in the lowest quartile of NFS was 2.17 (1.56, 3.03) for men (**Table 3**) and 2.86 (1.50, 5.46) for women (**Table 4**). We investigated the effects of combining fatty liver, IR and the highest quartile of FIB-4 on risk of incident diabetes at follow up. The aHR (95% CIs) for incident diabetes after combining fatty liver, IR

and highest quartile of FIB-4 was 2.63 (1.41, 4.92) for men (**Table 3**) and was 1.50 (0.32, 6.95) for women (**Table 4**) (although it should be noted that there were only two incident cases of diabetes amongst the 36 women with this combination of risk factors at baseline).

Discussion

The novel results of our study are that NAFLD is an independent risk factor for incident diabetes in normal weight, non-centrally obese subjects. When we considered the influence of fatty liver alone in this patient group, fatty liver as a single risk factor increased the risk of incident diabetes at follow up. Patients with NAFLD are often obese and insulin resistant and it is uncertain whether liver fat and IR are still risk factors for incident diabetes if subjects are not obese and importantly are non-centrally obese. Our data clearly show that the combination of fatty liver and IR remains as a powerful dual risk factor combination in these patients, increasing risk of diabetes approximately four fold in men and greater than six fold in women

In contrast, the relationship between FIB-4 levels as a single risk factor and incident diabetes is complex. Whereas there was an increase in risk of incident diabetes in the highest FIB-4 quartile in the unadjusted model, adjusting for age markedly attenuated this effect. Indeed, adjusting for age, the highest FIB-4 quartile was associated with decreased risk of developing diabetes at follow up. We are uncertain why there was a decreased risk of developing diabetes with increasing levels of FIB-4 but it is unlikely that the explanation for this finding is that subjects in the highest FIB-4 quartile had cirrhosis (and therefore decreased hepatic glucose output with a failing liver). Only 101/17,576 subjects in the highest FIB-4 quartile had a FIB level ≥2.67 (which is the FIB-4 threshold above which subjects are likely to have advanced liver fibrosis [17]. Additionally, we also noted that there was also a decreased risk of developing diabetes in the second and third quartiles of FIB-4 after adjustment for age but at present we are unable to explain the decreased risk of incident diabetes with increasing FIB-4 levels after adjustment for age and this finding requires verification in other cohorts.

When we investigated the effects of combining fatty liver, IR and the highest quartile of FIB-4 on risk of incident diabetes at follow up there were 12 incident cases of diabetes amongst 180 men with this combination of risk factors at baseline. In contrast, there were only two incident cases of diabetes amongst 36 women with this combination of risk factors. In the fully adjusted models, combining fatty

liver, IR and highest quartile of FIB-4 in men showed there was a 2.6 fold increase in risk of incident diabetes. Although there was a broadly similar non-significant 1.5 fold increase in incident diabetes with the same risk factor combination in women, it is likely our study lacked sufficient power in women because of the very small number of outcomes (n=2) and the small number of subjects (n=36) with this combination of exposures..

In recent findings from the largest and longest series of patients with biopsy-proven NAFLD to date (*n* = 646 subjects, mean follow-up time of 19.9 years), 19% of this patient group had a BMI <25kg/m^2) and were defined as 'lean NAFLD'. Patients in this "lean-NAFLD" group (*n* = 123 subjects) were older, had lower transaminases, and although patients with lean NAFLD had no increased risk for overall mortality, there was a ~2.7 fold increased risk for development of severe liver disease in this group, compared to subjects with a higher BMI [12]. Our data suggest that in men the combination of fatty liver, IR and highest quartile of FIB-4 (as combined indicators of potentially more severe liver disease) was associated with a similar increase in the HR for incident DM, than the presence of fatty liver alone. In the meta-analysis (previously mentioned), which analyzed the association between NAFLD and incident diabetes [14], the HR for incident diabetes among subjects with more severe NAFLD (defined by a combination of liver fat identified by ultrasound and biomarker scores for liver fibrosis) was 2.63 (95% CIs 1.57, 3.70). Coincidentally, this level of risk was the same as the estimate of risk that we observed (for men) i.e. aHR 2.63 (95% CIs 1.41, 4.92) for the association between fatty liver, IR and the highest quartile of FIB-4 combined, and incident diabetes (**Table 3**).

All of the major studies to date that have investigated the relationship between NAFLD and risk of type 2 diabetes have adjusted for BMI. To date, the association between NAFLD and risk of type 2 diabetes in non-obese subjects who are also not centrally obese has not been described. There is evidence to suggest that in non-obese subjects with NAFLD, there is increased prevalence of the PNPLA3 rs738409 genotype [8,20]. Although the PNPLA3 rs738409 genotype is associated with more severe liver disease in NAFLD, this genotype is not associated with increased risk of type 2 diabetes [21,22].

Unfortunately in our cohort. data on genotypes are not available and therefore we are unable to comment on the relationship between genotypes and risk of incident diabetes.

Although it has been suggested that hyperlipidemia and hyperglycemia may occur as frequently in non-obese as obese subjects with NAFLD [3,4,6,7] other studies have suggested that hyperlipidaemia and hyperglycaemia may occur even more frequently in non-obese than obese patients with NAFLD [23]. Consequently, large long-term follow-up cohort studies of NAFLD are needed to understand better the relationship between NAFLD (and NAFLD severity) and the development of incident diabetes, specifically in non-obese subjects [24].

Obesity and central obesity are very important and well recognised risk factors for type 2 diabetes. However, middle-aged asymptomatic non-obese and non-centrally obese subjects without a family history of type 2 diabetes are at low risk of developing type 2 diabetes. Importantly, our data suggest that identifying fatty liver and insulin resistance in asymptomatic subjects, may identify a sub group of non-obese individuals who are at increased risk of type 2 diabetes. In such subjects, implementation of lifestyle changes such as weight loss (if appropriate), or increases in physical activity, may reduce risk of type 2 diabetes. However, individuals who do not need to lose weight, or who are unable to increase their physical activity, may also benefit from pharmacological treatment to reduce risk of type 2 diabetes. Whereas both metformin [25] and pioglitazone [26] have been shown to decrease risk of type 2 diabetes, only pioglitazone has been recommended for the treatment of NASH in each of the US, European and UK guidelines [27-29] and this drug may be useful in this specific patient group.

There are a few strengths and limitations of our study that should be acknowledged. We have studied approximately 70,000 non-obese subjects with data on ultrasound-diagnosed fatty liver status at baseline and have identified 852 cases of incident diabetes at follow up. The limitations are that we have identified incident diabetes by either a self-reported history of diabetes, the use of glucose-lowering medications and/or HbA1c ≥6.5% (48 mmol/mol) or fasting glucose ≥ 126mg/dL (7 mmol/L). It was not possible to

undertake oral glucose tolerance testing and we do not have repeated measures of BMI or waist circumference, drug history or change in lifestyle during the follow up period; all of which could affect risk of incident diabetes at follow up [30-32]. We have also used HOMA-IR ≥2.0 as a marker of insulin resistance because more sensitive or specific measurements of insulin sensitivity were not available in this cohort. We have also used the highest quartile of FIB-4 to attempt to identify those subjects who were likely to have more severe liver disease but there were only 101 subjects in the whole cohort with a FIB level ≥2.67 in keeping with possible advanced fibrosis.

In summary, in normal weight, non-centrally obese men and women, the presence of fatty liver alone, and also fatty liver plus IR, increased risk of incident diabetes. In addition in men, the combination of fatty liver, IR and the highest quartile of FIB-4 (as a marker of increased liver disease severity) also increased risk of incident diabetes, and there was a non-significant trend in the same direction in women.

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Author Contributions

K.S takes full responsibility for the data collection and integrity of the analyses. CDB, SHW DCS, SJL, MYL and KS have written the manuscript and all authors have read and agree the manuscript as written.

Conflict of Interest

All the authors declared no competing interests.

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Tables

Table 1. Baseline characteristics of cohort of subjects with BMI <25kg/m^2 stratified by development of incident diabetes at follow up in non-obese subjects

	No diabetes at follow up	**Diabetes at follow up**	**p value**
N	69,451	852	<0.001
Age (years)	35.5± 6.0	40.2±7.4	<0.001
Female, *n* (%)	35,694 (51.4)	286 (33.6)	<0.001
BMI (kg/m^2)	21.7±2.0	22.8±1.8	<0.001
Education, *n* (%)			<0.001
≤high school	4,401 (6.3)	56 (6.6)	
>high school	33,168 (47.8)	304 (35.7)	
Unknown	31,882 (45.9)	492 (57.8)	
Exercise, *n* (%)			<0.001
<1 time per week	42,730 (61.5)	468 (54.9)	
≥1 time per week	26,156 (37.7)	379 (44.5)	
Unknown	565 (0.8)	5 (0.6)	
Smoking, *n* (%)			<0.001
Never/former	52,640 (75.6)	555 (65.1)	
Current	13,481 (19)	278 (32.6)	
Unknown	3,330 (4.8)	19 (2.2)	
Glucose (mg/dL)	92.0±7.7	104.6±10.5	<0.001
Insulin (μIU/mL)[a]	4.25 (2.96, 5.93)	5.12 (3.59, 7.29)	<0.001
HOMA-IR[a]	0.97 (0.66,1.38)	1.30 (0.90,1.88)	<0.001
LDL-C (mg/dL)	108.3±28.9	119.8±31.4	<0.001
Triglyceride (mg/dL)	81 (60, 115)	123 (86, 180)	<0.001

HDL (mg/dL)	58.6±13.8	51.9±12.5	<0.001
AST (IU/L)	19 (16, 23)	22 (19, 27)	<0.001
ALT (IU/L)	16 (12, 22)	22 (16, 32)	<0.001
Albumin (g/dL)	4.6±0.24	4.7±0.23	<0.001
Platelet (10^3/mm^3)	253.7±54.2	270.5±60.0	<0.001
hs-CRP (mg/dL)	0.04 (0.02, 0.07)	0.05 (0.03, 0.09)	<0.001
IR (HOMA-IR ≥2.0), *n* (%)	5,355 (7.7)	178 (20.9)	<0.001
Fatty liver, *n* (%)	8,899 (12.8)	340 (39.9)	<0.001
FIB4 Score [b]	0.72±0.29	0.78±0.38	<0.001
Family Hx of DM	10,125 (14.6)	203 (23.8)	<0.001

Data are mean ± standard deviation or median (interquartile range) unless otherwise specified.

[a] Median(interquartile)

[b] (age [yr]x AST [U/L]) / ((PLT [109/L]) x (√ALT [U/L]))

AST, aspartate transaminase; ALT, alanine aminotransferase; BMI, body mass index; FU, follow up; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment-Insulin resistance; hs-CRP, high-sensitivity c-reactive protein; IR, insulin resistance; LDL, low-density lipoprotein; NAFLD, non alcoholic fatty liver disease.

Table 2. Baseline characteristics according to fatty liver status in non-obese (defined by BMI) and non-centrally obese subjects (defined by waist circumference)

	BMI (<25 kg/m^2)			Normal waist (men <90cms; women <85cms)		
	No fatty liver	Fatty liver	p value	No fatty liver	Fatty liver	p value
N	61,064	9,239	<0.001	27,340	5,462	<0.001
Age (years)	35.3±5.93	37.2±6.73	<0.001	35.6±6.88	38.1±7.65	<0.001
Female, *n* (%)	34,396 (56.3)	1,584 (17.1)	<0.001	15,254 (55.8)	817 (15.0)	<0.001
BMI (kg/m^2)	21.4±2.00	23.4±1.24	<0.001			
Waist				76.4±6.61	83.7±4.27	<0.001
Education, *n* (%)			<0.001			<0.001
≤high school	3,978 (6.51)	479 (5.18)		2,702 (9.9)	451 (8.3)	
>high school	28,793 (47.1)	4,679 (50.6)		18,919 (69.2)	3,819 (69.9)	
Unknown	28,293 (46.3)	4,081 (44.2)		5,719 (20.9)	1,192 (21.8)	
Exercise, *n* (%)			<0.001			<0.001
<1 time per week	37,637 (61.6)	5,561 (60.2)		16,927 (61.9)	3,196 (58.5)	
≥1 time per week	22,919 (37.5)	3,616 (39.1)		10,016 (36.6)	2,200 (40.3)	
Unknown	508 (0.83)	62 (0.67)		397 (1.5)	66 (1.2)	

Smoking, *n* (%)			<0.001			<0.001
Never/former	47,186 (77.3)	6,009 (65.0)		21,119 (77.3)	3,600 (65.9)	
Current	10,809 (17.7)	2,950 (31.9)		4,199 (15.4)	1,593 (29.2)	
Unknown	3,069 (5.0)	280 (3.0)		2,022 (7.4)	269 (4.9)	
Glucose (mg/dL)	91.7±7.69	95.6±8.27	<0.001	90.1±7.83	94.2±8.47	<0.001
Insulin (μIU/mL)[a]	4.06 (2.84, 5.64)	5.81 (4.27, 7.79)	<0.001	4.04 (2.78, 5.66)	5.81 (4.18, 7.93)	<0.001
HOMA-IR[a]	0.92 (0.63, 1.30)	1.37 (0.99, 1.87)	<0.001	0.90 (0.61, 1.29)	1.34 (0.95, 1.89)	<0.001
LDL-C (mg/dL)	105.9±27.7	125.6±31.1	<0.001	110.0±28.4	131.7±30.8	<0.001
Triglyceride (mg/dL)	77 (58, 106)	130 (93, 182)	<0.001	73 (56, 100)	124 (90, 174)	<0.001
HDL (mg/dL)	59.9±13.7	49.4±10.7	<0.001	61.7±14.1	49.6±11.3	<0.001
AST (IU/L)	19 (16, 22)	22 (19, 27)	<0.001	18 (15, 21)	22 (18, 27)	<0.001
ALT (IU/L)	15 (12, 20)	26 (19, 37)	<0.001	15 (11, 20)	26 (19, 37)	<0.001
Albumin (g/dL)	4.59±0.24	4.69±0.23	<0.001	4.59±0.24	4.69±0.24	<0.001
Platelet (10^3/mm^3)	252.4±54.2	263.0±54.0	<0.001	241.4±49.9	247.2±48.2	<0.001
hs-CRP (mg/dL)	0.03 (0.02, 0.06)	0.06 (0.04, 0.10)	<0.001	0.03 (0.02, 0.06)	0.06 (0.04, 0.11)	<0.001
FIB4 Score[b]	0.73±0.29	0.67±0.29	<0.001	0.75±0.31	0.73±0.32	<0.001
IR (HOMA-IR ≥2.0), *n* (%)	3,617 (5.9)	1,916 (20.7)	<0.001	1,589 (5.8)	1,179 (21.6)	<0.001

Family Hx of DM	8,763 (14.4)	1,565 (16.9)	<0.001	4,322 (15.8)	997 (18.3)	<0.001

Data are mean ± standard deviation or median (interquartile range) unless otherwise specified.

[a] Median(interquartile)

[b] (age [yr]x AST [U/L]) / ((PLT [109/L]) x (√ALT [U/L]))

AST, aspartate transaminase; ALT, alanine aminotransferase; BMI, body mass index; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment-Insulin resistance; hs-CRP, high-sensitivity c-reactive protein; IR, insulin resistance; LDL, low-density lipoprotein; NAFLD, non alcoholic fatty liver disease.

Table 3. Hazard ratios for incident diabetes at follow-up for different combinations of baseline fatty liver, IR (HOMA-IR ≥2), and highest quartile (Q4) of FIB-4 in subjects without central obesity and BMI <25 kg/m^2 (Men)

	Incident diabetes (*n*) / Risk factor (*n*)	Crude	Model 1	Model 2 (*n*=16,731)	Model 3 (*n*=16,731)	Model 4 (*n*=16,731)	Model 5 (*n*=16,731)
Fatty liver	97 / 4645	3.02 (2.25-4.05)	2.82 (2.1-3.78)	2.61 (1.91-3.58)	2.52 (1.83-3.47)	2.49 (1.81-3.44)	2.17 (1.56-3.03)
FIB 4 Q4	76 / 4344	1.97 (1.46-2.65)	0.75 (0.51-1.09)	0.8 (0.55-1.16)	0.8 (0.55-1.16)	0.8 (0.55-1.16)	0.84 (0.58-1.22)
IR	50 / 1663	4.1 (2.96-5.69)	4.43 (3.16-6.21)	4.02 (2.83-5.71)	3.91 (2.75-5.56)	3.85 (2.7-5.49)	3.38 (2.35-4.88)
Fatty liver + FIB 4 Q4	37 / 1047	3.82 (2.66-5.48)	2.01 (1.37-2.96)	1.79 (1.21-2.65)	1.71 (1.16-2.53)	1.68 (1.13-2.49)	1.53 (1.03-2.27)
Fatty liver + IR	40 / 952	5.5 (3.87-7.82)	5.56 (3.88-7.96)	4.98 (3.41-7.26)	4.76 (3.25-6.97)	4.68 (3.19-6.87)	3.95 (2.64-5.9)
IR + FIB 4 Q4	14 / 306	5.62 (3.25-9.7)	3.01 (1.72-5.28)	2.61 (1.48-4.61)	2.54 (1.44-4.48)	2.46 (1.39-4.36)	2.15 (1.21-3.82)
Fatty liver + FIB 4 Q4 + IR	12 / 180	8.32 (4.63-14.96)	4.21 (2.31-7.67)	3.56 (1.94-6.53)	3.35 (1.82-6.16)	3.25 (1.76-5.99)	2.63 (1.41-4.92)

Adjustments Model 1: age, education, exercise, smoking and alcohol intake (g/day), Center, Year, family history of diabetes

Model 2: Model 1 + waist circumference

Model 3 Model 2 + BMI

Model 4 Model 3 + medication for hypertension and hyperlipidaemia

Model 5 Model 4 + triglyceride and LDL-C

BMI, body mass index; HOMA-IR, homeostatic model assessment-Insulin resistance; IR, insulin resistance.

Table 4. Hazard ratios for incident diabetes at follow-up for different combinations of baseline fatty liver, IR (HOMA-IR ≥2), and highest quartile (Q4) of FIB-4 in subjects without central obesity and BMI <25 kg/m^2 (Women)

	Incident diabetes (*n*) / Risk factor (*n*)	Crude	Model 1	Model 2 (*n*=16,071)	Model 3 (*n*=16,071)	Model 4 (*n*=16,071)	Model 5 (*n*=16,071)
Fatty liver	20 / 817	8.02 (4.76-13.51)	4.16 (2.35-7.33)	3.85 (2.11-7.04)	3.73 (2.01-6.89)	3.59 (1.92-6.7)	2.86 (1.5-5.46)
FIB 4 Q4	20 / 3857	1.29 (0.77-2.18)	0.35 (0.18-0.67)	0.37 (0.19-0.71)	0.37 (0.19-0.72)	0.39 (0.2-0.75)	0.41 (0.21-0.79)
IR	18 / 1105	5.3 (3.09-9.08)	4.56 (2.63-7.91)	4.23 (2.39-7.49)	4.11 (2.31-7.32)	4.08 (2.28-7.3)	3.36 (1.83-6.16)
Fatty liver + FIB 4 Q4	6 / 193	8.22 (3.55-18.99)	1.7 (0.67-4.35)	1.51 (0.59-3.88)	1.44 (0.56-3.7)	1.35 (0.52-3.49)	1.15 (0.45-2.98)
Fatty liver + IR	12 / 227	17 (9.11-31.75)	10.29 (5.42-19.54)	9.49 (4.77-18.9)	9.21 (4.53-18.73)	8.95 (4.35-18.43)	6.6 (3.05-14.27)
IR + FIB 4 Q4	5 / 175	9.71 (3.9-24.19)	2.68 (1.02-7)	2.39 (0.91-6.3)	2.33 (0.89-6.15)	2.28 (0.86-6.1)	1.82 (0.67-4.95)
Fatty liver + FIB 4 Q4 + IR	2 / 36	19.92 (4.87-81.5)	3.39 (0.8-14.44)	2.87 (0.67-12.3)	2.68 (0.62-11.53)	2.32 (0.52-10.35)	1.50 (0.32-6.95)

Adjustments Model 1: age, education, exercise, smoking and alcohol intake (g/day), Center, Year, family history of diabetes

Model 2: Model 1 + waist circumference

Model 3 Model 2 + BMI

Model 4 Model 3 + medication for hypertension and hyperlipidaemia

Model 5 Model 4 + triglyceride and LDL-C

BMI, body mass index; HOMA-IR, homeostatic model assessment-Insulin resistance; IR, insulin resistance.