Histological course of nonalcoholic fatty liver disease in Japanese patients: Tight glycemic control, rather than weight reduction, ameliorates liver fibrosis

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The histological course of nonalcoholic fatty liver disease in Japanese patients: Tight glycemic control, rather than weight reduction, ameliorates liver fibrosis

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Short running title: Glycemic control ameliorates NAFLD

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Abbreviations: α-GI, α-glucosidase inhibitor; AUC, area under the curve; FPG, fasting plasma glucose; hs-CRP, high-sensitivity C-reactive protein; HOMA-IR, homeostasis model assessment of insulin resistance; IGT, impaired glucose tolerance; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; PAI-1, plasminogen activator inhibitor type 1; p-III-p, procollagen III peptide; QUICKI, quantitative insulin-sensitivity check index; SU, sulfonylurea; TGF, transforming growth factor.

ABSTRACT

OBJECTIVE – The goal of this study was to examine whether metabolic abnormalities are responsible for the histological changes observed in Japanese patients with nonalcoholic fatty liver disease (NAFLD) who have undergone serial liver biopsies.

RESEARCH DESIGN AND METHODS – In total, 39 patients had undergone consecutive liver biopsies. Changes in their clinical data were analyzed, and biopsy specimens were scored histologically for stage.

RESULTS – The mean follow-up time was 3.0 years (range, 1.0–8.5 years). Liver fibrosis had improved in 12 patients (30.7%), progressed in 11 patients (28.2%), and remained unchanged in 16 patients (41%). Predictive variables for liver fibrosis progression included baseline aspartate aminotransferase activity and ΔA1C at follow-up (P=0.04 and 0.01, respectively). In a multiple regression analysis, only ΔA1C was an independent predictor of fibrosis progression in the liver (r = 1.23, P=0.04).

CONCLUSIONS – Tight glycemic control is necessary to prevent histological progression in Japanese patients with NAFLD.

Accumulating trans-sectional evidence suggests that the presence of multiple metabolic disorders, including obesity, diabetes, dyslipidemia, hypertension, and ultimately metabolic syndrome, are associated with nonalcoholic fatty liver disease (NAFLD) (1). However, it remains unclear which metabolic abnormalities are responsible for the pathological progression of NAFLD, especially in Japanese patients, who generally are not severely obese compared with Western patients.

We retrospectively compared clinical features with the histological changes in the livers of Japanese patients with NAFLD who had undergone serial liver biopsies.

RESEARCH DESIGN AND METHODS

Subjects

We recruited 195 patients with clinically suspected NAFLD who had undergone liver biopsies at Kanazawa University Hospital from 1997 through 2008. For details about the study subjects and the exclusion criteria, see Supplementary Figure 1. Of 178 patients diagnosed histologically as NAFLD, 39 had undergone serial liver biopsies.

Data collection

Clinical information, including age, gender, body measurements, and prevalence of

metabolic abnormalities, was obtained for each patient. Venous blood samples drawn for laboratory testing before the liver biopsies were obtained. All subjects had been administered a 75-g oral glucose tolerance test at baseline and at follow-up.

Liver biopsies

Biopsies were obtained after a thorough clinical evaluation and receipt of signed informed consent from each patient. All biopsies were analyzed twice and at separate times randomly by a single pathologist who was blinded to the clinical information and the order in which the biopsies were obtained. The biopsied tissues were scored for steatosis, stage, and grade as described (2), according to the standard criteria for grading and staging of nonalcoholic steatohepatitis (NASH) proposed by Brunt et al. (3).

For additional details on subjects, data collection methods, liver pathology, and statistical analyses, see Supplementary Methods.

RESULTS

The basal clinical and biochemical data from 39 patients with NAFLD are described in Supplementary Table 1. Prevalence of type 2 diabetes, hypertension, and dyslipidemia were 76, 35, and 64%, respectively. The mean follow-up period was 3.0 years (range,

1.0–8.5 years). Medications for diabetes and medication changes during the follow-up period are described in Supplementary Table 2. Seventeen patients treated with oral diabetic agents were switched to insulin therapy after the initial biopsy. No patients initiated pioglitazone during follow-up.

Liver fibrosis improved in 12 patients (30.7%), progressed in 11 patients (28.2%), and remained unchanged in 16 patients (41%). As shown in Table 1, fasting plasma glucose (FPG), A1C, insulin resistance indicators, and prevalence of metabolic disorders were not significantly different among the three liver fibrosis groups. Predictive values for the histological progression of the liver included the baseline aspartate aminotransferase activity and the change in A1C (ΔA1C) between the initial and final liver biopsies (*P*=0.04 and 0.01, respectively). In a multiple regression analysis, ΔA1C was significantly associated with fibrosis progression (r = 1.23, *P*=0.04), although basal aspartate aminotransferase was not significant, as shown in Supplementary Table 3. In a logistic regression analysis, ΔA1C was significantly associated with fibrosis improvement (odds ratio, 0.47; *P*=0.03) after adjustments for age, gender, BMI, diabetes, hypertension, dyslipidemia, treatment with angiotensin II receptor blockers, insulin and observation period (Supplementary Table 4).

CONCLUSIONS

In the present study, we showed that a change in glycemic control (ΔA1C), but not changes in insulin resistance indicators, was an independent predictor of the progression of liver fibrosis in Japanese patients with NAFLD. This is the first report identifying a change in A1C as a predictor of the histological course in the liver of patients with NAFLD. Two of five previous longitudinal studies have identified obesity, higher BMI, and homeostasis model assessment of insulin resistance (HOMA-IR) as predictors of liver fibrosis progression in Western populations (4; 5). The difference between those results and the results of the present study may be due in part to differences in the assessed severity of obesity and insulin resistance between the populations. We previously demonstrated that diabetes is an independent risk factor for the progression of liver fibrosis in hepatitis C (6) and that diabetes accelerates the pathology of NASH in the type 2 diabetic rat model OLETF (7).

Liver fibrosis is closely associated with two regulators of fibrosis: transforming growth factor-β (TGF-β) (8; 9) and plasminogen activator inhibitor type 1 (PAI-1) (8; 10). High glucose levels induce the expression of TGF-β (11) and PAI-1 (12). We previously reported that the expression of TGF family member genes, PAI-1, and genes involved in fibrogenesis are up-regulated in the livers of patients with type 2 diabetes (13; 14),

suggesting that a diabetic state increases the risk for liver fibrosis.

In the present study, only ΔA1C was associated with the progression of liver fibrosis, but not liver inflammation (data not shown). We speculate that the reduction of A1C inhibits the expression of master genes such as *TGF-β* and *PAI-1* that are involved in the regulation of fibrogenesis, rather than genes involved in liver inflammation, and thereby improves liver fibrosis in NAFLD.

The limitation of this study was small population size, and most of the study patients were diabetic. We could not evaluate the changes of liver histology according to the difference in detail characteristics such as treatment of diabetes. A large-scale prospective study is necessary to validate our results and to elucidate natural course of NAFLD.

In conclusion, ΔA1C is an independent predictor for liver fibrosis progression in Japanese patients with NAFLD, and tight glycemic control is necessary to ameliorate liver fibrosis.

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Table 1 – Basal and final clinical features and gradients of laboratory markers associated with changes in liver fibrosis in 39 patients with NAFLD

	The basal data				**The final data**			
	Improved (n=12)	Stable (n=16)	Progressed (n=11)	*P*	Improved (n=12)	Stable (n=16)	Progressed (n=11)	*P*
simple fatty liver:NASH, n	3:9	9:7	10:1		10:2	9:7	6:5	
Age (years)	51.5 (29-66)	48.5 (20-79)	51.5 (29-66)	0.9				
Gender (M : F)	5 : 7	12 : 4	5 : 7	0.3				
BMI (kg/m^2)	27.5 (23.2-34.1)	27.7 (22.5-44.4)	30.9 (23.4-37.7)	0.7	26.9 (22.8-31.2)	29.1 (24.3-44.8)	30.7 (24.1-36.3)	0.13
AST (IU/L)	70 (11-106)	29 (14-86)	32 (13-83)	0.04	23 (11-28)	26 (15-71)	24 (14-164)	0.2
ALT (IU/L)	71 (10-209)	48 (23-81)	40 (11-162)	0.1	21 (11-53)	36 (21-66)	31 (12-202)	0.1
FPG (mg/dl)	133 (96-207)	143 (87-414)	111 (76-167)	0.1	103 (93-220)	121 (83-198)	116 (88-199)	0.5
A1C (%)	8.2 (4.7-11.6)	8.0 (4.9-13.6)	6.2 (5.1-9.5)	0.2	6.0 (5.0-9.0)	6.2 (5.0-10.0)	7.0 (6.0-11.0)	0.1
HOMA-IR	3.9 (0.7-5.5)	3.4 (1.9-7.7)	3.9 (1.6-11.1)	0.9	3.1 (1.5-8.5)	3.4 (1.9-7.7)	3.9 (1.6-11.1)	0.7
QUICKI	0.32 (0.29-0.40)	0.31 (0.27-0.34)	0.31 (0.29-0.35)	0.5	0.33 (0.28-0.37)	0.32 (0.30-0.35)	0.31 (0.29-0.34)	0.5
Muscle insulin resistance	2.1 (1.5-4.0)	1.7 (0.3-3.3)	3.0 (2.1-4.4)	0.5	2.0 (1.3-5.9)	2.4 (1.6-4.5)	1.9 (1.3-4.5)	0.3
Hepatic insulin resistance (x10^6)	5.3 (2.3-10.2)	5.0 (2.3-10.0)	3.7 (1.4-10.6)	0.3	3.9 (1.4-9.8)	4.3 (1.9-15.9)	4.5 (2.3-8.8)	0.4
Total cholesterol (mg/dl)	191 (128-276)	187 (129-252)	206 (163-244)	0.6	192 (114-224)	195 (136-273)	194 (162-234)	0.7
TG (mg/dl)	111 (28-224)	114 (36-204)	96 (36-521)	0.7	104 (22-241)	115 (57-241)	131 (36-173)	0.6
HDL – cholesterol (mg/dl)	47 (35-82)	51 (31-73)	48 (20-74)	0.7	53 (40-71)	52 (39-64)	52 (36-79)	0.9
Platelets (x10^4/µl)	21.1 (9.4-30.8)	23.0 (7.0-38.2)	24.3 (20.2-41.2)	0.2	23.3 (14.5-27.6)	21.5 (6.3-31.8)	24.0 (15.2-32.6)	0.6
Ferritin (µg/dl)	185 (13-452)	397 (190-604)	46 (10-347)	0.1	74 (16-211)	162 (110-614)	62 (10-171)	0.05
hsCRP	0.40 (0.08-7.53)	0.14 (0.02-0.61)	0.06 (0.00-0.30)	0.2	0.09 (0.04-0.23)	0.10 (0.00-0.24)	0.09 (0.00-0.89)	0.8
Type IV collagen 7S (ng/dl)	5.1 (2.7-10.0)	4.1 (3.1-7.2)	3.7 (3.3-4.5)	0.2	3.5 (2.3-3.9)	8.3 (3.2-14.0)	4.0 (3.2-5.0)	0.2
HA (ng/dl)	20.6 (0.0-144.7)	25.5 (11.5-299)	30.4 (0.0-61.7)	0.8	32.8 (0.0-117.2)	24.5 (0.0-570)	24.3 (0.0-140.3)	0.6
P-III-P (U/ml)	0.6 (0.5-1.2)	0.6 (0.4-45.0)	0.5 (0.4-0.6)	0.1	0.6 (0.3-0.8)	0.5 (0.5-233.0)	0.6 (0.4-1.0)	0.9
Diabetes (%)	82	69	64	0.8	82	75	64	0.7
Dyslipidemia (%)	73	63	73	0.7	73	63	73	0.7
Hypertension (%)	64	18	36	0.2	64	18	36	0.2
Metabolic syndrome (%)	73	38	27	0.3	67	50	45	0.5
∆A1C					-1.9 (-6.0 - +0.4)	-1.2 (-6.1 - +4.4)	+0.3 (-1.8 - +7.1)	0.01
∆BW					-4.7 (-10.6 - +10.2)	+2.2 (-9.4 - +13.4)	-0.9 (-12.7 - +9.6)	0.05
∆ HOMA-IR					-1.3 (-4.4 - +1.2)	-0.3 (-4.3 - +3.3)	-0.7 (-6.1 - +1.8)	0.6

Data are median (range) or %. BW, body weight; HA, hyaluronic acid.

Electronic supplementary material

Additional details on methods

Subjects

We consecutively recruited 195 patients with clinically suspected NAFLD who had undergone liver biopsies at Kanazawa University Hospital from 1997 through 2008. Fatty liver was clinically diagnosed based on ultrasound examination showing an increase in hepatorenal contrast. Hepatorenal contrast, also known as "bright liver" (1), is defined as the ratio of hepatic to kidney echo levels of over 1.0. In each patient, all other liver disorders were excluded, including viral hepatitis B and C, primary biliary cirrhosis, autoimmune hepatitis, sclerosing cholangitis, hemochromatosis, Wilson's disease, drug-induced liver injury, and biliary obstruction. All patients reported drinking less than 20 g/day of ethanol. Liver biopsies were performed during hospitalization. Serum elevations in transaminase (>40 IU/L) were examined and metabolic disorders such as diabetes mellitus and obesity were treated. None of the patients were on medication (e.g., vitamin E or ursodeoxycholic acid), which could influence histological changes in the liver throughout the follow-up period.

Data collection

A diagnosis of diabetes mellitus was based on the American Diabetes Association criteria (2). Hypertension and dyslipidemia were defined according to metabolic syndrome definitions provided by the National Cholesterol Education Program–Adult Treatment Panel III (3).

Metabolic syndrome was defined as the presence of abdominal obesity (given as waist circumference: ≥85 cm for men, ≥90 cm for women) and included at least two of the following components: hypertriglyceridemia [≥150 mg/dl (≥1.69 mmol/l)] and/or low HDL-cholesterolemia [<40 mg/dl (<1.03 mmol/l)]; blood pressure (systolic ≥130 and/or diastolic ≥85 mmHg), and fasting plasma glucose (FPG) [≥110 mg/dl (≥6.11 mmol/l)] according to the Japanese diagnostic criteria for metabolic syndrome (4). Laboratory tests included liver enzymes, blood counts, fasting lipid profile, A1C, glucose, insulin, high-sensitivity C-reactive protein (hs-CRP), ferritin, liver fibrosis markers such as type IV collagen domain 7S, hyaluronic acid, and procollagen III peptide (p-III-p) . All tests were conducted and analyzed at the central clinical laboratory in our hospital. Insulin resistance indicators such as homeostasis model assessment of insulin resistance (HOMA-IR), quantitative insulin-sensitivity check index (QUICKI), and indices for muscle and hepatic insulin resistance were calculated based on the results of the 75-g oral glucose tolerance tests, using previously reported formulas: HOMA-IR = [fasting insulin (μU/ml) × fasting plasma glucose (μmol/l)]/22.5 (5), QUICKI = 1/[log (fasting insulin expressed in μU/ml) + log (fasting plasma glucose expressed in mg/dl)] (6). Index for

muscle insulin resistance = 10,000/log [fasting glucose (mg/dl) × fasting insulin (μU/ml)] × [mean glucose (mg/dl) × mean insulin during the oral glucose tolerance test (μU/ml)], and Index for hepatic insulin resistance = $glucose_{0–30}$ area under the curve (AUC) × $insulin_{0–30}$ AUC, respectively (7).

Liver biopsies

Sections were cut from a paraffin block and stained with hematoxylin and eosin, and Azan–Mallory, and silver reticulin impregnation. The biopsied tissues were scored for steatosis (from 0 to 3), stage (from 1 to 4), and grade (from 1 to 3) as described (8), according to the standard criteria for grading and staging of NASH proposed by Brunt et al. (9). All cases showed variable degrees of steatosis that histologically corresponded to NAFLD. NASH was histologically diagnosed based on the presence of ballooned hepatocytes with lobular hepatitis. In contrast, livers without ballooned hepatocytes were diagnosed as fatty liver. The scoring system according to Brunt classification (9) applies to NASH, but not fatty liver. Therefore, for fatty liver without any features of steatohepatitis, we assigned a score of stage 0 and grade 0. In addition, when histological activity or fibrosis varied within a biopsy, cases were scored as an average (i.e., 0.5 or 1.5) so that we could examine histological changes in detail.

The progression of liver histology was defined as an increased stage score in the final biopsy with

respect to the baseline; a decreased stage score was considered an improvement.

Statistical analyses

Continuous data are presented as the medians (ranges) and categorical data are presented as a number (%). A Kruskal–Wallis test and a χ^2 test were applied for the analysis of differences in continuous and categorical variables. The independent influence of significant variables on fibrosis progression was assessed using a multiple regression analysis. The odds ratio was calculated for assessment of relative risks using a logistic regression.

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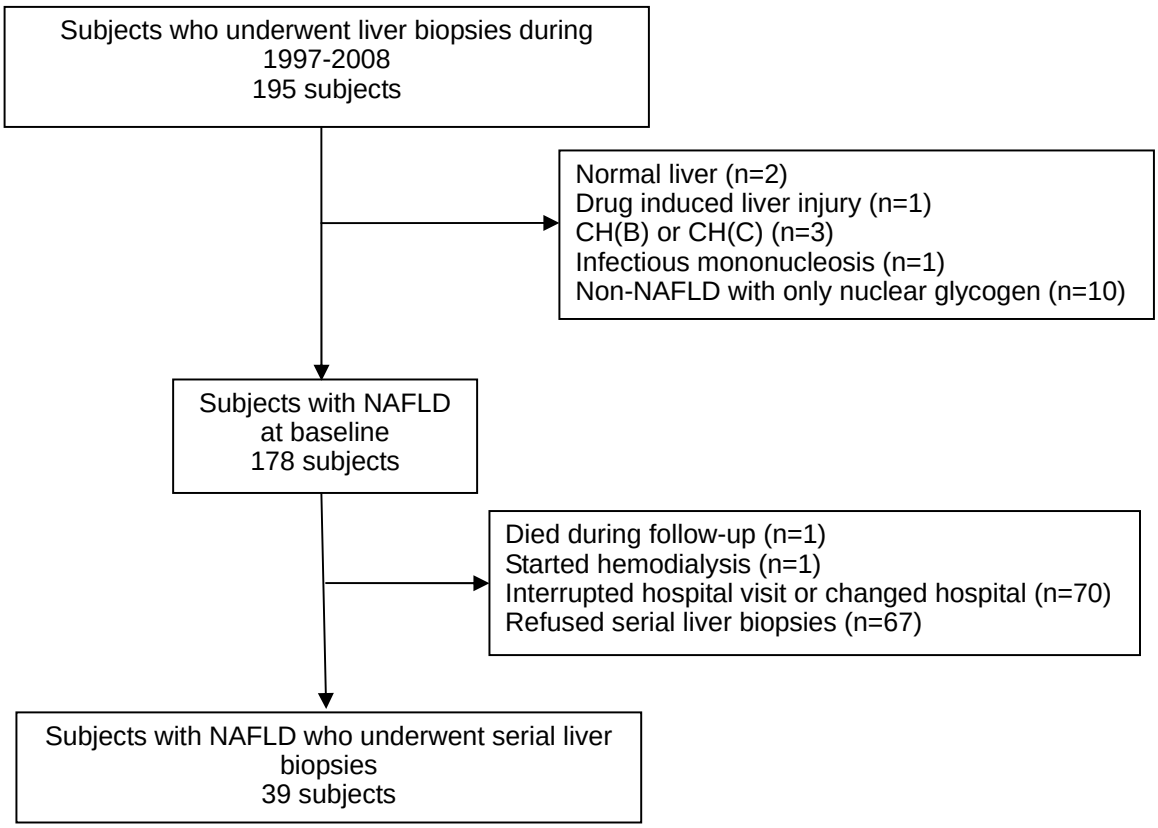
Supplementary Figure 1 – Description of study subjects and exclusion criteria



Supplementary Table1 – Basal clinical and biochemical data of 39 patients with NAFLD

		normal range
Age (years)	47 (20-79)	
Gender (M : F)	20 : 16	
BMI (kg/m^2)	27.8 (22.5-44.4)	18-25
AST (IU/L)	40 (11-106)	10-48
ALT (IU/L)	54 (10-209)	3-50
FPG (mg/dl)	128 (76-414)	70-110
HbA_{1c} (%)	6.6 (4.7-13.6)	4.3-5.8
HOMA-IR	3.9 (0.7-11.1)	<2.0
Total cholesterol (mg/dl)	199 (128-276)	132-220
TG (mg/dl)	112 (28-521)	32-150
HDL – cholesterol (mg/dl)	48 (20-82)	40-97
Platelets (x10^4/μl)	22.8 (7.1-41.2)	13-35
Ferritin (μg/dl)	185 (13-5-640)	6.2-138
hsCRP	0.17 (0-7.53)	<0.2
Type IV collagen 7S (ng/dl)	4.1 (2.7-10.0)	<6.0
HA (ng/dl)	24.7 (0-299)	<50
P-III-P (U/ml)	0.6 (0.4-45.0)	0.3-0.8
Diabetes (%)	30 (76.9)	
Dyslipidemia (%)	25 (64.1)	
Hypertension (%)	14 (35.8)	
Metabolic syndrome (%)	15 (38.4)	

Data are median (range) or numbers of patients; n=39; HA, hyaluronic acid.

Supplementary Table2 – Changes of pathological score and the treatment for diabetes and hypertension in 39 patients with NAFLD

Case	Initial diagnosis	Final diagnosis	Stage	Grade	Steatosis	DM	Treatment for DM	HT	ARB
Improved group									
1	NASH	FL	3 → 1	3 → 1	2 → 1	+	diet → diet	–	–
2	FL	FL	2 → 1	0 → 0	3 → 3	-		–	–
3	NASH	FL	2 → 1	1 → 1	2 → 1	+	diet → insulin	+	+
4	NASH	FL	2 → 1	2 → 0	2 → 2	+	diet → insulin	–	–
5	NASH	FL	1 → 0.5	1 → 1	3 → 2	+	SU → insulin	–	–
6	FL	FL	2 → 1	0 → 0	2 → 1	+	diet → insulin	–	–
7	NASH	FL	3 → 1	2 → 0	2 → 1	+	diet → diet	+	+
8	NASH	NASH	1 → 0.5	2 → 1	2 → 2	+	diet → insulin	+	+
9	NASH	FL	3 → 2	2 → 1	3 → 2	-		+	–
10	NASH	FL	3 → 2	2 → 0.5	3 → 2	+	diet → diet	–	–
11	FL	FL	1 → 0	0 → 0	1 → 1	+	diet → insulin	–	–
12	NASH	NASH	4 → 3	2 → 0.5	2 → 1	+	diet → insulin	+	–
Progressed group									
13	FL	FL	1 → 2	0 → 0	1 → 1	+	diet → insulin	+	+
14	FL	NASH	1 → 3	0 → 2	3 → 3	+ → IGT	diet → diet	–	–
15	FL	FL	1 → 2	0 → 1	1 → 1	+	diet → diet	+	+
16	FL	NASH	1 → 2	0 → 1	1 → 1	+	α–GI → SU, Met	+	+
17	FL	NASH	1 → 2	0 → 1	1 → 2	+	diet → α-GI	–	–
18	FL	FL	1 → 1.5	0 → 0.5	1 → 2	-		–	–
19	FL	NASH	1 → 2	0 → 3	3 → 2	+	diet → insulin	+	+
20	FL	FL	1 → 2	0 → 0	1 → 2	+	Met → Met, α-GI	+	–
21	FL	FL	1 → 1.5	0 → 0.5	3 → 2	+	diet → insulin	+	+
22	FL	FL	1 → 1.5	0 → 0.5	2 → 2	-		+	–
23	NASH	NASH	1 → 1.5	1 → 1.5	3 → 2	IGT	diet → diet	–	–
Stable group									
24	NASH	NASH	3 → 3	3 → 3	1 → 1	+	Met, SU → Met, SU	–	–
25	FL	FL	1 → 1	0 → 0	2 → 1	+	α–GI → insulin	+	+
26	FL	FL	1 → 1	0 → 0	2 → 1	+	diet → diet	–	–
27	FL	FL	1 → 1	0 → 0	2 → 1	+	Met, SU → insulin	–	–
28	FL	FL	1 → 1	0 → 0	1 → 1	+	diet → insulin	+	+
29	FL	FL	1 → 1	0 → 0	2 → 1	- → IGT	diet	–	–
30	FL	FL	1 → 1	0 → 0	1 → 2	- → IGT	diet	–	–
31	NASH	NASH	4 → 4	2 → 2	2 → 1	+	diet → insulin	–	–
32	FL	FL	1 → 1	0 → 0	3 → 3	+	diet → insulin	–	–
33	FL	FL	1 → 1	0 → 0	2 → 3	+	diet → diet	+	–
34	NASH	NASH	1 → 1	1 → 1	1 → 1	+	SU, Pio → insulin	–	–
35	NASH	NASH	1 → 1	1 → 1	3 → 3	+	diet → diet	–	–
36	FL	FL	2 → 2	0 → 0	3 → 3	+	diet → diet	–	–
37	NASH	NASH	2 → 2	1 → 1	2 → 2	+	diet → insulin	–	–
38	NASH	NASH	4 → 4	3 → 3	1 → 1	IGT	diet → diet	–	–
39	NASH	NASH	4 → 4	3 → 2	3 → 1	- → DM	diet	–	–

Patients with histological improvement (Cases 1–12), progression (Cases 13–23), or stability (Cases 24–39) are indicated in the Improved, Progressed, and Stable groups, respectively. Presence of diabetes and hypertension, and the treatment with ARB are indicated by +. α-GI, α-glucosidase inhibitor; ARB, angiotensin II receptor blocker; FL, fatty liver; IGT, impaired glucose torelance; Met, metformin; NASH, nonalcoholic steatohepatitis; Pio, pioglitazone; SU, sulfonylurea.

Supplementary Table3 –A multivariate analysis of the association between fibrosis progression and independent predictors

variable	*r*	*t* -statistic	*p*
the gradient of HbA_{1c}	1.23	2.48	0.04
AST (at baseline)	-9.79	-2.14	0.08

Variables were adjusted by age, gender, and BMI.

Supplementary Table4 –A logistic regression analysis of factors associated with liver fibrosis improvement

Factor	Odds ratio	95% CI	*p*
Age (years)	1.01	0.91-1.13	0.75
Gender（male)	2.66	0.18-38.5	0.47
BMI (kg/m^2)	0.92	0.63-1.35	0.69
Diabetes	0.23	0.00-16.4	0.5
Hypertension	18.2	0.58-566.8	0.09
Dyslipidemia	0.5	0.04-5.74	0.57
Observation period (years)	1.02	0.97-1.07	0.38
Treatment with ARB	0.4	0.00-69.5	0.73
Insulin	0.52	0.00-50.0	0.78
△A1c	0.47	0.21-1.01	0.04

Multivariate model is adjusted for all variables for the model.