

Nonalcoholic Fatty Liver Disease Is a Risk Factor for Type 2 Diabetes in Middle-Aged Japanese Men

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OBJECTIVE — To determine the association between nonalcoholic fatty liver disease and the risk for development of diabetes.

RESEARCH DESIGN AND METHODS — We conducted an observational cohort study in male workers ≥ 40 years old in a Japanese company from 1997 to 2005. We excluded workers with alcohol intake ≥ 20 g/day and those with impaired glucose tolerance by a 75-g oral glucose tolerance test. The remaining 3,189 workers were classified into fatty liver (FL) and non-FL group based on the findings of abdominal ultrasonography. Both groups were followed for the development of diabetes. Hazard ratio (HR) was determined in Cox proportional hazard analysis. A nested case-control study was conducted to determine the odds ratio (OR).

RESULTS — The average age of participants was 48.0 years at the entry, and the average follow-up period was 4.0 years. The incidence of diabetes in the FL group was 2,073 per 100,000 person-years (65 cases), whereas 452 per 100,000 person-years (44 cases) in the non-FL group. The age- and BMI-adjusted HR of diabetes associated with FL was 5.5 (95% CI 3.6–8.5, $P < 0.001$). In the nested case-control analysis, the OR adjusted for age and BMI was 4.6 (3.0–6.9, $P < 0.001$).

CONCLUSIONS — Nonalcoholic fatty liver disease significantly increases the risk of diabetes in middle-aged Japanese men.

Diabetes Care 30:2940–2944, 2007

Nonalcoholic fatty liver disease (NAFLD) has become the most common disease of chronic liver damage, with increased prevalence of obesity, diabetes, and metabolic syndrome in the U.S. (1–3). The prevalence of NAFLD is increasing in Japan because of the westernization of the lifestyle, such as a high-fat and high-calorie diet and less physical activity (4). The high prevalence of fatty liver in association with type 2 diabetes has been reported (5,6).

NAFLD is characterized by significant lipid deposition in hepatocytes in patients without history of excessive alcohol intake and is often associated with obesity

(7), type 2 diabetes (8,9), dyslipidemia (10), and hypertension (11). Although they are often categorized as the insulin resistance syndrome or the metabolic syndrome (12), each of these individual abnormalities carries a risk of cardiovascular disease. In addition, diabetes, insulin resistance, and increased plasma fatty acids are considered to increase the risk for NAFLD (13,14), and each of these metabolic factors is also characteristic of type 2 diabetes. It has been reported that NAFLD influences severity of hepatic insulin resistance in type 2 diabetes (15). Moreover, NAFLD was correlated with hepatic insulin resistance independently of obe-

sity and intra-abdominal adiposity among nonobese men without type 2 diabetes (16).

Increased prevalence of NAFLD in relation to the development of diabetes has been reported in a cross-sectional study (17), and a close relationship between liver enzymes and diabetes has been reported in cohort studies (18–23). However, the former does not prove a causal relationship, and the latter does not directly pay attention to NAFLD. There is no cohort study about the direct relationship between NAFLD and incidence of diabetes. The causal relationship between NAFLD and diabetes is not clear, but NAFLD might play an important role as a confounding factor. The present study was designed to clarify the significance of NAFLD on glucose intolerance in an observational cohort study.

RESEARCH DESIGN AND METHODS

We conducted an observational cohort study with a nested case-control analysis in a telecommunication company in Kyushu, Japan. The Industrial Safety and Health Law in Japan requires the employer to conduct annual health examinations of all employees, and employees are required by law to participate. Moreover, the workers ≥ 40 years old are required by law to undergo biochemical examinations such as fasting plasma glucose and liver function test.

In this company, in addition to these annual screenings, all applicants ≥ 40 years of age can undergo more detailed medical checkups such as a 75-g oral glucose tolerance test, abdominal ultrasonography, and questionnaires on lifestyle characteristics every year from 1980. All data are compiled into a database for individual health maintenance and management from 1997, and an observational cohort study to clarify risk factors for metabolic disorders had prospectively started at the same time. All applicants received for annual medical checkups were informed, and we got the informed consent that their data will be used for scientific study, when they registered to the study. This study is an ongoing cohort study, and applicants were

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Received for publication 24 April 2007 and accepted in revised form 25 July 2007.

Published ahead of print at <http://care.diabetesjournals.org> on 31 July 2007. DOI: 10.2337/dc07-0792.

Abbreviations: FL, fatty liver; NAFLD, nonalcoholic fatty liver disease.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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registered at any time when they agree. Furthermore, when the Japanese Personal Information Protection Law came into effect in April 2005, we reconfirmed approval of both employees and employers for research.

Study population

Between April 1997 and May 2005, 6,798 male workers were registered with the observational cohort study. We excluded 1,405 workers with daily alcohol intake of ≥ 20 g at the time of registration and those with < 1 year of follow-up. Of 5,393 potential participants, 2,204 were excluded because of the following reasons: 1,728 had impaired glucose tolerance, impaired fasting glucose, or diabetes on a 75-g oral glucose tolerance test based on the criteria of the American Diabetes Association; 394 were using medications for hypertension, dyslipidemia, liver disease, or thyroid disease; 246 were positive for markers of viral hepatitis (hepatitis B surface antigen or hepatitis C antibody); 14 had a history of either coronary heart disease or stroke; and 72 had a history of gastrectomy at the time of registration. The remaining 3,189 employees constituted the study cohort. From the study cohort, those with fatty liver (diagnosed by abdominal ultrasonography as described below) were categorized as the fatty liver (FL) group ($n = 802$) and those without FL as the non-FL group ($n = 2,387$). The outcome of interest was the incidence of diabetes during follow-up.

Nested case-control analysis

We conducted a nested case-control analysis to facilitate multivariate analysis as well as assessment of any duration-response effect. All cases ($n = 109$) of diabetes from the cohort study were included as the cases. Control subjects were randomly selected from the employees in the study cohort who did not develop diabetes in the follow-up period using incidence density sampling (24). Each case was matched with up to 10 control subjects on the year of birth (± 1 year), duration of follow-up (± 6 months), and BMI (± 0.5 kg/m²). Control subjects were assigned the same index date as the matched case subject.

Measurements

The annual clinical examination consisted of a medical history, physical examination, blood pressure measurement, anthropometric measurements, and questionnaire on lifestyle characteristics,

such as physical activity, the number of cigarettes smoked, and daily alcohol consumption. Trained nurses took all measurements. Employees were asked to fast for 12 h and to avoid smoking and heavy physical activity for at least 2 h before the examinations. After a 5-min rest in a quiet room, systolic and diastolic blood pressure was measured twice at an interval of a few minutes on the right arm with a standard mercury sphygmomanometer. Anthropometric measurements, including height, weight, and percentage of body fat, were measured. The BMI was calculated as the weight in kilograms divided by the height in meters squared. Blood samples were drawn from an antecubital vein. Serum concentrations of total cholesterol, HDL cholesterol, triglyceride, plasma glucose, A1C level, and serum enzyme activities of aspartate aminotransferase, alanine aminotransferase, γ -glutamyltransferase, and alkaline phosphatase were determined by standard laboratory procedures (7,23). FL was diagnosed based on the results of abdominal ultrasonography by one gastroenterologist without reference to any of the employee's other individual data. Employees with hepatorenal echo contrast and liver brightness were diagnosed as FL. Employees were asked about the type and weekly frequency of leisure-time physical activity. Physical exercise was

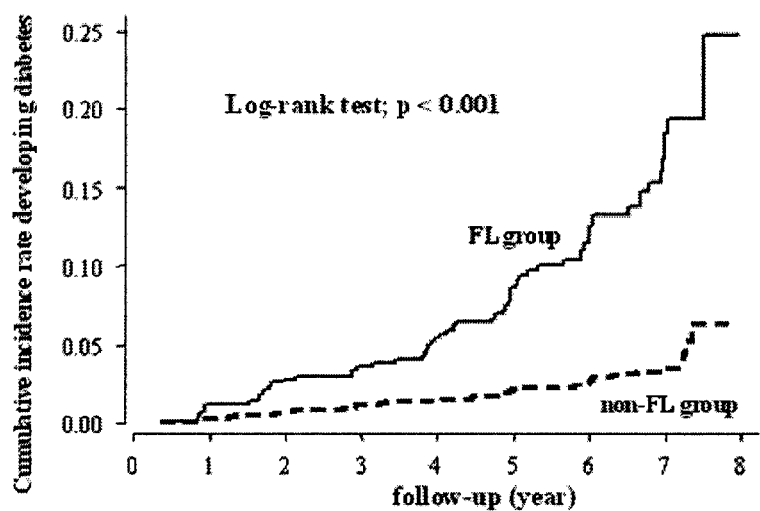
defined as participation in any physical activity such as walking, jogging, swimming, or football that was performed long enough to work up a sweat. The questions related to alcohol intake included items about the type of alcoholic beverage, the frequency of alcohol consumption per week, and the usual amount of daily consumption. Weekly alcohol intake was calculated and then converted to daily alcohol consumption (grams of ethanol per day). Subjects were categorized based on cigarette smoking habit into never, ex, or current smokers; ex or current smokers were required to specify the number of cigarettes smoked per day and years of smoking.

Diagnosis of type 2 diabetes

Employees with fasting plasma glucose level ≥ 7.0 mmol/l and 2-h postload plasma glucose level ≥ 11.1 mmol/l on a 75-g oral glucose tolerance test were diagnosed as having type 2 diabetes. Because of the age range of the study population, all cases of diabetes were diagnosed after the age of 40 years and were thus classified as type 2 diabetes.

Diagnosis of the metabolic syndrome

The Japanese Society of Internal Medicine proposed the following criteria for the diagnosis of the metabolic syndrome (25): 1) abdominal obesity (abdominal circum-



Year of follow-up	0	1	2	3	4	5	6	7	8
FL	802	743	543	470	397	336	251	121	1
non-FL	2,387	2,208	1,605	1,422	1,259	1,103	905	498	6

Figure 1—Cumulative incidence rate of individuals who developed diabetes during the follow-up among the FL and non-FL groups. The table shows the number of uncensored patients left in the cohort at the beginning of each year of follow-up.

Table 1—Multivariate Cox proportional hazards analysis of time to developing diabetes for the FL group and non-FL group

	n	Diabetes [n (%)]	Adjusted*		Adjusted†	
			HR (95% CI)	P	HR (95% CI)	P
Non-FL	2,387	44 (1.8)	1.0		1.0	
FL	802	65 (8.1)	4.8 (3.3–7.1)	<0.001	5.5 (3.6–8.5)	<0.001

*Adjusted for age. †Adjusted for age and BMI.

ference ≥ 85 cm for men and ≥ 90 cm for women), 2) elevated serum triglyceride (≥ 1.70 mmol/l) or decreased HDL cholesterol (< 1.04 mmol/l) concentrations, 3) elevated arterial blood pressure (systolic/diastolic blood pressure $\geq 130/85$ mmHg), and 4) an elevated fasting plasma glucose level (≥ 6.11 mmol/l). In our study, we substituted BMI instead of waist circumference because waist circumference was not measured. A BMI ≥ 25 kg/m² has been proposed as a cutoff for the diagnosis of obesity in Japan. In our study, individuals with obesity and two or more of the above-mentioned criteria were considered to have the metabolic syndrome.

Statistical analysis

The incidence of diabetes was calculated for the FL and non-FL groups. The hazard ratio (HR) of diabetes associated with FL was calculated using Cox proportional hazard regression after adjusting for age. For the nested case-control analysis, we used logistic regression (26) to calculate odds ratio (OR) and 95% CIs as estimates of the relative risk of diabetes associated with FL. Data were analyzed using the SPSS/PC statistical package (SPSS, Chicago, IL). The unpaired *t* test and χ^2 test were used to analyze statistical differences among clinicopathological features of study participants at the time of registration. All reported *P* values are two-tailed, and those < 0.05 were considered statistically significant.

RESULTS— The FL group accumulated 3,135 person-years of follow-up, and the non-FL group was followed for 9,744 person-years. The duration of follow-up in the FL group was 3.9 ± 2.4 years (range 1–8 years) and non-FL group was 4.1 ± 2.5 years (range 1–8 years). BMI of the FL group was higher than that of the non-FL group (24.8 ± 2.5 vs. 22.5 ± 2.3 kg/m², $P < 0.0001$). The incidence of diabetes in the FL group was 2,073 per 100,000 person-years (65 cases), whereas that in the non-FL group

was 452 per 100,000 person-years (44 cases). The cumulative incidence of diabetes of both groups is illustrated in Fig. 1. The age-adjusted HR of diabetes in the FL group was 4.8 (95% CI 3.3–7.1, $P < 0.0001$, Table 1). Additional adjustment for BMI had a large effect (HR 5.5 [95% CI 3.6–8.5, $P < 0.001$, Table 1]).

In the nested case-control analysis, the 109 case subjects who developed diabetes in the cohort study were matched with 1,044 control subjects. We were able to identify at least one control subject for each of all case subjects, and nine control subjects for $> 90\%$ of case subjects. In addition to the matching variables, the case and control subjects were also similar with regard to age, duration of follow-up period, prevalence of metabolic syndrome, smoking and physical activity sta-

tus, BMI, and blood pressure (Table 2). The prevalence of FL was significantly higher in case subjects than in control subjects, but the prevalence of metabolic syndrome was similar in the two groups. Employees with FL had about four times higher risk of diabetes (unadjusted OR 4.4 [95% CI 2.9–6.7, $P < 0.001$], Table 3) compared with non-FL. In addition, the adjusted OR for age and BMI was 4.6 (95% CI 3.0–6.9, $P < 0.001$, Table 3).

CONCLUSIONS— In the present cohort study, we have demonstrated that NAFLD is a strong risk factor for developing diabetes in middle-aged healthy Japanese men. This relationship is statistically significant even after adjustment for age and BMI.

The public health implications of the emerging pandemic of obesity are dire in light of the growing list of associated metabolic consequences. This list includes dyslipidemia, hypertension, type 2 diabetes, and cardiovascular disease (27). Previous studies have reported the cross-sectional associations between diabetes and NAFLD (17,28). Jimba et al. (17) demonstrated that the prevalence of NAFLD in healthy middle-aged Japanese individuals increases with impairment of

Table 2—Characteristics of case and control subjects

	Case subjects	Control subjects	P
n	109	1,044	
Age (years)	48.5 \pm 4.6	48.0 \pm 4.8	0.42
Duration of follow-up (years)	4.1 \pm 2.0	4.1 \pm 2.5	0.81
NAFLD (%)	59.6	25.0	<0.001
Metabolic syndrome (%)	7.3	6.1	0.62
Current smoker (%)	55.0	53.1	0.69
Regular physical activity at least once a week (%)	54.1	57.9	0.45
BMI (kg/m ²)	23.5 \pm 2.4	23.4 \pm 2.5	0.83
Body fat (%)	21.4 \pm 4.9	21.6 \pm 4.5	0.72
Systolic blood pressure (mmHg)	127 \pm 16	126 \pm 15	0.58
Diastolic blood pressure (mmHg)	78 \pm 11	79 \pm 11	0.43
75-g oral glucose tolerance test			
Fasting plasma glucose (mmol/l)	5.2 \pm 0.4	5.2 \pm 0.4	0.38
1-h plasma glucose (mmol/l)	9.5 \pm 1.9	7.9 \pm 1.9	<0.001
2-h plasma glucose (mmol/l)	6.3 \pm 1.0	6.1 \pm 1.0	0.02
A1C (%)	5.0 \pm 0.4	4.9 \pm 0.4	0.001
Total cholesterol (mmol/l)	5.1 \pm 0.8	5.2 \pm 0.8	0.35
HDL cholesterol (mmol/l)	1.3 \pm 0.4	1.4 \pm 0.4	0.002
Triglyceride (mmol/l)	1.5 \pm 0.7	1.4 \pm 0.9	0.49
Aspartate aminotransferase (IU/l)	25 \pm 9	23 \pm 7	0.03
Alanine aminotransferase (IU/l)	30 \pm 17	27 \pm 16	0.03
γ -Glutamyltransferase (IU/l)	44 \pm 30	40 \pm 26	0.08
Alkaline phosphatase (IU/l)	151 \pm 41	166 \pm 51	0.002

Data are means \pm SD or percent of subjects.

Table 3—Unadjusted and adjusted ORs and 95% CIs for diabetes risk for FL and non-FL groups

	Case subjects [n (%)]	Control subjects [n (%)]	Unadjusted		Adjusted	
			OR (95% CI)	P	OR (95% CI)	P
Non-FL	44 (59.6)	261 (25.0)	1.0		1.0	
FL	65 (40.4)	783 (75.0)	4.4 (2.9–6.7)	<0.001	4.6 (3.0–6.9)	<0.001

glucose tolerance: 27% in normal fasting glucose, 43% in impaired fasting glucose, and 62% in newly diagnosed diabetes (17). However, a cross-sectional relationship alone does not prove a causal relation. We have shown in the present cohort study that participants with NAFLD have about five times higher risk for future diabetes.

A number of cohort studies have indicated the relationship between liver injury aspartate aminotransferase and diabetes (18,20–23,29). In the CARDIA (Coronary Artery Risk Development in Adults) study, aspartate aminotransferase was significantly correlated.

In contrast, Nakanishi et al. (20) have reported that alanine aminotransferase or aspartate aminotransferase was not associated with diabetes risk in male Japanese workers. Previous studies have shown that elevated liver enzymes reflect fatty changes in the liver, which associate with insulin resistance (30–34). However, in these studies, ultrasonography, computed tomography, or liver biopsies were not performed to evaluate FL changes. Moreover, most of these reports included heavy alcohol drinkers, and they calculated the relationship by adjusting the dose of alcohol intake. What is important in the present study design was that we excluded those individuals with daily alcohol intake ≥ 20 g, and we estimated the direct relationship between NAFLD and diabetes.

Intra-abdominal fat accumulation plays an important role in the metabolic syndrome, and previous studies have related FL to waist circumference or central abdominal adiposity (13,14,30,35–37). The “two-hit” theory of steatohepatitis was proposed under the observation that not all patients with steatosis progress to steatohepatitis (38,39). The first hit is the accumulation of excess fat in the hepatic parenchyma. The second hit is the progression from FL to steatohepatitis. It is generally accepted that oxidative stress causes the peroxidation of lipids in the hepatocyte membrane, which can initiate fibrosis via the proinflammatory cyto-

kines and activation of hepatic stellate cells. The first hit links to insulin resistance, including obesity, diabetes, and dyslipidemia. At this step, hepatic insulin resistance is the most important factor in whole-body insulin resistance.

Metabolic syndrome is a primary phenomenon of type 2 diabetes associated with insulin resistance. In our nested case-control study, the prevalence of metabolic syndrome was low, but NAFLD was high (case vs. control subjects, 7.3 vs. 6.1% with metabolic syndrome, 59.6 vs. 25.0% with NAFLD). We propose that NAFLD is an important phenomenon in the development of diabetes and is expressed earlier than the metabolic syndrome.

A few potential limitations need to be considered. First, misreports of the self-reported information including alcohol intake could be a source of bias. Second, ultrasonography has a sensitivity of 89% and a specificity 93% in detecting FL, but it may give an incorrect diagnosis in 10% of case subjects (40). Moreover, ultrasonography cannot distinguish steatohepatitis from simple steatosis and does not distinguish between NAFLD and alcohol-related liver disease. Third, we used a cutoff level of <20 g daily ethanol consumption to define a nonalcoholic person. Although the ideal cutoff level is not known (41), <20 g ethanol per day did not seem to increase the risk for the disease.

In conclusion, we found that NAFLD is a risk of diabetes in middle-aged Japanese men.

Acknowledgments—The authors thank the employees and the medical staff of the telecommunication company for participating and cooperating in this study.

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